Antioxidant Activity Of *Padina boergesenii* Allender & Kraft

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ABSTRACT
The antioxidant potential of the marine seaweed *Padina boergesenii* was evaluated. Two different solvents viz., ethanol and ethyl acetate were used for the study. The antioxidant activity was determined by DPPH, Hydroxyl radical scavenging and Total antioxidant assays. The results of the study showed that the selected seaweed possess significant antioxidant activity in the tested assays. The antioxidant potential was found to be maximum in the ethanol extract. Dose depended activity was noticed in all the tested samples.

Keywords: Antioxidant, bioactive compounds, free radicals, brown seaweed

INTRODUCTION

The Oxidative stress due to reactive oxygen species (ROS) are considered as the harmful products of usual metabolic processes in living organisms (Rout *et al*., 2022). Oxidative stress has been related with many multifactorial diseases like cancer, diabetic, cardiovascular diseases (Sachidanandame *et al.*, 2005), Parkinson's and Alzheimer disease, post-ischemic and neural degradation disease (Pirian *et al.*, 2017) and inflammatory disorders (Bodamyali *et al.*, 2000).
This oxidative stress caused by the productions of oxidants including antioxidant systems and ROS. The excess amount of ROS (reactive oxygen species) present in the body includes lipid peroxidation can react with or alter the structure of biomolecules in the body causing cellular disorders or cell death (Arive *et al*., 2017). Moreover, the number of free radicals present in our environment has increased making antioxidant intake necessary to counteract the effects of free radicals on human health (Arive *et al*., 2017). Free radicals are the main cause of various chronic diseases, such as cardiac, inflammation, hypertension, diabetes mellitus and neoplasia (Bhadury and Wright, 2004; Kolanjinathan and Stella, 2009; Badea *et al*., 2009). The damage subsequently leads to degenerative diseases (Fayaz *et al.*, 2005; Rojas *et al.*, 2002).
Many numbers of natural compound have been proven to exhibit antioxidant activity and be applicable for treatment of oxidative-damage related diseases. Antioxidants are the compounds that stop or interrupt the oxidation process in cells by scavenging free radicals and hence prevent or mitigate diseases (Kokabi *et al.*, 2013).
There are many artificial antioxidant substances, yet with unsafe effects. The drug discovery using natural products such as medicinal plants or marine organism still as an important target for recent research. Thus, exploring new natural antioxidants have been a challenge target. In this concern, marine seaweeds have been known as traditional sources of natural antioxidants (Samaraweera *et al.*, 2012).
Several researchers have reported the antioxidant properties of brown seaweeds from across the globe (Nakayama *et al*., 1999; Ismail and Hong, 2002; Lim *et al*., 2002; Heo *et al*., 2005; Kuda *et al*., 2005; Yuan *et al*., 2005; Duan *et al*., 2006).
Based on the above facts the effect of the extract of *Padina boergesenii* for its potential antioxidant property were evaluated. This study results are expected to provide baseline information for further development of Brown seaweed *Padina boergesenii* as a source of antioxidant ingredients.

MATERIALS AND METHODS

Preparation of seaweed extract

Padina boergesenii was collected, cleaned and cut into small pieces and dried under shade at room temperature. The dried materials were ground to fine powder using a mechanical blender and passed through 24 mesh sieves. The powdered sample were kept in close containers until used. The sample was further used to make the different extraction using ethanol and ethyl acetate by following standard methods (Pizzale *et al.*, 2002). The extracts thus obtained was directly used for the determination of DPPH radical scavenging assay, hydroxyl radical scavenging activity and Total antioxidant activity.

Antioxidant Activity

DPPH Radical Scavenging Assay

Different concentrations of sample such as 12.5µg/mL- 200µg/mL from stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. (Chang *et al*., 2001).

Hydroxyl Radical Scavenging Activity

various concentration such as 125-2000µg from a stock concentration of 10mg/mL were mixed with 500µl reaction mixture ((2 deoxy 2 ribose (2.8mM), FeCl3 (100µm), EDTA (100µm), H_2O_2 (1.0mM), ascorbic acid (100µm) in KH_2PO_4 - KOH buffer (20 mM pH 7.4)) was made up to a final volume of 1 ml. After incubation for 1 hour at 37°C, add 1ml of 2.8% TCA, then 1ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution (Elizabeth and Rao, 1990).

