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Evaluation of antibacterial and antioxidant activities of seaweeds from Pondicherry coast

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ABSTRACT

Seaweeds with their diverse bioactive compounds have opened up potential opportunities in pharmaceutical and agri-food processing industries. The present study designated to evaluate antibacterial and antioxidant potential of eight seaweeds (Enteromorpha compressa, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, Chaetomorpha antennina, Padina gymnospora, Grateloupia lithophila, and Hypnea valentiae) from Pondicherry coast. Brown seaweed P. gymnospora showed maximum zone of inhibition against all the five pathogens examined with maximum activity against K. pneumonia (14.7±0.5 mm). In this study, antioxidant activities were tested using five different assays, total phenolic content, total antioxidant activity, reducing power, Hydrogen peroxide radical scavenging assay, DPPH radical scavenging activity and maximum activity was observed in P. gymnospora.

Keywords: Antimicrobial activity; Antioxidant activity; Total phenolic content; Total antioxidant activity; Reducing power; Hydrogen peroxide radical scavenging assay, DPPH radical scavenging activity

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INTRODUCTION

Algae have become very useful in many different industries. Aside from its uses in the food manufacturing industry, it is also being studied for its components which are believed to have medicinal effects. According to Abou-Elela *et al.,* 2009 marine organisms are rich source of structurally biologically active metabolites and studies suggested that some bioactive compounds isolated from marine organisms had shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities. Several algae have been found to have secondary metabolites and most of which are phenolic compounds, which have medicinal potentials (Aliyu *et al*., 2009) and are important in developing new pharmaceutics (Menelo *et al.*, 2012).

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. Due to the increase in bacterial resistance against commercial antibiotics there is a growing need, for new antibacterial compounds that are active against pathogenic bacteria. In addition, most of the existing antibiotics are occasionally associated with adverse effects to the host, including hypersensitivity, immune suppression and allergic reactions. These developments demand that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Extracts of marine algae were reported to exhibit antibacterial activity (Siddhananta *et al.*, 1997).

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage that in turn leads to geriatric degenerative conditions, cancer and a wide range of other human diseases (Aruoma, 1999; Borek, 1993; Reaven & Witzum, 1996). Carotenoids, the natural pigments from plant origin react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration (Akoh & Min, 1997). Furthermore, antioxidants from natural sources increase the shelf-life of foods (Schwarz *et al.*, 2001). Therefore, consumption of antioxidant and/or addition of antioxidant in food materials protect the body as well as foods against these events (Chandini *et al.*, 2008).

Many researchers have reported various types of antioxidants in different kinds of higher plants (Larson, 1988; Shon *et al.*, 2003). More recent reports revealed seaweeds to be a rich source of antioxidant compounds (Duan *et al.*, 2006; Kuda *et al.*, 2005; Lim *et al.*, 2002; Park *et al.*, 2004). Having the advantages of low cost raw material, high amounts of secondary metabolites, and no secondary pollution, this alga may be used to be a natural source of antibacterial and antioxidant agents. This study aimed to document antibacterial and antioxidant activity of eight seaweeds collected from Pondicherry coast.

MATERIALS AND METHODS

2.1. Sample collection and preparation

Fresh thallus of *Enteromorpha compressa*, *Enteromorpha intestinalis*, *Ulva fasciata*, *Ulva lactuca*, *Chaetomorpha antennina*, *Padina gymnospora*, *Grateloupia lithophila*, and *Hypnea valentiae* were collected from the intertidal regions of Pondicherry coast, India. The seaweeds were washed thoroughly with to remove extraneous materials. Washed samples were shade dried and ground with the help of electric mixer. The seaweed powdered samples were then stored in refrigerator for further use.

2.2. Preparation of crude extract

The seaweed powders were extracted with acetone in soxhlet extractor for 12 h. The extracts were then concentrated under reduced pressure using a rotary flash evaporator. The crude extracts obtained were stored in dark at 4ºC for further use.

2.3. Antibacterial activity

2.3.1. Bacterial strains

Human pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* and *Klebsiella pneumonia* were obtained from the Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamilnadu, India. The bacterial stock cultures were maintained in nutrient agar slant at 4°C.

2.3.2. Disc Diffusion assay (Kartnig *et al.*, 1991)

Whatmann filter paper (No.1) discs of 6mm diameter were impregnated with 10 µl of the solution containing crude extracts obtained from the seaweeds (at a concentration of 100mg/ml) and these discs were evaporated at 37°C for 24 h. Reference standard discs were prepared with streptomycin (50 µg/ml) to compare the antibacterial activity of seaweed extracts. After drying, the discs with seaweed extract and standard streptomycin were placed on Muller Hinton agar (MHA) already swabbed by bacterial stock cultures and incubated at 37°C for 24 h. After incubation, plates were examined for clear zone around the discs. A clear zone with diameter more than 2 mm was taken as a antibacterial activity. All the experiments were carried out in triplicate and the mean values were recorded.

