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STUDIES ON THE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *PADINA BORYANA* THIVY

KEY WORDS: Seaweed, DPPH, Padina boryana, Phytochemical Screening.

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ABSTRACT

Objective: Phytochemical is naturally present in the seaweeds which biologically play a significant role. The intention of this study was designed to screen the phytochemical constituents and antioxidant activity of *Padina boryana* collected from Mandapam coast, India.

Methods: The present study investigated the presence of phytochemical constituents of the brown seaweed *Padina boryana,* which were extracted with various solvents both polar and non polar like methanol, ethanol, acetone and water and screened for antioxidant potential by DPPH method (1,1-diphenyl-2-picrylhydrazyl) with standard procedure (Molyneux, 2004).

Results: The different extracts of *Padina boryana* showed the presence of alkaloids, steroids, tannins, saponins, flavonoids and phenols. The highest percentage of DPPH radical scavenging of *Padina boryana* was observed in methanol extract (56.02%) at a concentration of 1mg/L and at room temperature.

Conclusion: The species studied show interesting phytochemical constituents and antioxidant activities which can be used to prevent oxidative stress. The seaweed extract manifest preferable antioxidant activities, hence in the future, it would be good if it is further taken for treatment of human diseases.

INTRODUCTION

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from them (Domettila *et al.*, 2013). Over the past decades, seaweeds have been used by humans as medicine and food and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential.

Seaweeds are the reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B_1, B_{12}, C, D and E (Skulberg, 2000). Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected human from various diseases (Savithramma *et al.*, 2011). So, phytochemical analysis of the seaweeds will be a good preliminary approach to reveal its secondary metabolite constituents and the resultant medicinal values. Seaweeds are a known source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Bansemir *et al.*, 2006). Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential (Shyamala *et al.*, 2013). Macroalgae produce a wide variety of chemically active metabolites including alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities.

Antioxidant activity is important in various pharmacological activities such as anti-aging, anti-inflammatory, and anti-cancer activities (Lee *et al.*, 2004; Middleton *et al.*, 2000). Antioxidant activity is claimed to be present in most of the nutraceuticals and cosmeceuticals. However, numerous synthetic antioxidants are produced, but are quite unsafe and their toxicity is of concern (Madhavi *et al.*, 1995). On the other hand, Natural products with antioxidant activity are used for human consumption because of their safety. A wide range of studies has described the high antioxidant capacity of a range of edible seaweeds. This capacity is endowed by the presence of sulphated polysaccharides, Polyphenolic compounds and antioxidant enzymes.

Free radicals are molecules with an odd or unpaired electron in the outer orbital of their atomic structures, which makes them very reactive. These radicals are produced during cellular respiration and the human body owns a way to neutralize them. However, it happens that the production of these molecules becomes uncontrolled and settles what is called oxidative stress (Beaudeux *et al.*, 2006). In this case, free radicals cause degradation of important biological substrates such as DNA, proteins and lipids, leading to the appearance of pathogens of degenerescence such as cancers and cardiovascular diseases (Sadi *et al.*, 2010).

Based on the above facts, the present study aims to screen different solvents of *Padina boryana* qualitatively for the phytochemicals and antioxidant activity.

MATERIALS AND METHODS

Chemicals required

Distilled water, Dragendorff's reagent, Sodium hydroxide, ferric chloride, Lead acetate, concentrated sulphuric acid, Millon's reagent, ammonia, 1,1 – diphenyl -2- picrylhydrazyl (DPPH), Dimethylsilphoxide (DMSO), Butylated hydroxytoluene (BHT)

Sample collection

The seaweeds were collected from Mandapam coast, Rameshwaram India. Algal sample was handpicked, washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags, and put in an ice box, then transferred to the laboratory immediately until the experimental work was done. Then seaweeds were blotted on the blotting paper, shade dried at ambient temperature and the samples were grounded into a fine powder using tissue blender. The powdered samples were then stored in the refrigerator for further use. The algae collected was identified as *Padina boryana* Thivy. and was authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The specimen has been submitted to the institute for preservation.

Preparation of seaweed extracts

Ten grams of powdered samples were packed in Soxhlet

apparatus and extracted with (1:10) solvents like methanol, acetone, and ethanol and aqueous for 8 h, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

Phytochemical analysis

The phytochemical screening of different algal extracts was assessed by standard method as described by Raman (2006). Phytochemical screening was carried out to identify the major natural chemical groups such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts tested.

