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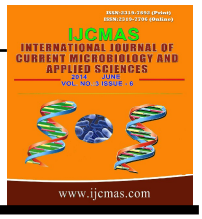

Original Research Article

Antimicrobial and Hemolytic activity of seaweed *Padina gymnospora* from South Andaman, Andaman and Nicobar Islands of India

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A B S T R A C T

To evaluate *In vitro* antimicrobial and hemolytic activity of seaweed *Padina gymnospora* collected from Wandoor marine beach, South Andaman. The seaweeds was collected from Wandoor area, south Andaman, Andaman and Nicobar Islands and crude extract was screened for antimicrobial and hemolytic activity. Methanol extract was tested against selected human pathogens, *E.coli, S. aureus, S. epidermidis, B.cereus, S. typhi, S. flexneri, P. aeruginosa, K. pneumoniae, P. mirabilis, Candida albicans, Aspergillus niger*. Crude extract was also analysed for hemolytic activity to study the toxicity. Maximum of 21.67 ±1.15 mm inhibition zone was observed against *S. aereus* and the minimum 12.3 ± 0.58 mm against *P. mirabilis* among the studied bacterial strains. *P. gymnospora* also showed activity against the fungal pathogens. The results confirms that seaweeds *P. gymnospora* can be further studied for purification of antimicrobial compounds.

Keywords

P. gymnospora, Antimicrobial activity, Human pathogens, Hemolytic assay, Relative Percentage Inhibition.

Introduction

The interest in marine organisms as a potential and promising source in marine organisms has increased during the last decades (David et al., 2003). Seaweeds have been used as food stuff in Asian countries from centuries as it contains carotenoids, dietary fibres, proteins, vitamins and minerals. Red and brown algae are mainly used as human food sources (Manivannan et al., 2011). Marine algae are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan. Seaweeds also know to possess valuable medicinal components such as anticoagulants, antiangiogenic and

antiadhesive activities (Albana Cumashi et al., 2007). Recently seaweeds have received significant attention for their potential as natural antioxidants, anti-bacterial and cytotoxic properties (Neeta et al., 2012, Kayalvizhi et al., 2012, Mayalen et al., 2007). In ever-growing antimicrobial resistant world, the prevention and treatment infectious diseases by marine seaweeds appears to be a possible alternative resource.

Seaweeds or marine algae are primitive non-flowering plants without true root stem and leaves. There are quite a lot of reports on antibacterial activity of solvent extracts from marine algae. Several compounds from the seaweeds have shown pharmacological activities, primarily for treating deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS), arthiritis etc. (Hiroomi Funahashiet al., 2004; M. Witvrouw et al., 1994;Fauziah et al., 2013), while some compounds have been used to treat inflammation etc (Albana Cumashi et al., 2007).

Andaman Islands marine ecosystem is unique, isolated and understudied compared to other marine ecosystem,may have potential of rich source of antimicrobial compounds. Therefore, it was worthwhile to investigate the antimicrobial and hemolytic activity of marine seaweed *Padinagymnospora (P. gymnospora)* against human pathogenic bacteria and fungi that often cause of infectious disease in human beings.

Materials and Methods

Collection of seaweeds

The seaweeds *Padina gymnospora* was collected in bulk quantity from Wandoor area, south Andaman, Andaman and Nicobar Islands, India. Seaweeds were collected in dispobags (Himedia) along with the seawater and brought to the laboratory. Seaweeds were washed thoroughly with sterile seawater to remove attached debris and sand particles. The final washing was done using double distilled water and dried under shade. The dried seaweeds were grinded to fine powder using the mechanical grinder.

Extraction process

The chemical extraction was done as per the method followed by Salem et al. (2011) with minor modifications. 25gms of dried seaweed powder was suspended in 250ml of 95% methanol for one week with intermediate shaking at room temperature. The extract was filtered with Whatman No. 1 paper and evaporated under reduced pressure at 45^{0}C using the rota evaporator (Eppendorff Concentrator 5301, Germany). The resulting crude was stored at 4^{0}C in the refrigerator for further study.

