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ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant Potential of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

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ABSTRACT
The consumption of sea algae is widespread around the world. Brown algae are one of the seaweeds that have been approved for human consumption. These plants have a wide range of possible biological actions and contain essential phytochemical elements. Bioactive compounds from marine algae have been studied in a variety of locations across the world. Polysaccharides and their sulfated derivatives known as carrageenan, which are utilised as antigens and anticoagulants, were among the first bioactive ingredients discovered in marine algae. Sargassum polycystum is one of the important species belonging to the genus Sargassum and a wide range of bioactive properties have been reported. The present study investigated the presence of phytochemical constituents and antioxidant activity of Sargassum polycystum on the DPPH, FRAP and ABTS Assay. The extraction of the water soluble crude Sulfated polysaccharide (WSCSPs) from Sargassum polycystum was performed as described by with minor modifications. Phytochemical screening of the extracts was carried out according to the standard methods. WSCSPs from Sargassum polycystum were investigated for their DPPH radical scavenging activity, FRAP reducing power activity and ABTS radical scavenging activity was performed at the concentration ranging from 50 – 250 µg/ml. From the phytochemical screening, it was observed that the WSCSPs from Sargassum polycystum revealed the presence of alkaloids, flavonoids, steroids, tannins, phenols, terpenoids and saponins. WSCSPs from Sargassum polycystum exhibit remarkable antioxidant potential through DPPH, FRAP and ABTS Assay. These findings show that the phytoconstituents in the extracts are responsible for free radical scavenging capacity. Seaweeds have gotten a lot of attention as a source of natural antioxidants, and they've been found to be a good source. The results of the present study confirmed that Sargassum polycystum may be rich sources of phytoconstituents which can be isolated and further screened for various biological activities.
Keywords: Phytochemical screening, Antioxidant activity, DPPH, FRAP and ABTS

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INTRODUCTION

Herbal medicines have been utilized to treat sickness symptoms since ancient times. Despite the significant improvements in modern medicine over the last few decades, plants continue to play a vital role in health care. However, medicinal plants and seaweed have sparked a lot of attention due to their long history of usage in folk medicine along with their preventive effects, particularly in developing nations [1]. Antioxidant capabilities have been studied in a large range of seaweed. Natural antioxidants, whether in the form of raw extracts or chemical components, are extremely effective in preventing oxidative stress-related damage. Exogenous substances and endogenous metabolic activities in the human body produce free radicals, or highly reactive oxygen species. These are capable of oxidizing biomolecules such as nucleic acids, proteins, lipids, and DNA, and can cause neurological problems, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative diseases [2]. Antioxidants are substances that stop free radicals from attacking cells and thereby lessen the chance of disease. Almost all organisms are protected from free radical damage to some extent by antioxidant substances such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes such as superoxide dismutase and catalase. Natural antioxidants are currently receiving a lot of interest as a way to protect the human body, particularly brain regions, from oxidative damage caused by free radicals [3]. Seaweed extract exhibits various biological activities as a result of its phytochemical content of bioactive compounds like antioxidants, anti-cancer agents, antiproliferative, antimicrobial properties, and treatments for cardiovascular diseases and ailments like atherosclerosis, hyperlipidemia, hypertension, and thyroid diseases [4-5]. The present study has been conducted to evaluate the antioxidant activity of *Sargassum polycystum*.

Sargassum species can be found in tropical and subtropical areas of the world, and they are known to produce metabolites of various structural classes, including terpenoids, polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides, and others, which have a variety of therapeutic properties. The metabolites meroterpenoids, phlorotanins and fucoidans are isolated from *Sargassum* sp. *Sargassum polycystum* is a marine alga with powerful antioxidant and anticancer properties. Chloroform extract of *Sargassum polycystum* demonstrated the strongest cytotoxicity against cervical HeLa cells which is potential to be developed as a candidate for new anticervical cancer agents [6]. Therefore, the present investigation was undertaken to elucidate the antioxidant activity of water soluble crude sulfated polysaccharide of *Sargassum polycystum* on the DPPH, FRAP and ABTS Assay.