2.4. Antioxidant assay

2.4.1. Evaluation of antioxidant activity

The lyophilized seaweed extracts were dissolved in distilled water at a concentration of 10 mg ml^{-1}. The free radial scavenging activity of the seaweed extracts was evaluated using standard procedures and Gallic acid was used as the reference compound. All analysis were run in triplicates and averaged.

I. Total phenolic content

Phenolic contents of crude extracts were estimated by the method of Taga *et al.* (1984). Briefly 100 µl of aliquot sample was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation,

100 µl of 50% Folin Ciocalteau's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Phenolic content are expressed as Gallic acid equivalent per gram).

II. Total antioxidant activity

Total antioxidant activity was measured following the method of Prieto *et al*. (1999). To 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml distilled water and labeled as Total Antioxidant Capacity (TAC) reagent. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

III. Reducing power

Reducing power of different crude extract was determined by the method prescribed by Oyaizu (1986). 1.0 ml of different solvent extract containing different concentration of samples was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50ºC for 20 min. After incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 min. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance indicates increased reducing power.

IV. Hydrogen peroxide radical scavenging assay

The ability of seaweed extract to scavenge hydrogen peroxide was determined by following the standard procedure (Gulçin *et al*., 2004). Hydrogen peroxide 10 mM solution was prepared in the phosphate buffer saline of 0.1 M, pH 7.4. 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide) after 10 min of incubation at 37ºC. The percentage of scavenging of hydrogen peroxide was calculated using the following formula

% scavenging (H_2O_2) = (A_0-A_1/A_0) X 100

A_0 - absorbance of control
A_1 – Absorbance of sample.

V. DPPH radical scavenging activity

The Scavenging effects of crude methanol extract and fractions were determined by the method of Yen and Chen (1995). Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The Scavenging effect (%) was calculated by using the formulae given by Duan *et al*. (2006).

Scavenging effect (%) = [1 - (A sample - A blank) / A control] × 100

A control - absorbance of the control (DPPH solution without sample)
A sample - absorbance of the test sample (DPPH solution + Test sample)
A blank - absorbance of the sample only (sample without DPPH solution).

RESULTS

3.1. Antimicrobial activity

Eight seaweed extracts were examined for antibacterial activity against five bacterial pathogens (*E. coli*, *S. aureus*, *P. aeruginosa*, *V. parahaemolyticus* and *K. pneumonia*). Brown seaweed *P. gymnospora* showed maximum zone of inhibition against all the pathogens examined with maximum activity against *K. pneumonia* (14.7±0.5 mm) and minimum activity against *E. coli* (10.7±0.6 mm). *P. aeruginosa* strain showed resistance and thus minimum activity was recorded to extracts of *E. compressa*, *E. intestinalis*, *U. fasciata*, *U. lactuca*, and *C. antennina* while maximum zone of inhibition was recorded as 13.8±0.9 mm against *P. gymnospora* which was higher than the value of positive control streptomycin (10.0±1.0 mm). *E. coli* also showed resistance against *U. fasciata*, *U. lactuca*, and *C. antennina*

while maximum zone of inhibition was recorded as 10.7±0.6 mm against *P. gymnospora*. The results are shown in **Table 1**.

Table 1: Antibacterial activity of seaweeds from Pondicherry coast

S.No	Bacterial Pathogen	Zone of Inhibition (mm)								
		E. compressa	***E. intestinalis***	***U. fasciata***	***U. lactuca***	***C. antennina***	***P. gymnospora***	***G. lithophila***	***H. valentiae***	**Streptomycin (+ ve cont.)**
1	***E. coli***	**3.0±0.5**	**3.4±0.6**	**–**	**–**	**–**	**10.7±0.6**	**5.1±0.4**	**6.4±0.7**	**13.3±1.1**
2	***S. aureus***	**2.9±0.3**	**3.1±0.7**	**4.1±0.9**	**3.8±0.4**	**3.2±0.8**	**13.6±0.6**	**5.3±0.7**	**5.2±0.6**	**22.7±1.4**
3	***P. aeruginosa***	**–**	**–**	**–**	**–**	**–**	**13.8±0.9**	**12.0±0.9**	**12.6±0.4**	**10.0±1.0**
4	***V. parahaemolyticus***	**4.7±0.2**	**5.1±0.7**	**–**	**–**	**6.7±0.7**	**12.2±0.7**	**8.3±0.7**	**9.0±0.8**	**12.0±0.9**
5	***K. pneumoniae***	**3.2±0.3**	**3.5±0.9**	**3.6±0.8**	**3.7±0.2**	**4.8±1.0**	**14.7±0.5**	**8.6±0.8**	**8.6±0.5**	**11.4±1.1**

