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EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF POLYSACCHARIDE EXTRACTED FROM *SARGASSUM* SPECIES

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Abstract

Sargassum species were used to study their phytochemical and antioxidant properties. *Sargassum species* were obtained during low tides along the shore of Raigad district, Shrivardhan region. The algal extracts were analysed for phytochemicals using standard procedures. The various extracts of *Sargassum species* contained flavonoids, alkaloids, glycosides, saponins, steroids, tannins, reducing sugar, etc. The antioxidant properties of *Sargassum species* were assessed using DPPH radical scavenging activity and metal ion chelating ability. The crude extracts of *Sargassum species* had a 56.13± 0.36 % free radical scavenging activity when compared to ascorbic acid, which was the reference control and had a 72.71± 0.26 % free radical scavenging activity. The present study confirmed that *Sargassum species* are rich in polysaccharides and may serve as active antioxidants.

Keywords: DPPH, *Sargassum species*, Ferrous Ion-Chelating (FIC), Polysaccharides.

1. Introduction

The body's billions of cells generate free radicals and reactive oxygen species (ROS) throughout metabolism [3]. Oxidative Stress is caused by an imbalance of free radical generation that surpasses the body's antioxidant capacity and damages cells, including DNA, proteins, and lipids. Exogenous antioxidants are essential for mitigating and preventing oxidative damage. Exogenous antioxidants can come from both synthetic and natural components. According to many studies, seaweeds are a rich source of natural antioxidants [4]. Scientists have studied antioxidants that prevent ROS-induced oxidation processes for years. Antioxidants are widely utilised as food additives. Long-term usage of synthetic antioxidants might have negative effects, highlighting the importance of natural alternatives. Algal extracts from various species can play a major role in this field. Over 50 brown algae species from across the world have been found to exhibit considerable antioxidant activity [6].

Marine brown algae are a major source of sulfated polysaccharides. Given their health advantages, marine polysaccharides are well-known and potential candidates in the medical, nutraceutical, and pharmaceutical industries. Seaweeds are becoming increasingly popular in the functional food market due to their availability, active qualities, and low processing costs [1]. *Sargassum* species, a plant of the Phaeophyceae family, is widely used for medicinal purposes [4]. *Sargassum species* cell walls are made up of an amorphous matrix of acid

polysaccharides, including sulphated polysaccharides and alginic acid, connected by proteins, providing structural integrity and flexibility to the seaweed [9]. Polysaccharides account for 5-10% of dry algal biomass; however, this varies depending on the species and season. Algal polysaccharides exhibit a wide variety of structural variations, and their functioning is primarily determined by their structural divergence [6].

2. Materials and Methods

2.1 Collection and Preparation of *Sargassum species* extract

Fresh *Sargassum species* were collected at low tide along the coast of Raigad district, Maharashtra, and then manually cleaned from sand, epiphytes, and animal waste. The samples were then rinsed with seawater to remove associated debris, plankton, or bacteria. Morphologically different thalli of algae were placed individually in fresh polythene bags, maintained in an ice box, and transferred to the laboratory. Furthermore, the material was properly rinsed with tap water to remove the salt from the samples' surfaces, and the water was drained from the seaweed and spread on blotting paper to remove any extra water. The dried samples were washed again with sterile distilled water to eliminate any remaining salt on the surface. The *Sargassum species* samples were shade-dried for 5 days, then oven-dried at 50°C for an hour before being pulverised in an electric mixer for 1 hour. Finally, 300 mg of powdered seaweed sample was collected and kept in the refrigerator at 4°C for future use.

2.2 Preparation of *Sargassum species* extract

Sargassum species extract was studied for the phytochemical screening using the method given by Harborne (1973). Weigh and homogenise 5 grams of *Sargassum species* powder with 50 ml of each solution: methanol, Hexane, and water. The extract was boiled, then cooled and filtered. The filtrate was stored in individual sterile glass containers for later use.