MATERIAL AND METHODS

Seaweed sample collection

The brown seaweed *surgassum polycystum* was collected from the Mandapam, Gulf of Mannar (Lat.09° 17'N; Long.79° 08'E), Rameshwaram, Tamil Nadu, India. The collected seaweed sample was washed first in seawater and then followed by distilled water to remove the unwanted debris present over the seaweed sample. Then washed seaweeds were shad dried at room temperature. The dried seaweeds were cut into small pieces and make into powdered in a mixer grinder for further extraction process.

Extraction of Fucoidan

The extraction of the water soluble crude Sulfated polysaccharide **(**WSCSPs) was performed as described by with minor modifications [7]. 100 grams of *Sargassum polycystum* sample was treated with a liter of ethanol and stirred with a mechanical stirrer for about 12 h at room temperature in order to remove proteins and pigments, centrifuged at 3000 rpm for 10 min. Then the residue was left to dry at room temperature. Five grams of the biomass was taken and extracted with 100 ml of acidic water pH 2.0 at 90ºC with stirring for a 3 h. The extraction was done twice and the extracts were pooled. The combined extracts were centrifuged at 15,000 rpm for 10 min and the supernatant was collected. Then the supernatant was mixed well with 1% of $CaCl_2$ and the solution was kept at 4ºC overnight to precipitate alginic acid. The solution was then centrifuged at 15,000 rpm for 10 min and the supernatant was collected. Ethanol (99%) was added into the supernatant in order to arrive upon the final ethanol concentration of 30% and the solution was positioned at 4ºC for 4h. The obtained solution was centrifuged at 15,000 rpm for 10 min and the pellet was collected. Ethanol (99%) was added into the supernatant in order to reach the final ethanol concentration of 70% and the solution was kept at 4°C overnight.

Qualitative analysis of the water soluble crude sulfated polysaccharide of *Sargassum polycystum* [8 -10]

Phenols2 ml of plant extract was taken in a test tube and add 1% lead acetate solution. Formation of white precipitate indicates the presence of phenolic compounds

Tannins

Take 2 ml of plant extract and add few drops of 0.1% ferric chloride solution in it, formation of brownish green color indicates the presence of tannins

Flavanoids

2ml of plant extract was treated with 2ml of 10% Lead acetate solution. Appearance of yellowish green color indicated the presence of flavanoid

Saponins

To about 1ml of each extract was added to 2ml of distilled water in a test tube and shaken vigorously with few drops of olive oil. Foam which persisted was taken as an evidence for the presence of saponins

Terpenoids

2ml of each extract of plant samples was mixed with 2ml of chloroform. Then allow evaporating and adding 2ml of concentrated sulfuric acid, then heat for 2 minutes. Greyish color indicates the presence of terpenoids

Alkaloids

Take 2 ml of plant extract and add 2ml Wagner's reagent. Test tubes were observed for the appearance of reddish brown precipitate

Glycosides

To 100 µl of plant extract 2ml of concentrated H2SO4 was added. Formation of reddish brown color indicates the presence of glycosides

Quinones

2ml of plant extract was mixed with 3 or 4 drops of concentrated HCl. Formation of yellow color indicated the presence of quinones

Fatty Acids

0.5 ml of extract was added to 5ml of ether and allowed it to evaporate on filter paper. Then the filter paper was dried and the appearance of transparency on filter paper is the indication of presence of fatty acids

Phytosterols

To 2ml of plant extract, 2ml of chloroform and 2ml of concentrated H2SO4 was added and shaken well. If the chloroform layer appeared red and acid layer appeared greenish yellow fluorescent indicates the presence of sterols

Antioxidant activity of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

DPPH Radical Scavenging Assay

The WSCSPs of *S. Polycystm* was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH) at varying concentrations such as 50, 100, 150, 200, and 250µg/ml and phenytoin various concentrations such as 6.25, 12.5, 25, 50, and 100 and 250µg/ml to check their donor capacity at dark conditions. Ascorbic acid was used as standard. The readings were taken in UV-Spectrometer at 517nm. The experiments were performed in triplicates [11].