Microbial test cultures

In-vitro antimicrobial activity was evaluated against 11 pathogenic microorganisms *viz. Escherichia coli*(MTCC 443), *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 737), *Bacillus cereus* (MTCC 1272), *Klebsiella pneumoniae* (MTCC 129),*Staphylococcus epidermidis* (MTCC 3615), *Shigella flexneri* (MTCC 1457), *Pseudomonas aeruginosa* (MTCC 1688),*Proteus mirabilis* (MTCC 425),*Candida albicans* (MTCC 227) and *Aspergillus niger*(MTCC 282). All these microorganisms were procured from IMTECH, MTCC, Chandigarh. Bacterial strains were maintained on Nutrient Agar

slants at 4^0C, while fungal strains were maintained on Sabourand Dextrose Agar (SDA) slants. The fresh cultures were obtained by growing the test strains overnight at 37^0C for bacteria while fungi were grown at 28^0C for 48 hours.

Antimicrobial assay

Antimicrobial assay of crude extracts was performed against 11 pathogenic strains by disc diffusion method (Ghassan et al., 2008). The suspension ($1x10^8$ cells/ml) of each tested isolate was spread on the surface of complete media plate using cotton swab. Wells of 8 mm in diameter were made using cork borer in the media, 100µl of each crude extract was added in respective wells. DMSO, Amoxyclav (for bacteria) and Fluconazole (for fungi) was used as controls. The bacterial cultured plates were incubated at 37^0C for 24 hours and fungal cultured plates were incubated at 28^0C for 72 hours. The radius for the zone of inhibition was measured. Experiments were carried out in triplicates and repeated twice to confirm the results.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula (Gaurav et al., 2010).

Relative percentage inhibition of the test extract=

$$\frac{(X-Y)}{(Z-Y)} \times 100$$

Where,

X: total area of inhibition of the test extract

Y: total area of inhibition of the solvent

Z: total area of inhibition of the standard

Hemolytic activity

In vitro hemolytic activity was performed as described previously (EP, P.B. Mccray Jr et al., 2000) with modifications. Blood was collected from a healthy volunteer in heparinized tubes and washed three times in PBS. A volume 1 ml of 10% RBCs suspension was mixed with 1 ml of crude extracts (125, 250, 500, 1000µl) were dispensed in dried, clean glass tubes. Distilled water and PBS were served as positive and negative control respectively. After 1 hour incubation, cell suspensions were centrifuge for 10 min at 1500 X g and supernatants were transferred to a flat bottom 96-well plant. The absorbance (A) was read at 492 nm by ELISA reader (Tecan). Experiment was performed in triplicates at each concentration. The level of percentage haemolysis was calculated using the formula,

$$\text{Haemolysis (\% of control)} = \frac{(\text{A of sample} - \text{A of blank})}{(\text{A of positive control} - \text{A of blank})} \times 100$$

Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analysed statically by using Microsoft Excel 2013.

Results and Discussion

Antimicrobial assays

The antimicrobial activity of methanol extract of the *P. gymnospora* was presented in the Table 1. The extract of the *P. gymnospora* shows a strong inhibition in the growth of tested bacteria compare to the standard used in the study. The maximum zone of inhibition was observed against *S.aureus* and minimum in *P. mirabilis.*