Antioxidant activity–DPPH method

Free radical scavenging inhibition assay it is used to determine the antioxidant capacity of the of seaweed extract. The antioxidant activity of *Padina boryana* extract was determined using the DPPH free radical scavenging assay according to the method described by Molyneux (2004). The assay was carried out in triplicate and the mean value was recorded. Freshly prepared solution of 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10°C in the dark. Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm. The ability to scavenge the DPPH radical was calculated using the follow equation given by (Duan *et al*., 2006)

$$\% \text{ Antioxidant activity} = \frac{(\text{Acontrol})-(\text{Asample})}{(\text{Acontrol})} \times 100$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical screening of different chemical compounds (alkaloids, steroids, tannins, saponins, flavonoids, phenols, proteins and anthraquinones) were tested in four different extracts. In the present study, the phytochemical screening was performed with aqueous, ethanol, acetone and methanol extracts of *Padina boryana* (Table 1).

Among the four different extracts, ethanol extract showed the presence of maximum number (five) of compounds. Next to that, aqueous extracts showed four compounds. methanol and acetone extracts showed only three compounds. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction. Alkaloids were found in ethanol extract. Alkaloids have cytotoxic activity that is due to the presence of microtubule interfering agents that can bind to beta tubulin, thus inhibiting the formation of the mitotic spindle fibre required for cell division (Solanki *et al*., 2008).

Steroids were found only in aqueous extracts. Steroids of seaweeds are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine and cosmetics (Okwu, 2001). Tannins were found in all four extracts. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (Kolodziej and Kiderlen, 2005). Saponins did not show any positive result in any extract of *Padina boryana*.

Flavonoids showed its presence in all tested extracts. Flavonoids have antimicrobial, antiviral, antioxidant and spasmolytic activity. Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. Phenols showed its presence in ethanol extract. In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions (Aliyu *et al*, 2009).

Proteins showed its presence in all tested extracts. Proteins are used to treat various diseases such as diabetes, cancer, infectious disease, haemophilia and anemia. Anthraquinones did not show any positive result in any extract of *Padina boryana*.

Antioxidant activity–DPPH method

DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compound (Molyneux, 2004, Prabhakar *et al*, 2006 and Wangensteen *et al*, 2004).

DPPH radical scavenging activities of *Padina boryana* is summarized in Table 2. The highest percentage of DPPH radical scavenging of *Padina boryana* was observed in methanol extract (56.02%) at concentration 1mg/L and at room temperature. These results showed that compounds with medium polarity have the strongest radical scavenging activity. Almost all fractions (except aqueous fraction) showed greater activity which ensures the need for purification fractionation of crude extract. Indeed this is probably due to interactions between the compounds present in the extract that can exert an antagonistic effect between them. The correlation between the concentration of the extract and the percentage of inhibition was studied: The percentage of inhibition increases with the concentration of the extract in all samples so the antioxidant activity is dose-dependent.

CONCLUSION

The present study concluded that different extracts of brown seaweed, *Padina boryana* possess several chemical compounds including alkaloids, steroids, tannins, saponins, flavonoids and phenols but lacks saponins. The species studied show interesting antioxidant activities and can be used to prevent oxidative stress. The seaweed extract manifest preferable antioxidant activities, hence in the future, it would be good if it is further taken for treatment of human diseases.

The results obtained in the present study clearly demonstrate that the methanol extract derived from *Padina boryana,* fairly active fractions for *in vitro* DPPH free radical scavenging activity. The findings of the current work appear useful for further research aiming to isolate and identify the specific compounds which is responsible for higher antioxidant activity.

Table 1: Phytochemical screening of P.boryana extracts

S. No	Phytochemical Test	Aqueous Extract	Ethanol Extract	Methanol Extract	Acetone Extract
1.	Alkaloids	-	+	-	-
2.	Flavonoids	+	+	+	+
3.	Tannins	+	+	+	+
4.	Phenols	-	+	-	-
5.	Saponins	-	-	-	-
6.	Steroids	+	-	-	-
7.	Proteins	+	+	+	+
8.	Anthraquinones	-	-	-	-

Table 2 : Antioxidant Activity of *P.boryana* extracts

S. No	CONCENTRATION (µg/ml)	Aqueous Extract	Ethanol Extract	Methanol Extract	Acetone Extract

1.	**200**	2.408	13.343	26.648	24.161
2.	**400**	5.882	17.923	32.964	29.174
3.	**600**	7.698	22.542	40.071	34.662
4.	**800**	11.685	25.858	49.388	37.583
5.	**1000**	15.515	30.872	**56.020**	41.176

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