Ferric Reducing Antioxidant Power Assay

The WSCSPs of *S. Polycystm* and phenytoin was tested. The FRAP assay was performed according to the references with minor modifications (Nishaa et al., 2012). Various concentration WSCSPs and Phenytoin such as 50, 100, 150, 200, and 250µg/ml and phenytoin various concentrations such as 6.25, 12.5, 25, 50, and 100 250 µg/ml mixed with 2mL of the FRAP reagent and added with distilled water up to 1 mL. After 30 minutes of incubation. Absorbance was measured at 593 nm against blank [12].

ABTS Assay

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Assay. Free radical scavenging activity of the extract was also determined by ABTS (Sigma-Aldrich) radical cation decolorization assay [13]. ABTS radical cation was generated by mixing 20 mM ABTS solution with 70 mM potassium peroxodisulphate and allowing it to stand in dark at room temperature for 24 hours before use. 0.6 ml of extract (0.25 mg) was mixed with 0.45 ml of ABTS reagent and absorbance of these solutions was measured at 734 nm after 10 min.

Statistical Analysis

Statistical analysis of was done by one-way ANOVA followed by Student's t test. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The present study exemplified the antioxidant potential of water soluble crude sulfated polysaccharide of *Sargassum polycystum*. Seaweeds are known as medicinal plants, rich in metabolites and have been extensively studied and used in the pharmaceutical industry. Phytochemical analysis of water soluble crude sulfated polysaccharide of *Sargassum polycystum* exhibits various phytoconstituents viz., phenols, tannins, flavonoids, saponins, terpenoids, alkaloids and phytosterols (Table 1). Plants, especially seaweeds, contain phenolic compounds, which have been shown to have a wide range of biological activities, including antioxidant properties [14 -15]. According to the reports, phenolic compounds are one of the most powerful antioxidants found in brown algae. Our results were akin with Nazarudin et al. [16] reported that the *S. polycystum* exhibits various secondary metabolites Tannins, which may bind to adhesives and play roles in enzyme inhibition, substrate deprivation, and membrane disruption, have been discovered to have antibacterial effects [17]. Saponins have specific biological activities such as anticancer, anti inflammatory, antimicrobial and antioxidant properties [18]. Flavonoids are phenolic compounds that have been hydroxylated and have been studied for their antioxidant properties [19]. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals, and lipids is thought to be the reason for their action. Peroxy radicals are essential for preventing illnesses caused by oxidative cell, membrane, and protein damage. Yadav and Agarwala [20] reported that the steroids have been possess antibacterial properties and they are very important compounds due to their relationship with compounds like sex hormones. Terpenoids have been reported to possess cytotoxicity against a variety of cancer cells and cancer prophylactic [20]. Hence the presence of secondary metabolites from *S. polycystum* suggests that, it can be used as various biological properties that have great medicinal values and extensively used in the drug and pharmaceutical industry.

Table 1 Qualitative analysis of the water soluble crude sulfated polysaccharide of *Sargassum polycystum*

S. No	Phytochemical test	WSCSP
1	Phenols	Present
2	Tannins	Present
3	Flavonoids	Present
4	Saponins	Present
5	Terpenoids	Present
6	Alkaloids	Present
7	Glycosides	Absent
8	Quinones	Absent
9	Fatty acids	Absent
10	Phytosterols	Present

Table 2 DPPH Radical Scavenging Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

S. No	Concentrations µg/ml	WSCSPs
1	50	32.10 ± 0.09
2	100	43.16 ± 0.04
3	150	51.08 ± 0.06
4	200	76.13 ± 0.06
5	250	92.10 ± 0.09