Figure 1: *Sargassum species* extract

2.3 Phytochemical analysis

The phytochemical contents in the Methanol, hexane, and water extracts of the *Sargassum species* were identified using the standard approach given by Harborne (1973)

2.3.1 Alkaloids: 10ml of methanol was added to 200 mg of seaweed extract and boiled and filtered; 1% HCl was added to the filtrate, and a few drops of Dragendorff reagent were added; a brownish-red precipitate appeared, indicating the presence of alkaloids.

2.3.2 Tannins: To 200 mg of seaweed extract, 10ml of distilled water was added, which was then heated and filtered. Drops of $FeCl_3$ were added to the filtrate. A blue-black precipitate shows the presence of tannins.

2.3.3 Saponins (Frothing test). To 0.5 mg of seaweed extract, add 5 mL of distilled water and vigorously shake for 2 minutes. Durable foam shows the presence.

2.3.4 Cardiac Glycosides (Keller-Kiliani test): 1 mL of glacial acetic acid and a few drops of $FeCl_3$ were mixed into 2 mL of seaweed extract. The mixture was treated with concentrated H_2SO_4, resulting in a greenish-blue colour

2.3.5 Steroids (Liebermann-Burchard reaction): 10 mL of chloroform and acetic anhydride were added in a 1:1 ratio to 200 mg of seaweed extract, resulting in the indication of a blue-green ring.

2.3.6 Flavonoids: To 5 mL of dilute ammonia, followed by a concentrated solution of H_2SO_4, was added to the seaweed extract, resulting in the formation of a yellow colour, indicating the presence.

2.3.7 Terpenoids (Salkowski test): When 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated sulfuric acid (H_2SO_4) were added to 200 mg of seaweed extract, a reddish-brown colour developed, indicating the presence of terpenes.

2.3.8 Reducing Sugars: A few drops of Fehling's solution A and B were added to the seaweed extract; an orange-red precipitate showed the presence of reducing sugars.

3. Extraction of Polysaccharide From *Sargassum species*

The method described by Ye *et al.* (2009) was utilised, with slight modifications, to study the effects of EDTA-Na on the yield of seaweed fucoidan extraction. 15 g of *Sargassum species* powder was dissolved in 450 ml of 0.5% EDTA-Na (w/v) and heated at 70°C with agitation for 3 hours. The mixture was centrifuged at 5000 g for 20 minutes at 4°C. The supernatant was neutralised with 0.1 M NaOH and adjusted to pH 7. The extracts were centrifuged and treated with $CaCl_2$. Sulphated polysaccharide was precipitated by adding 80% (v/v) absolute ethanol, then incubated at 4 °C overnight. The sulphated polysaccharide was then oven-dried until a consistent mass was achieved. After drying, sulphated polysaccharide was placed in a desiccator for about 4 hours before being stored in a cool, dry place.

4. Screening of DPPH free radical scavenging activity

The antioxidant activity of *Sargassum* species extracts was measured using the DPPH free radical scavenging activity. To begin, 1 mL of 0.1mM DPPH solution was taken, and 1mL of different concentrations (20-100µg/mL) of sulphated polysaccharides was added. The reaction mixture was incubated in the dark for 12 hours, and absorbance was measured at A 517nm.

The fraction of DPPH free radical scavenging activity was calculated using the method given below.

% DPPH radical scavenging activity = ((A0-A1) / A0) X 100

Where, A0 is the absorbance of the control, and A1 is the absorbance of the sample. All assays were performed three times, and the results show the average and standard deviation (SD) of all three tests. The antioxidant properties of the *Sargassum species* extract were compared to ascorbic acid.

5. Metal ion Chelating ability

The Ferrous Ion-Chelating (FIC) ability was evaluated using the methods of Wang *et al.* (2009). Mix 1 mL of seaweed extract solution (3.33 mg/mL) with 0.05 mL of 2 mM $FeCl_2$, 0.2 mL of 5 mM ferrozine, and 2.75 mL of distilled water. The absorbance of the iron ions-ferrozine complex was measured at 562 nm after 10 minutes of shaking at room temperature in the dark. EDTA was used as a positive control.

