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ANTIBACTERIAL, ANTI-DIABETIC AND ANTI-INFLAMMATION PROPERTY OF THE SEA WEED, *PORTERESIA COARCTATA*, COLLECTED FROM MANGROVE FRINGED MUDFLAT OF SUNDARBAN COAST, WEST BENGAL

Harekrishna Jana[1]* and Keshab Chandra Mondal[2]
[1]Department of Microbiology, Panskura Banamali College,
Midnapur (E) 721152, West Bengal, India
[2]Department of Microbiology, Vidyasagar University,
Midnapur (W) 721102, West Bengal, India

ABSTRACT

Porteresia coarctata (Syn = *Oryza coarctata*) is a perennial halophytic wild grass, relative of rice, member of Poaceae and acts as a pioneer species in the succession process of mangrove formation along the estuaries of India. The sequestering carbon, fertilizer in aquaculture and salt tolerance property of this mangrove associate has been dealt with by a number of workers earlier**.** But, the present study was to evaluate the antibacterial property of aqueous, acetone, ethanol and methanol extracts of *Porteresia coarctata* collected from the Matla river of Indian Sundarban delta. Collected sea weeds were screened for their antibacterial studies against gram positive bacteria including *Staphylococcus aureus, Streptococcus fecalis* and *Bacillus subtilis* and gram-negative bacteria including *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Proteus vulgaris* using disc diffusion method. Present study shows that ethanol extracts of *Porteresia coarctata* has maximum antibacterial activity against *E.coli* (1.2±0.01 mm) and *Streptococcus fecalis* (1.4±0.01 mm) at an MIC of 700 µg/mL and 500 µg/mL, respectively. Along with the antimicrobial activities, seaweeds also showed anti-diabetic activity and but have no anti-inflammation activity. Therefore, the results suggest that these sea weeds could be exploited in the management of various infectious diseases and can be used as for pharmaceutical purpose.

Key word: *Porteresia coarctata, Antibacterial, Sundarban, Anti-diabetic, Anti-inflammation*

*Corresponding Author: Email: hkjmicro@gmail.com

INTRODUCTION

Seaweeds are large algae (macroalgae) that grow in saltwater or marine environment. Seaweeds are plants (Thallophytes), although they lack true stems, roots, and leaves. However, they possess a blade that is leaf-like, a stipe that is stem-like and a holdfast that resembles a root. Like land plants, seaweeds contain photosynthetic pigments (similar to chlorophyll) and use solar energy to produce food and oxygen from carbon dioxide and water. Seaweeds are the rich source for biodiversity and it is a rich source of medicine because it produces a host of biomolecules, most of which is probably involved as chemical against predation or infection (Yan *et al.,* 2002). Seaweeds are the source of important bioactive compounds and also possess various properties like antiviral, cytotoxic, antihelminthic, antioxidant, haemolytic, antifungal and antibacterial activities. They are also used as food, feed and fertilizer. They are the active ingredients of many life saving drugs for treatment of cancer, arthritis, *etc.* Marine seaweeds are rich in polyunsaturated acids, carotene, sulphated polysaccharides and sterols. The chemical substance may be a multitude of compounds like glycoside, alkaloids, terpenes, essential oils, steroids, hormones, vitamins, enzymes, plant acids, sugars, starches, fats, waxes, oleoresins, oleo gum-resins, resins, balsams, *etc.* The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anticancer, antiviral, antibacterial and cardio protective activities. Antioxidants may play a role in these health promoting activities (Yan *et al.*, 2002). Natural products which contain antioxidant properties, such as, phenolics and include flavonoids and phenolic acids (Klimczak *et al.*, 2007), carotenoids and vitamins (Rupasinghe and Clegg, 2007). Scientific studies of plants used in ethno medicine led to the discovery of many valuable drugs, including taxol, comptothecin, vincristine and vinblastine (Gupta and Roy 2004). Current interest in them stems from their antioxidants, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities (Thompson, 2000).