The results of the antioxidant characteristics of water soluble crude sulfated polysaccharide of *Sargassum polycystum*, estimated by the DPPH scavenging activity, Ferric reducing antioxidant power assay and ABTS assay. The free radical scavenging antioxidant assay 2,2-diphenyl-1-picrylhydrazyl (DPPH) has been widely used to assess the antioxidant capacity of extracts. Sachindra et al. [21] opined that the radical molecule is stable and does not need to be manufactured, the DPPH test is regarded a valid, accurate, simple, and cost-effective approach for evaluating antioxidant radical scavenging activity. A novel methodology for assessing antiradical efficiency against DPPH has been developed, which has several advantages over existing methods. In the present study, water soluble crude sulfated polysaccharide of *Sargassum polycystum* depicted higher tendency to scavenge the DPPH radicals in a dose dependent manner and steadily increased with increase in extract concentrations. At 50 µg/mL of water soluble crude sulfated polysaccharide of *Sargassum polycystum* possessed 32.10 % and the maximum level of DPPH reduction was observed at 250 µg/mL and it was found to be 92.10% which suggested that the polysaccharide of *Sargassum polycystum* possess more potential to scavenge DPPH free radicals due to the higher concentration of phenolic content (Table 2 and Fig.1). Previous studies reported that ethanolic and aqueous extracts of Sargassum spp. including *S. horneri, S. macrocarpum* and *S. siliquastrum* produced more than 60% of DPPH radical scavenging activity [22-23] Seaweed containing antioxidant molecules can diminish DPPH free radicals by attacking them and adding hydrogen atoms or electrons to them, changing the colour of the DPPH solution from purple to yellow [24]. In compared to red and green seaweeds, brown seaweed rich in natural bioactive components such as carotenoid, fucoxanthin, phenolic, and flavonoids has been shown to have better antioxidant capacity [25].

Antioxidants in the sample reduce the ferric (III) to ferrous (II) in a redox-linked colourimetric reaction involving single electron transfer in the FRAP assay. The reducing power of antioxidant chemicals shows that they are electron donors that can reduce the oxidised intermediate of the lipid peroxidation process, allowing them to function as primary and secondary antioxidants [26]. The reducing capacity of the samples were analyzed by the FRAP method measuring the absorbance at 593 nm and antioxidant power was calculated. The highest FRAP values was found to be 89.12 % at 250 µg/mL of polysaccharide of *Sargassum polycystum*. Similarly, Giriwono et al. [27] reported that the lipid-soluble fraction of *Sargassum cristaefolium* possessed free radical scavenging activity. The antioxidant compounds reducing power suggests that they are electron donors and can reduce the oxidised intermediates of the lipid per oxidation process, allowing them to function as primary and secondary antioxidants. Using the FRAP assay, we found that the brown seaweed *Sargassum polycystum* exhibits better FRAP activity.

The ABTS assay is a simple indirect approach for evaluating natural antioxidant activity. ABTS radical is rather stable in the absence of phenolics, but it interacts energetically with an H-atom donor such as phenolics, resulting in a non-colored type of ABTS [28]. The antioxidant activity rises with increasing

concentrations, according to the ABTS radical cation-scavenging assay. The maximum scavenging effect was shown by the polysaccharide of *Sargassum polycystum* at 250 µg/ml and it was found to be 91.06 % and it was statistically significant ($p<0.05$) (Table 4 and Fig.3). The lower concentration of extract was sufficient to nullify free radical mediated chain reactions in this assay. Parthiban et al. [29] reported that the strongest anti-oxidant activity in the ABTS method was achieved by the compound phenols. The results of the present study correlated with the result of Sroka and Cisowski [30] that they were confirmed the strong quench ABTS radical is related with the more phenol contents of the experimental alga. The capacity of seaweed extracts to scavenge free radicals may account for their antioxidant action. Seaweeds, with their multiple polyphenols and polysaccharides enriched in a hydroxyl group and carbonyl group on ring C, have several sites for metal complexation able to chelate metal ions. Furthermore, the antioxidant activity of seaweed extracts appears to be mediated by phenolic substances. *Sargassum polycystum* can be employed for a variety of beneficial chemo-preventive effects, according to the findings. As there is a growing trend of disease and an increased requirement for medicine or drugs, this study recommends that macroalgae being an underutilized bioresource could be exploited in a sustainable manner for the welfare of mankind. The findings of this study support the fact that some seaweed commonly consumed in India are promising sources of potential antioxidants.