Table.1 Antimicrobial activity of *Padina gymnospora*

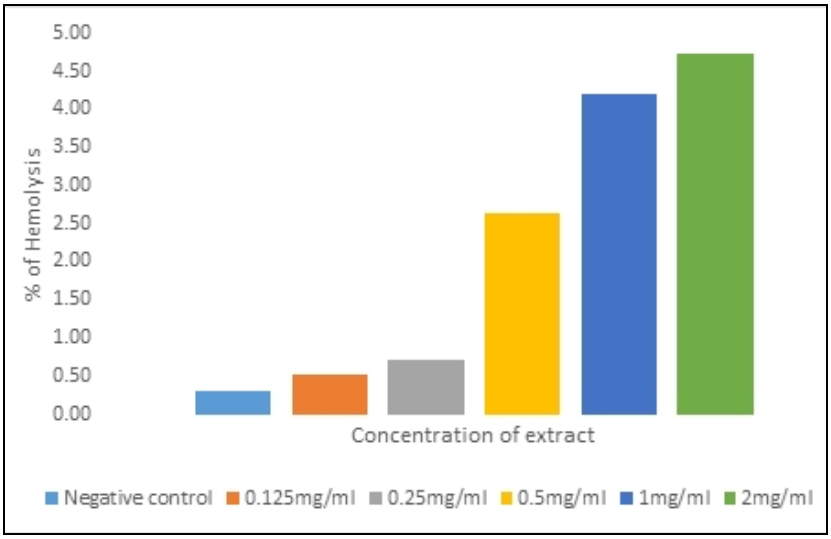
Test Organism	Inhibition zone diameter (mm)		
	ME	PC	NC
E.coli	17.67±0.58	12.33±0.58	-
S. aureus	21.67±1.15	16.67±0.58	-
S. epidermidis	13.33±1.53	27.33±1.15	-
B.cereus	19.67±0.58	22.00±0.00	-
S. typhi	18.67±0.58	20.67±0.58	-
S. flexneri	15.33±1.15	12.33±0.58	-
P. aeruginosa	14.33±0.58	12.67±0.58	-
K. pneumoniae	14.33±1.15	15.33±1.53	-
P. mirabilis	12.33±0.58	13.67±0.58	-
C. albicans	14.33±0.58	17.33±0.58	-
A. niger	11.00±1.73	18.67±0.58	-

ME: Methanol extract, PC: Positive control, NC: Negative control

Table.2 Relative percentage inhibition of *Padina gymnospora* compare to standard antibiotics

Test organisms	Relative percentage of inhibition (%)
E.coli	143.24
S. aureus	130.00
S. epidermidis	48.78
B.cereus	89.39
S. typhi	90.32
S. flexneri	124.32
P. aeruginosa	113.16
K. pneumoniae	93.48
P. mirabilis	90.24
C. albicans	82.69
A. niger	58.93

Figure.1 Hemolytic activity of *Padina gymnospora*



*C. albicans*was found to be more susceptible among the fungal strains used in the study.

Relative percentage inhibition

The result of antimicrobial activity of crude extract was compared with the positive control for evaluating their relative percentage inhibition (Table 2). The methanol extract exhibits maximum relative percentage inhibition against *E.coli* (143.24%) followed by *S.aureus* (130.0%), *S. flexeneri* (124.32%), *P. aeruginosa* (113.16%) respectively.

Hemolytic activity

Limitation of extracts to be used in therapy is their potential to cause damage to mammalian cells. In order to assess, we examined the ability of crude extract of *P. gymnospora* against normal human erythrocytes. Extracts exhibited low hemolytic effect towards human erythrocytes. Hemolytic activity of crude extract is expressed in % hemolysis. Methanolic extract (at dose 2000 µg/ml) possess maximum hemolytic activity (4.73%) with an IC_{50} value 21142 µg/ml which is much higher than the concentrations show antibacterial potential. Hemolytic percentage was found to be increasing with increase in concentration (Figure 1).

Seaweeds are considered as source of bioactive compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory antiviral activities have been detected in green, brown and red algae (Mayer AMS and Hamann MT 2002). In the present work methanolic extract of *P. gymnospora* inhibited most of the tested bacteria such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Klesiella pneumoniae*, *S. epidermidis*, *S. flexneri*, *P. aeruginosa*, *P. mirabilis*. The test algae shows greater zone of inhibition against some pathogens when compared with positive control. The results also showed seaweed possess very less hemolytic activity and further it may be used for the isolation of bioactive compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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