Ferrous ion-chelating ability (%) = ((A0-A1) / A0) X 100

where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

6. Result and discussion

Terpenoids, phenolics, flavonoids, and tannins, reducing sugar were observed in the preliminary phytochemical analysis. Our findings confirm Bandoniene and Murkovie's 2002 study, which found that macroalgae contain antioxidant compounds such as terpenoids, polyphenols, phenolic acids, reducing sugar, and flavonoids. Marine algae are an excellent source of unsaponifiable, nontoxic sterols with therapeutic benefits (Rajasulochana *et al.*, 2009). The presence of phenolics, alkaloids, flavonoids, steroids, and saponins in Seaweed methanolic extracts suggests that the seaweeds can be used as antimicrobial (antiviral, antifungal, and antibacterial), antiparasitic, anti-inflammatory, anti-feeding, antioxidant, antiallergenic, anti-thrombin, anti-carcinogenic, and anti-ulcer agents in the near future.

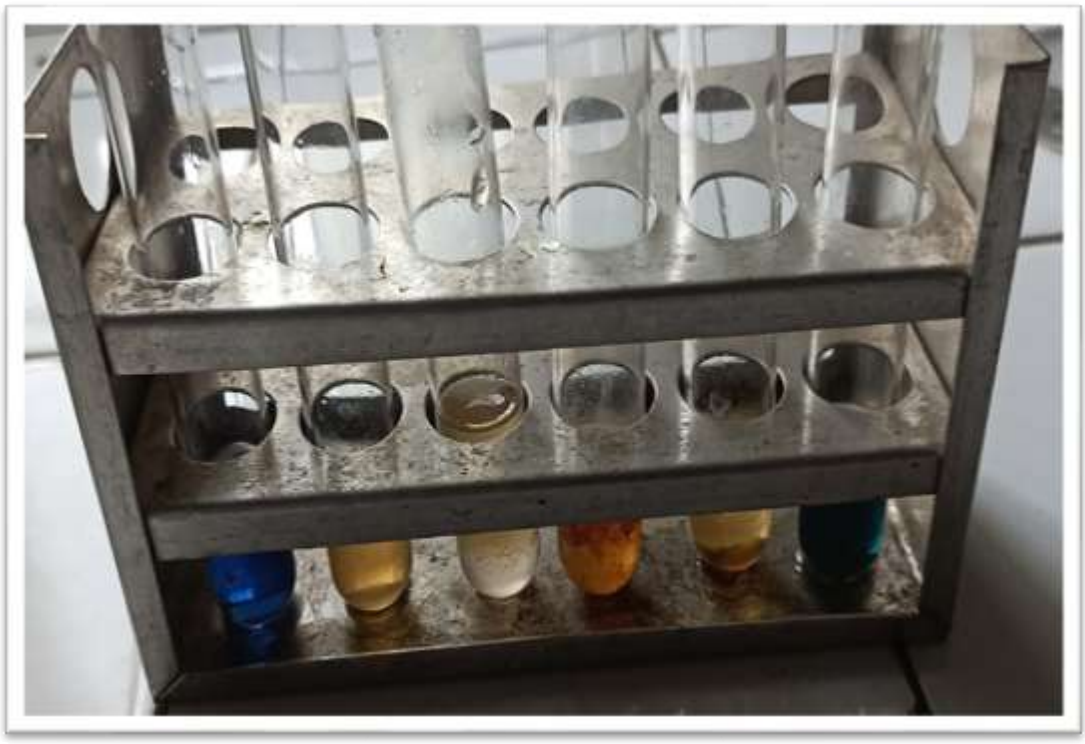

Figure No. 2. Phytochemical analysis of different extracts of *Sargassum species*