3.2. Antioxidant activity

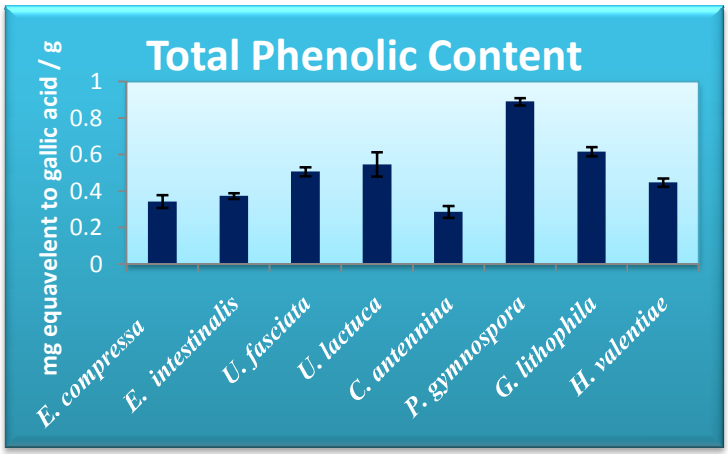
3.2.1. Total phenolic content

Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant activity. Acetone extract of *P. gymnospora* followed by *G. lithophila* exhibited higher phenolic content of 0.89±0.02 and 0.61±0.02 mg GAE/g of seaweed extract respectively (**Fig. 1**), as compared to other seaweeds analysed. Minimum phenolic content of 0.28±0.03 mg GAE/g was recorded in *C. antennina*.

3.2.2. Total antioxidant activity

Total antioxidant activities of eight seaweeds are presented in **Fig. 2**. Higher activity of 1.92±0.05, 1.54±0.07 and 1.27±0.05 mg ascorbic acid equivalent/g of seaweed was observed in *P. gymnospora*, *G. lithophila*, and *H. valentiae* respectively. Minimum activity of 0.64±0.03 mg ascorbic acid equivalent/g of seaweed was observed in *C. antennina*.

Fig. 1: Total phenolic content of seaweeds from Pondicherry coast



3.2.3. Reducing power

Reducing power of the seaweeds analysed are presented in the **Fig. 3**. The maximum (2.678±0.03) reducing power value was observed in 1 ml concentration of *P. gymnospora* and minimum (0.69±0.02) was obtained in *E. compressa*.

3.2.4. Hydrogen peroxide radical scavenging assay

Hydrogen peroxide radical scavenging activity of *P. gymnospora* was recorded in highest percentage (91%) followed by *G. lithophila* (54%) and *H. valentiae* (54%) respectively. The lowest scavenging activity was recorded in *C. antennina* (28%), *E. compressa* (31%) and *E. intestinalis* (31%) respectively which was shown in **Fig. 4**.