Total Antioxidant Activity

Different concentrations such as 125µg/mL -2000µg/mL from a stock concentration of 10mg/mL was obtained with 3ml of reagent solution (0.6ml H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate). The tube containing the reaction solutions were incubated at 95^0C for 90 minutes. The absorbance of the solution was measured at 695nm against blank Ascorbic acid (10mg/mL DMSO) was used as reference. The antioxidant activity is expressed as number of gram equivalent of ascorbic acid (Prieto *et al*., 1999).

RESULTS AND DISCUSSION

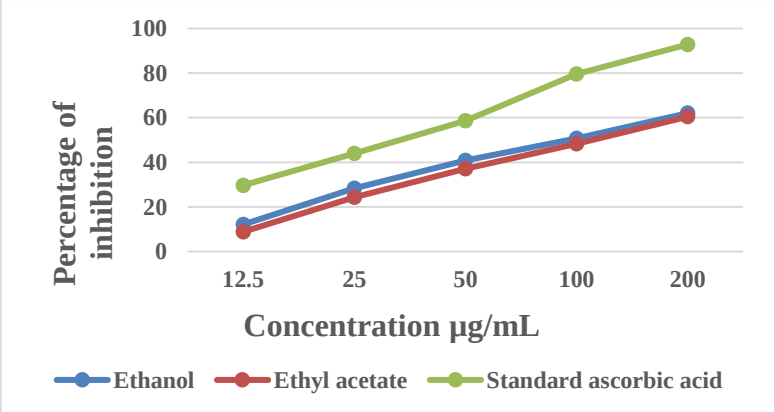
The antioxidant potential of seaweed *Padina boergesenii* was evaluated using three assays. Although several studies across the world have demonstrated the antioxidant capabilities of seaweed in the last two decades, there is scanty information regarding the antioxidant potential of the brown seaweed *Padina boergesenii* from south Tamil Nadu.

The antioxidant activity was determined by using two different solvents such as ethanol and ethyl acetate. The Free radical scavenging activity was analysed by DPPH radical scavenging assay, Hydroxyl radical scavenging assay and Total antioxidant activity. The extracts showed differences in their antioxidant activity based on concentration. Different concentrations like 12.5, 25, 50, 100, 200 µg/mL were used for the DPPH assay study. the ascorbic acid is used as control.

Table 1: DPPH radical scavenging activity of *Padina boergesenii*

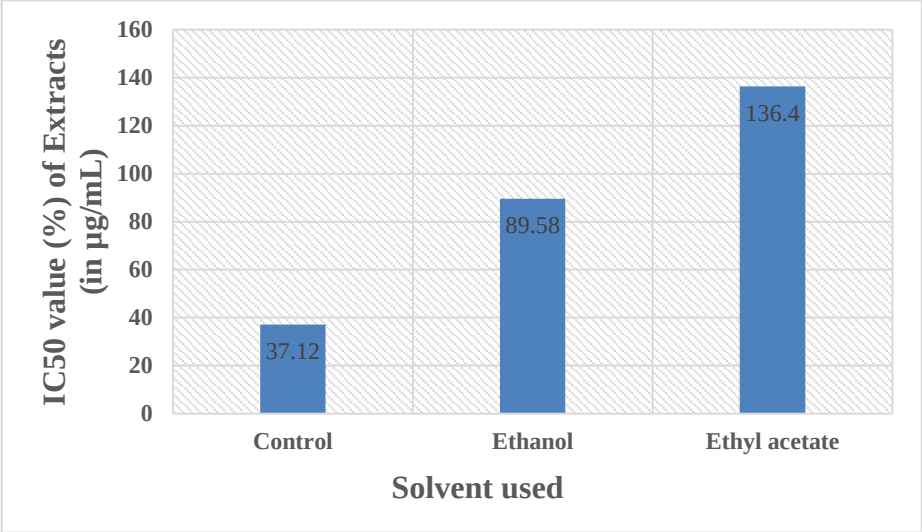
Concentration µg/mL	Percentage of inhibition		
	Ethanol	Ethyl acetate	Standard ascorbic acid
12.5	12.08	8.82	29.69
25	28.35	24.35	43.98
50	40.78	37.04	58.58
100	50.76	48.23	79.57
200	62.03	60.42	92.85