Table 1. Phytochemical screening of different extracts of *Sargassum species*

Sr.No.	Compound Name	Methanol	Hexane	water
1	Alkaloid	+	+	+
2	Tannin	+	-	-
3	Saponin	+	-	+
4	Cardiac Glycosides	-	+	-
5	Steroids	+	+	+
6	Flavonoids	+	+	+
7	Terpenoids	+	+	+
8	Reducing sugar	+	+	+

(+) **Presence** (-) **Absence**

6.1 DPPH free radical scavenging activity

Sargassum species extracts showed antioxidant activity when tested using free radical scavenging assays. Increasing algal extract concentration resulted in enhanced activity. *Sargassum species* at 100µg/mL achieved the highest inhibition. The results showed that the crude extracts of *Sargassum* had a 56.13± 0.36 % free radical scavenging activity when compared to ascorbic acid, which was the reference control and had a 72.71± 0.26% free radical scavenging activity.

Table 2: % DPPH radical scavenging activity of *Sargassum species*		
Sr.No.	**Sample conc.**	**% inhibition**
1	20	20.89 ± 0.21
2	40	29.18± 0.15
3	60	36.47± 0.10
4	80	43.73± 0.20
5	100	56.13± 0.36

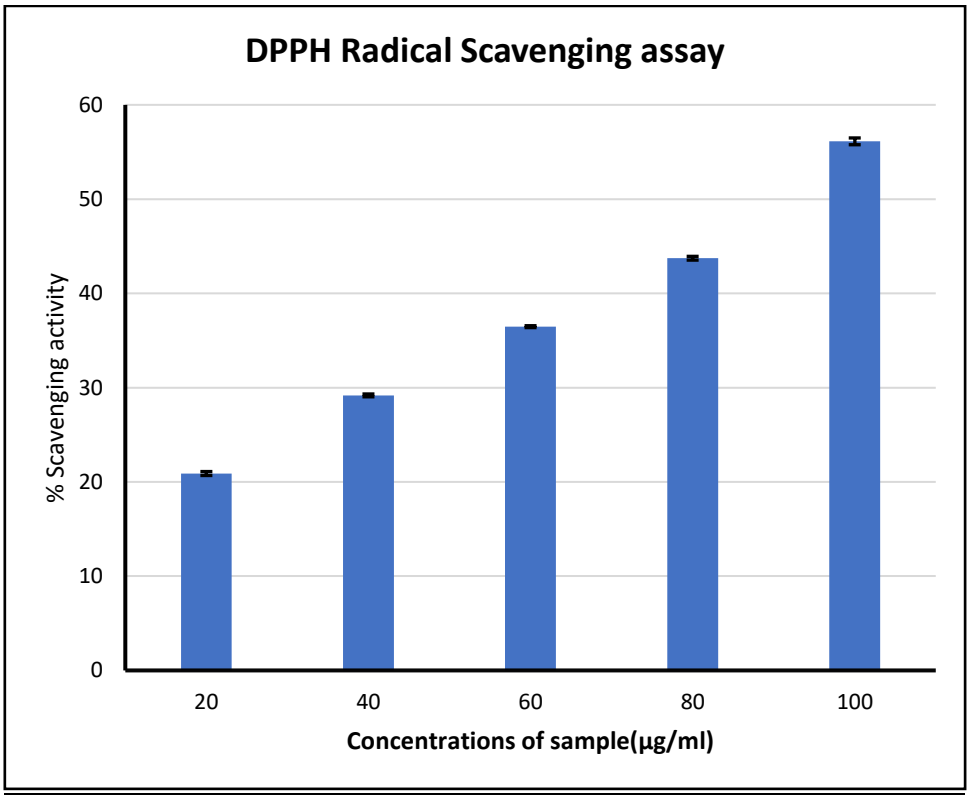

Figure 3: DPPH radical scavenging activity of *Sargassum species*

Table 3: % DPPH radical scavenging activity of ascorbic acid.		
Sr.No.	**Sample conc.**	**% inhibition**
1	20	47.81± 0.73
2	40	56.89± 0.49
3	60	60.97± 0.52
4	80	67.32± 0.32
5	100	72.71± 0.26