Fig. 2: Total antioxidant activity of seaweeds from Pondicherry coast

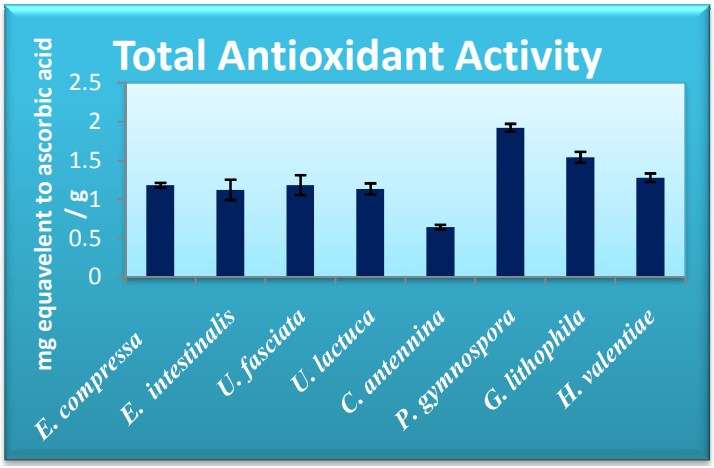


Fig. 3: Reducing power of seaweeds from Pondicherry coast

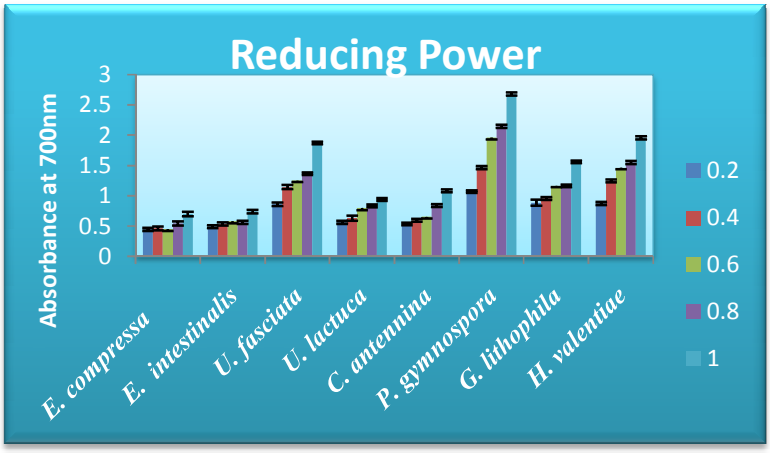
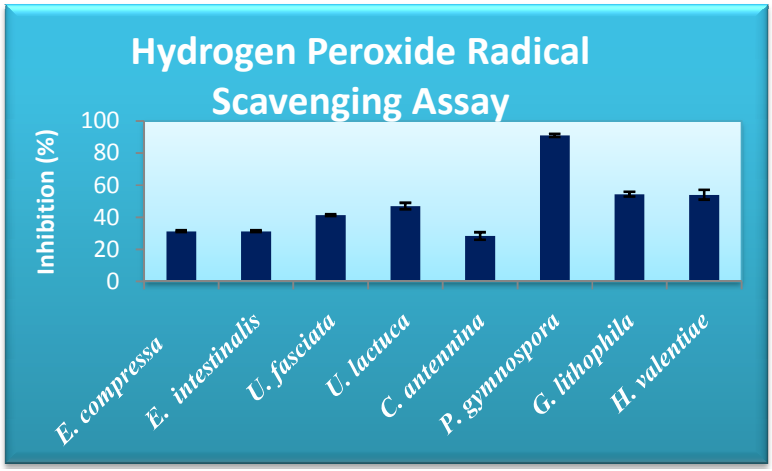


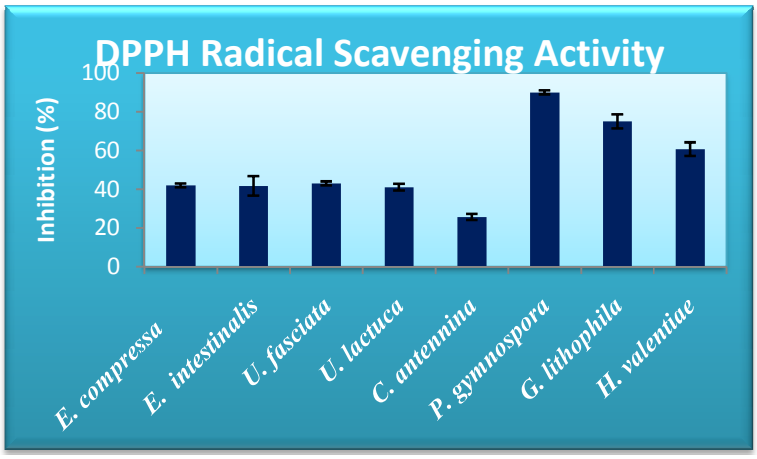
Fig. 4: Hydrogen peroxide radical scavenging assay of seaweeds from Pondicherry coast



3.2.5. DPPH radical scavenging activity

Free radical scavenging ability of seaweeds were expressed in percentage (%) and shown in **Fig. 5** Maximum activity was found in *P. gymnospora* (90%) followed by *G. lithophila* (75%) and *H. valentiae* (60%) respectively and the lowest activity was recorded in *C. antennina* (25%), *U. lactuca* (41%) and *E. intestinalis* (41%).

Fig. 5: DPPH radical scavenging activity of seaweeds from Pondicherry coast



DISCUSSION

Antimicrobial activities found in seaweeds were considered to be an indication of synthesis of bioactive secondary metabolites. The marine macroalgae have an effective antibacterial activity aginst most of the human bacterial pathogens. It was reported that 151 species of macroalgal crude extracts showed inhibitory activities against pathogenic bacteria (Hornsey and Hide, 1985). There have been a number of reports that demonstrate the antimicrobial activity of marine plants (Zampini *et al.*, 2009), marine algae or seaweeds (Devi *et al.*, 2008; Haliki *et al.*, 2005; Nair *et al.*, 2007; Sasidharan *et al.*, 2010), Mangrove flora (Chandrasekaran *et al.*, 2009) and seagrass (Kumar *et al.*, 2008; Kannan *et al.*, 2010). Still, in India only limited information is available on marine algae.