Fig 1: DPPH radical scavenging assay



IC_{50} Value of the DPPH radical scavenging activity

Solvent used	IC_{50} value (%) of Extracts
Control	37.12 µg/mL
Ethanol	89.58 µg/mL
Ethyl acetate	136.4 µg/mL



As the concentration of the extract increases the scavenging activity was also found to increase showing that these properties are dose dependent. The higher scavenging activity of *P. boergesenii* may be attributed to hydroxyl groups in the phenolic compounds, which might provide the essential component (Singh *et al*., 2006). The earlier works on *P. boergesenii* was found that the scavenging effect increased with the increase in concentration of the sample (Jeevitha *et al*., 2014). The previous studies on *Padina gymnospora* also showed that the difference in the scavenging activity was in a concentration-dependent manner (Balakrishnan and Pandina Devi, 2016). The influence of the number of bioactive substances might be responsible for higher antioxidant activity with the increase in concentration (Sobuj *et al*., 2021).

DPPH is an organic chemical compound 2,2-diphenyl-1-picrylhydrazyl and the abbreviated form is DPPH. It is a stable free radical molecule with a dark-coloured crystalline powder. Cotelle *et al*., (1996) reported that the DPPH had been used enormously as a free radical chemical to evaluate reducing substances. The salient feature of DPPH is the purple colour, usually disappeared while an antioxidant compound is present in the medium. (Subramanian *et al*., 2020). The results of the DPPH assay showed that the highest antioxidant activity was obtained by the ethanol extracts (62.03%) at 2000µg/mL concentration and the IC_{50} value was found to be 89.58 µg/mL. This was followed by ethyl acetate extracts that showed 60.42% percentage of inhibition with the IC_{50} value of 136.4 µg/mL. Similar such results were obtained by Cagalj *et al*., (2021) where the ethanol extract of *P. povonica* showed better antioxidant activity in ethanol extract.

The results of present study showed that the ethanol extracts of *P. boergesenii* showed better radical scavenging activity than ethyl acetate extract. In previous reports the ethanolic seaweed extracts showed less antioxidant potential (Duffy & Power, 2001). The differential effect of the antioxidant activity was strongly related to the type of extraction methods used (Yangthong *et al*., 2009; Widowato *et al*., 2014).

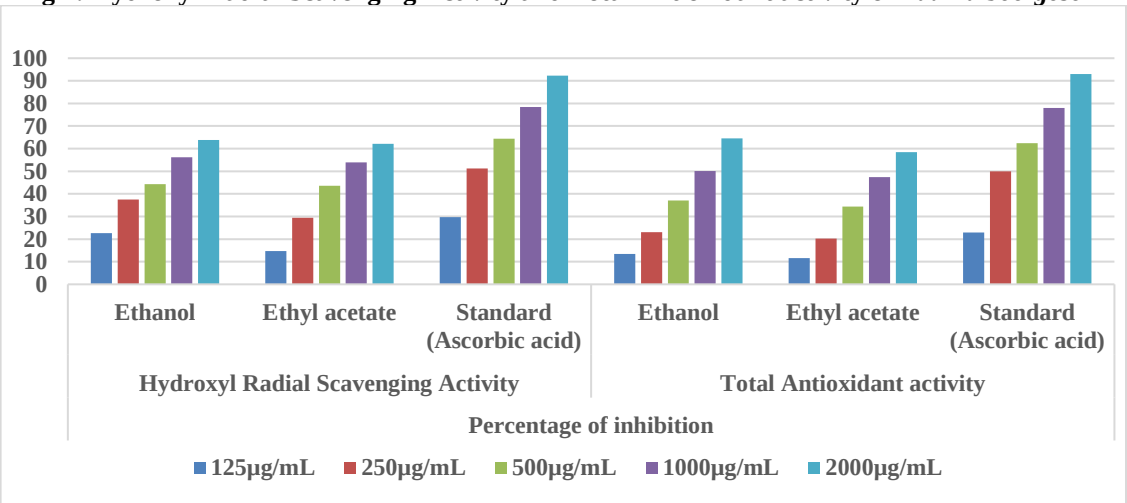
Due to the complex nature of the extracts, the antioxidants activity cannot be evaluated by a single method. In this context, different standard methods were used to validate nature of plant extract in terms of antioxidants (Cook and Samman, 1996).