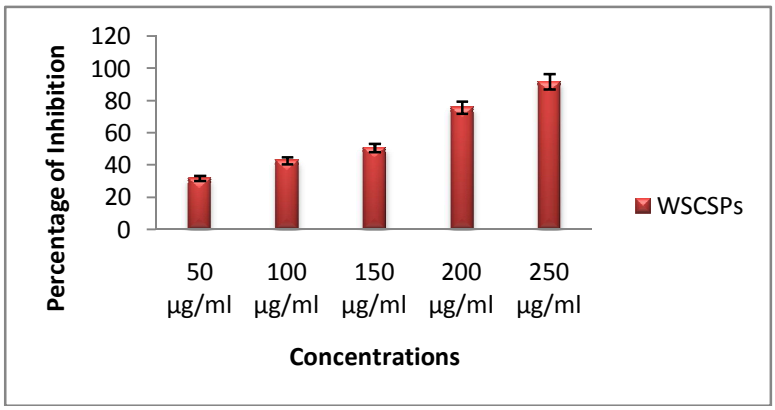

Fig.1 DPPH Radical Scavenging Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

Table 3 Ferric Reducing Antioxidant Power Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

S. No	Concentrations µg/ml	WSCSPs
1	50	37.03 ± 0.12
2	100	46.08 ± 0.1
3	150	55.21 ± 0.21
4	200	69.08 ± 0.28
5	250	89.12 ± 0.32

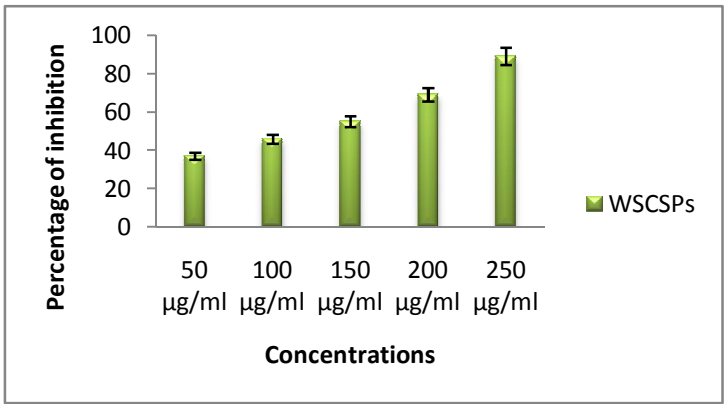

Fig. 2 Ferric Reducing Antioxidant Power Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

Table 4 ABTS Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

S. No	Concentrations µg/ml	WSCSPs
1	50	33.11 ± 0.11
2	100	44.05 ± 0.17
3	150	58.98 ± 0.26
4	200	74.19 ± 0.32
5	250	91.06 ± 0.40

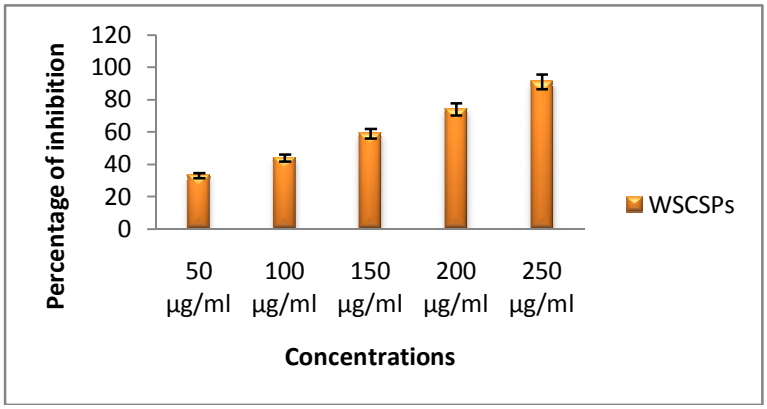

Fig.3 ABTS Assay of water soluble crude sulfated polysaccharide of *Sargassum polycystum*

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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