The Sundarban estuary regions of West Bengal have many different species of seaweeds. The stressful extreme habitat involving daily changes in pH of soil and water, humidity, salinity, temperature and tidal cycles may be possible reasons for many of these plants to synthesize a large number of different bioactive compounds. Many of these compounds have been found to have wide use in industry and human health care examples of which are : *Laminaria digitata* and *Ascophyllum* are even used as animal feed as it includes zinc, molybdenum, nickel, tin, vanadium, fluoride and iodine. (Bandaranayake, 2002). Brown seaweed, *Macrocystis pyrifera* and the red seaweed *Gracilaria edulis* are used as fish feed, (Sayyed *et al.*, 2008) and as biomass for fuel, as cosmetics and even used in aquaculture (Rajkrishnan and Ponnusamy, 2006). Some scattered studies have also been carried out earlier by different authors on these seaweeds for the purpose of identifying various activities related to human health and other industrial uses. For example, Roome *et al.*, (2008) have studied antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Ecklona maxima, Lessonia flavicans* and *Durvillaea potatorum* extracts at very preliminary levels. There is a need to development of new seaweeds as drugs because of the resistance developed to existing antibiotics by pathogen. Hence there is a

need to search and design new alternative drugs from seaweed product to control microbial infection. Seaweed is the best choice to isolate bioactive natural product against bacteria and fungi. So, the aim of the present study was to investigate the antimicrobial, anti-diabetic and anti-inflammatory activity of *Porteresia coarctata,* collected from Indian Sundarban.

Aims and Objectives

The floral species in mangrove ecosystem can be categorized into true mangroves and mangrove associates. Example of mangrove associate species are *Porteresia coarctata*, *Ipomoea pes-caprae*, *Sesuvium portulacastrum* and several seaweeds like *Enteromorpha intestinalis*, *Ulva lactuca*, *Catenella repens, etc*. Mangrove associate floral species are also used for preparing fish feed. Feed prepared from P. *coarctata* and *Enteromorpha intestinalis* have been found to boost up the growth *Macrobrachium rosenbergii*. Keeping the importance of sea weeds into consideration we investigate the pharmaceutical importance of sea weeds. Therefore the aim of this work is –

1. To determine the antibacterial properties of sea weeds like *Porteresia coarctata.*
2. To determine the anti- diabetic property of sea weeds.
3. To determine the anti-inflammation property of sea weeds.

These sea weeds could be exploited in the management of various infectious diseases and extracts might have roles as pharmaceuticals property.

MATERIALS AND METHODS

Sample collection

The seaweed *Porteresia coarctata* was collected from Indian Sundarban.

Preparation of the seaweed extract

The seaweeds were collected, cut into small pieces and air dried in shade. The seaweeds were shade dried for 15 days and then pulverized into fine powder using sterile pestle and mortar. Using the fine powder, different extracts are prepared as follows:

Preparation of aqueous extract

10 gm of powdered seaweeds materials were extracted with 10 ml of sterilized d. H_2O and incubated in a rotary shaker for 72 hours at 37ºC. Thereafter, it was filtered with the help of Whatman no. 1 filter paper and centrifuged at 5000 for 15 min (Amirkaveei *et al.*, 2011). The supernatant was collected and was stored at 4ºC for the test against microorganisms.

Preparation of acetone extract

10 gm of powdered seaweeds materials were extracted with 10 ml of acetone and incubated in a rotary shaker for 72 hours at 37°C. Thereafter, it was filtered with the help of Whatman No.1 filter paper and centrifuged at 5000 rpm for 15 minutes (Amirkaveei *et al.*, 2011). The supernatant was collected and tested against microorganisms and was stored at 4°C for further use.

Preparation of methanol extract

An amount (10 g) of crushed material was taken separately into 10 ml of methanol and kept on a rotary shaker at 120 rpm for 72 h. After shaking, it was filtered with the help of Whatman no 1 filter paper, centrifuged at 5000 rpm for 15 min (Amirkaveei *et al.,* 2011). The supernatant was collected and tested against microorganisms and was stored at 4°C for further use.

Preparation of ethanol extract

10 gm of powdered seaweeds materials were extracted with 10 ml of ethanol and incubated in a rotary shaker for 72 hrs. at 37°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper and centrifuged at 5000 rpm for 15 minutes (Amirkaveei *et al.*, 2011). The supernatant was collected and tested against microorganisms and was stored at 4°C for further use.