So, the hydroxyl radical scavenging assay was also determined in different concentration. The maximum percentage of inhibition was recorded in 2000 µg/mL concentration. The ethanol extracts showed 63.90 % of inhibition with the IC_{50} value 758.54 µg/mL. The ethyl acetate showed the percentage inhibition of 62.20% and the IC_{50} value recorded was 823.43µg/mL. The results of present study showed that the ethanol extracts of *Padina boergesenii* showed high hydroxyl radical scavenging activity.

Table 2: Hydroxyl Radial Scavenging Activity and Total Antioxidant activity of *Padina boergesenii*

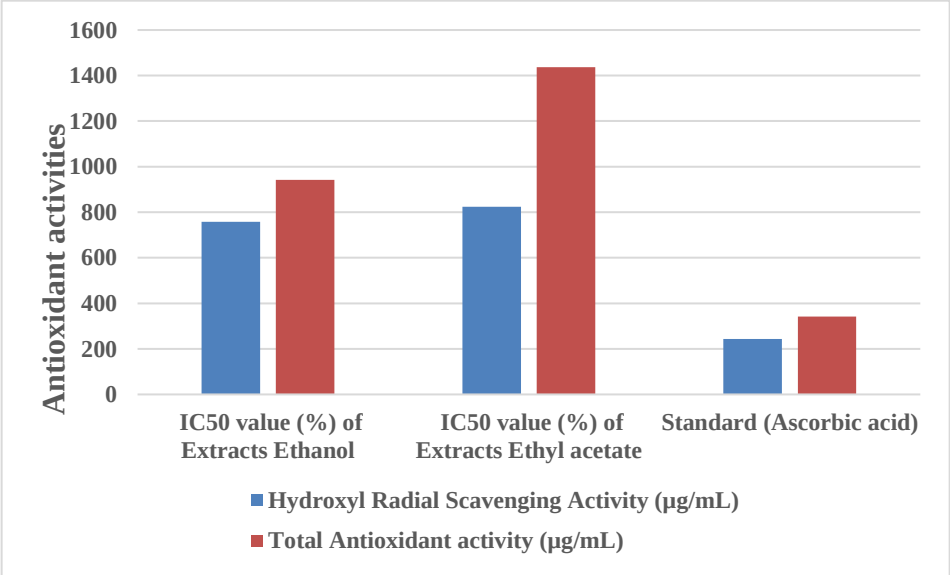
Concentration µg/mL	Percentage of inhibition					
	Hydroxyl Radial Scavenging Activity			Total Antioxidant activity		
	Ethanol	Ethyl acetate	Standard (Ascorbic acid)	Ethanol	Ethyl acetate	Standard (Ascorbic acid)
125	22.69	14.78	29.72	13.51	11.66	22.98
250	37.54	29.4	51.18	23.13	20.27	49.9
500	44.34	43.57	64.41	37.07	34.45	62.37
1000	56.17	53.89	78.42	50.11	47.35	77.97
2000	63.9	62.2	92.3	64.58	58.49	93.07

Fig 2: Hydroxyl Radial Scavenging Activity and Total Antioxidant activity of *Padina boergesenii*



IC_{50} Value Hydroxyl Radial Scavenging activity and Total Antioxidant activity

Antioxidant activities	IC50 value (%) of Extracts		Standard (Ascorbic acid)
	Ethanol	Ethyl acetate	
Hydroxyl Radial Scavenging Activity (µg/mL)	758.54	823.43	243.12
Total Antioxidant activity (µg/mL)	942.06	1437.3	342.27



Likewise, the ethanol extracts recorded high Total antioxidant (64.58%) activity with an IC_{50} value of 942.06 µg/mL. The ethyl acetate that showed the percentage inhibition of 58.49% with an IC_{50} value of 1437.3µg/mL. The results of all the study showed that the ethanol extracts of *Padina boergesenii* was more effective than ethyl acetate extracts.

This proves that the *Padina boergesenii* extract contains some active constituents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity (Gangwar *et al*., 2014).

On the basis of the above experiments, it is concluded that the ethanolic extract of *Padina boergesenii* Allender & Kraft showed high antioxidant and free radical scavenging activities. These in vitro assays of antioxidant activity indicated that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

CONCLUSION

This study demonstrates that *Padina boergesenii* possess antioxidant activity properties. More studies are required in order to correlate the specific factors responsible for the biological activity. In general, these finding highlight the potential antioxidant activity of the ethanol extracts at higher concentration. The finding further justifies the potential use of local. *Padina boergesenii* in further products development.

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