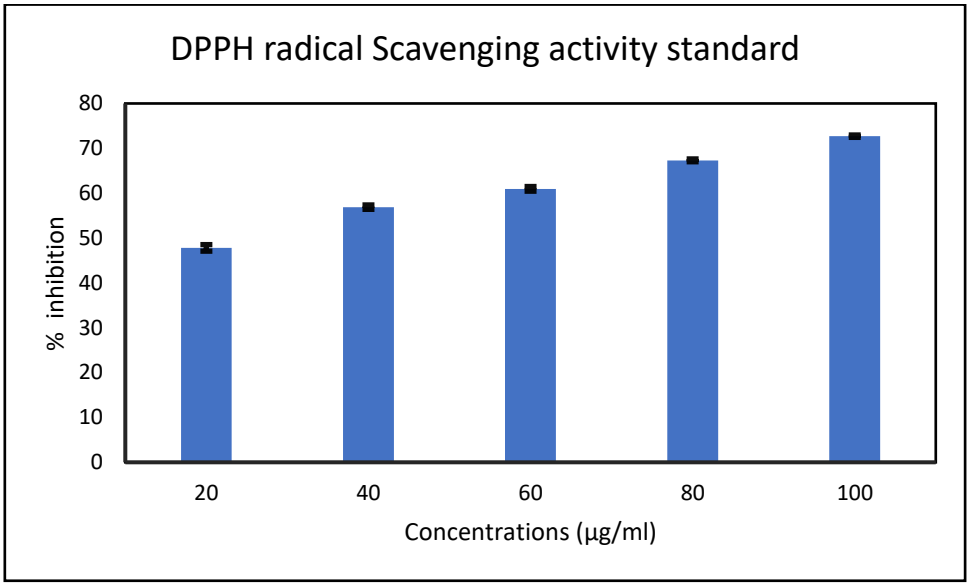

Figure 4: % DPPH radical scavenging activity of ascorbic acid.

The results of DPPH radical scavenging activity showed that *Sargassum species* extracts had antioxidant activity. The DPPH test determines the reactivity of the test compounds with stable free radicals.

6.2 Metal ion chelating ability

According to B. Halliwell (1996), the Fenton reaction accelerates the generation of hydroxyl radicals, which can cause a variety of disorders. It has been found that chelating compounds that establish bonds with metal ions are excellent secondary antioxidants because they diminish the redox potential, stabilising the metal ion's oxidised condition.

Table 4. Metal ion chelating ability

Sr. No.	Seaweed extract	% Chelating activity
1	*Sargassum cinctum (dried)*	22.05
2	*Sargassum cinctum* (after alginate extraction)	38.23
3	*Sargassum wightii* (dried)	19.11
4.	*Sargassum wightii* (after alginate extraction)	42.64

The chelating activity of *Sargassum cinctum* increased after alginate extraction by 38% compared to dried *Sargassum cinctum*, whereas the activity of shade-dried *Sargassum wightii*

after alginate extraction was 42.64 % Wang *et al.* (2009) found that polysaccharides, proteins, and peptides in extracts were more efficient in chelating ferrous ions than phenolic substances.

7. Conclusions

The study showed for the first time that the antioxidant activities of *Sargassum species* from Shrivardhan, Raigad district of Maharashtra (India) were influenced by various types of extracts, harvest seasons, harvest sites, and species. *Sargassum species* collected during the dry season showed the highest antioxidant activity. Antioxidants are known to have a key mechanism for scavenging proton radicals. Overall, the *Sargassum species* extract was more effective at stabilising DPPH radicals and metal ion chelating ability.

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