Qualitative antibacterial assays

Antibacterial sensitivity of the sea weeds extracts was tested by the agar well diffusion method using Nutrient agar media. The agar diffusion method was employed for the determination of antibacterial activities according to the method described by Bauer *et al.* (1966). The compounds under investigation were dissolved in respective solvent to a final concentration of 1000 µg/mL. Eight species of pathogenic bacteria, namely, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus fecalis, Salmonella typhi, Proteus vulgaris, Pseudomonas aeruginous* and *Bacillus subtilis* were used to screen the antibacterial activity of the seaweed extracts (Nostro, 2000). Pathogenic bacterial strains were incubated in sterile nutrient broth and incubated at 37°C for 24 h. The pathogen were swabbed (Inoculums size was adjusted so as to deliver a final inoculums of approximately 10^6 CFU/mL) on the surface of nutrient agar media and petri dishes containing 20 ml of Mueller–Hinton Agar with 100 µL inoculums of bacterial strain and media was allowed to solidify. Wells were cut into solidified agar media with the help of sterilized cup-borer. 100 µL of each sample solution was poured in the respective wells and the plates including control were incubated overnight at 37°C for bacteria. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each compound was expressed in terms of the mean diameter of zone of inhibition (cm) produced by the respective compound.

Determination of MIC value

Minimum inhibitory concentration was determined using inhibitory concentration in diffusion (ICD) method (Guerin-Faublee *et al.*, 1996). The minimal inhibitory concentration (MIC) values, which represent the lowest concentration of the compound that completely inhibits the growth of microorganisms, were determined by a micro-well dilution method (Wade, 2001). The inoculums of each bacterium were prepared and the suspensions were adjusted to 10^6 CFU/ml. For making this dilution, each lyophilized materials were dissolved at a concentration and serially diluted in distilled water, acetone, methanol and ethanol to obtain seaweeds extracts concentration of 1000 µg/ml, 800 µg/ml, 700 µg/ml, and 500 µg/ml were prepared. 100 µl of each seaweed extracts of different concentration was poured in the respective wells and the plates were incubated.

Determination of anti-inflammatory property of sample

The reaction mixture consisted of extracts at concentration 1 µg/ml and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted to 6.5 using 1(N) HCl and incubated at 37ºC for 20 minutes and then heated at 57ºC for 30 minutes. The denaturation process is stopped by cooling the samples and finally the turbidity was measured using colorimeter 660 nm. Aspirin was used as the reference standard and the control was taken without the extract (Suganya *et al.,* 2014).

Determination of anti-diabetic property of sample

The alpha-amylase inhibitory activity for seaweed extract was determined by the colorimetric assay. 1% of potato starch was soluble in 100 ml distilled water by boiling for 15 minutes. The enzyme solution was prepared by mixing 0.001 g of alpha amylase in 100 ml of 20 mM phosphate buffer (pH 6.9) contains 6.7 mM sodium chloride. The colour reagent was prepared by mixing 3, 5-dinitrosalicycllic acid and 5.31(M) sodium potassium tartarate in 2M sodium hydroxide (8 mL) with 12 ml distilled water. One ml of sample and one ml enzyme solution were mixed in a test tube and incubated at 25ºC for 30 minutes. Then, one ml colour reagent was added and tube was placed at 85ºC in water bath. Then, 1 ml starch solution was added and incubates at 25ºC for 30 minutes. After 15 minutes, reaction mixture was removed from the water bath, cooled and diluted with 9 ml distilled water. The absorbency was determined at 540 nm (Suganya *et al.,* 2014).

Statistical analysis

Each sample was analyzed in triplicate and data were represented as mean ±SD. Analysis was done by using MS excel.

RESULTS

Results of the present study are shown in Tables 1–4.

Table 1. Antimicrobial activity of specific concentration (1000 µg/ml) of different seaweed extracts compared with control by agar well diffusion method

Sea weeds	Extracts	Zone of Inhibition (cm)							
		Bacillus sp.	***Pseudomonas sp.***	***Proteus sp.***	***E.coli ATCC***	***S.typhi ATCC***	***Staphylococcus sp.***	***K. pneumonia***	***Streptococcus sp.***
	Acetone	0.9±0.05	1.0±0.03	0.9±0.05	0.9±0.05	1.0±0.04	0.9±0.05	0.9±0.05	1.0±0.03
	Methanol	1.0±0.04	0.9±0.05	0.9±0.05	0.9±0.05	1.0±0.05	0.9±0.05	0.9±0.05	0.9±0.05
	Ethanol	0.9±0.03	0.9±0.05	1.0±0.04	1.2±0.03	1.1±0.04	1.0±0.04	1.0±0.04	1.4±0.04
	Water	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05

Table 1 contd.