Kandhasamy and Arunachalam (2008) had studied the Chlorophyceae members and it showed high antibacterial activity than other members. The current study inferred with Phaeophyceae members showed higher antibacterial activity than Rhodophyceae and Chlorophyceae. The brown algae have naturally high secondary metabolites compared to red and green. The results shows that the brown algae *P. gymnospora* a strong antimicrobial activity against with streptomycin standard. Extracts of marine brown algae have been reported to exhibit antibacterial activity (Kim *et al.*, 2007; Kamenarska *et al.*, 2009) and antimicrobial activity (Tringali, 1997; Funahashik *et al.*, 2001).

In the present study, brown seaweed *P. gymnospora* showed maximum zone of inhibition against all the pathogens examined with maximum activity against *K. pneumonia* (14.7±0.5 mm) and minimum activity against *E. coli* (10.7±0.6 mm). The present results agreed with the findings of Rao and Parekh (1981) and Padma Kumar and Ayyakkannu (1997) that organic extract of Indian seaweed exhibit antimicrobial activity against gram negative and gram positive biomedical pathogens. In the present findings we have immense potential on the control of clinical pathogens, since the strains used in the study were collected from hospital sources and most of the strains appeared as multi drug resistant and cannot be controlled with commercially prescribed antibiotics (Adwan and Abu-Hasan, 1998).

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds are also rich in natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007). The presence of phytoconsituents, such as phenols, flavanoids and tannins in seaweeds and seagrasses indicated a possible role that its extracts may

have antioxidant activity. This activity was believed to help in preventing a number of diseases through free-radical scavenging activity (Anggadiredja *et al*., 1997; Ruberto *et al.*, 2001).

In the present study, the total phenolic content ranged from 0.28±0.03 to 0.89±0.02 mg GAE g^{-1} with minimum in the green seaweed *C. antennina* and maximum in the brown seaweed *P. gymnospora*. Similarly, Jimenez *et al.* (2001) reported that brown seaweeds contain higher phenolic content than the red seaweeds. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yukimoto, 2003) and also known to contain phlorotannins and fucoxanthin as major active compounds (Yan *et al.*, 1996; 1999). Higher total antioxidant activity of 1.92±0.05 mg ascorbic acid equivalent/g of seaweed was observed in *P. gymnospora*, and minimum activity of 0.64±0.03 mg ascorbic acid equivalent/g in *C. antennina*.

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. The DPPH assay method is based on the reduction of DPPH, a stable free radical. With the odd electron, the free radical DPPH gives a maximum absorption at 517 nm by visible spectroscopy (purple color). As the odd electron of the free radical comes paired off in the presence of a hydrogen donor, e.g. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolourization (yellow colour) is stoichiometric with respect to the number of electrons captured (Blois, 1958). In the present study, the maximum percentage of radical scavenging activity was observed in brown seaweed *P. gymnospora* (90%) and minimum in *C. antennina* (25%). Similarly, Ganesan *et al.* (2008) noticed higher percentage DPPH radical scavenging activity in methanol extract of brown seaweed *Sargassum polycystum*. Hydrogen peroxide radical scavenging activity of *P. gymnospora* was recorded in highest percentage (91%) and lowest in *C. antennina* (28%). Parthiban *et al*. (2014) studied on the antioxidant activities of selected seaweeds from Tuticorin coast and found that brown seaweed *T. conoides* exhibited maximum antioxidant activity than red and green seaweeds analyzed.

In the reducing power assay, antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox linked colourimetric reaction (Li *et al*., 2006) that involves single electron transfer, the reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). Concentration dependency of antioxidant activity was investigated as a function of reducing power as this gave a general view of reductones present in the sample. Reducing power increased with increasing concentrations in all the samples. In the present study also reducing power increased with increasing concentration, maximum reducing power value was observed in 1 ml concentration of *P. gymnospora* (2.678±0.03). The present results are in agreement with Matsukawa *et al*. (1997), who found that the antioxidant activity of brown algae was superior to that of red or green groups.

CONCLUSION

Overall results showed that brown algae *P. gymnospora* exhibits maximum antibacterial and antioxidant activity. The antibacterial and antioxidant activities could be attributed to the presence of different secondary metabolites such as phenolic compounds, and carotenoids and the mechanism of action might be due to their individual or collective participation. The experimental findings envisaged *P. gymnospora* extract as a good candidate in developing new antibacterial and antioxidant agents.

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