Sea weeds	Extracts	Zone of Inhibition (cm)							
		Bacillus sp.	***Pseudomonas sp.***	***Proteus sp.***	***E.coli ATCC***	***S.typhi ATCC***	***Staphylococcus sp.***	***K. pneumonia***	***Streptococcus sp.***
Control	Acetone	0.9±0.02	0.9±0.05	0.9±0.05	1.0±0.04	1.0±0.02	1.0±0.04	0.9±0.05	0.9±0.05
	Methanol	1.0±0.04	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	1.0±0.04	1.0±0.04	1.0±0.04
	Ethanol	0.9±0.05	0.9±0.05	1.0±0.03	1.0±0.02	0.9±0.05	0.9±0.05	1.0±0.04	0.9±0.05
	Water	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.05

Table 2. Minimum Inhibitory Concentration (MIC) value of different seaweeds against sensitive pathogenic bacteria (Please *vide* Fig. 1)

Name of Bacteria	Concentration (µg/ml)	Antimicrobial zone(mm)
Streptococcus sp.	800	13.5±0.1
	700	12.0±0.1
	500	11.0±0.1
	400	–
E. coli	1000	12±0.1
	800	11±0.1
	700	10±0.1
	500	–

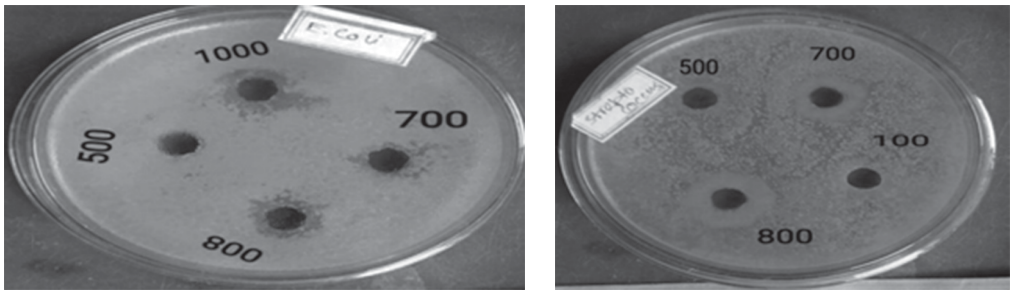

Fig. 1. MIC value of ethanol extracts of *Porteresia coarctata* against sensitive pathogenic bacteria

Table 3. Determination of anti-inflammatory activity of different seaweed extract.

Seaweeds	Extracts	OD value at 660nm at different concentration (µg/ml)		
		Blank	Test	Control
Porteresia coarctata	Ethanol	0	0.05	0.07

Table 4. Determination of anti-diabetic activity of different seaweed extract.

Seaweeds	Extracts	OD Value at 600nm at different concentration		
		Blank	Test	Control
Porteresia coarctata	Ethanol	0	0.08	0.10

DISCUSSION

The results of antimicrobial assay of *Porteresia coarctata* extracts showed formation of zone of inhibition surrounding the discs. In our study, it was found that the different solvent extract of the seaweed *Porteresia coarctata* showed antibacterial activity against *Escheirichia coli, Salmonella typhimurium* ATCC, *Klebsiella pneumonia, Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aurenginosa, Proteus vulgaris and Bacillus subtilis* (Table 1). But, maximum inhibitory effects of ethanol extracts showed against *E.coli* (12 mm) and *Streptococcus faecalis* (14 mm). The MIC value of ethanol extracts are summarized in the Tables 3 and 4. Present result indicates that the ethanol extracts is more effective against Gram positive bacteria than Gram negative. The differences in inhibitory effect may be due to the variants of cell wall structure of Gram-positive and Gram-negative, or it might be due to the permeability barrier in the cell wall. In addition, unlike gram-positive bacteria, the lipopolysaccharides layer along with proteins and phospholipids are the major components of the outer layer of Gram-negative bacteria. So, the outer lipopolysaccharides layer may hinder access of antibacterial compounds to the peptidoglycan layer of the cell wall (Abeysinghe, 2010). So, sea weeds extracts can be used as a folk medicine for the coastal people. Therefore, further phytochemical analysis is required to find the bioactive compound present in sea weed, responsible for antimicrobial activity.

CONCLUSION

It can be concluded that the ethanol extracts of the *Porteresia coarctata* has antimicrobial activities against different pathogenic bacteria and can be regarded as a new source of antibacterial compounds. However, further research needs to be done on the identification of the bioactive compounds present in the extract. In future studies, other bioactive compounds of *Porteresia coarctata* may be analyzed qualitatively and also quantitatively with different solvent extracts. Therefore, the results suggested that these sea weeds could be exploited in the management of various infectious diseases and can be used as for pharmaceutical purpose.

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