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PHYTOCHEMICAL CHARACTERIZATION AND INVITRO ANTIOXIDANT EFFICACY OF *SARGASSUM WIGHTII*

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ABSTARCT

Marine seaweeds contain many active components that are identified by the phytochemical analysis. Sea weeds of genus *Sargassum* has been widely used as a resource for food and medicine. In medicine it heals to cure many diseases. *Sargassum Wightii* was used for the study of phytochemical analysis. The extractions of phytochemicals from *Sargassum wightii* were carried out with different solvents such as Ethanol, Methanol and Water. The preliminary screening revealed the presence of tannin, saponin, flavonoids, steroids, terpenoids triterpenoids, alkaloids, carbohydrate, protein, polyphenol and glycosides with varied degree. It was found that the aqueous extract of shows better results than the other. The total phenol and flavanoid determination were 271 and 209 mg/g. The invitro antioxidant activity of *Sargassum wightii* was measured by DPPH radical scavenging assay, superoxide anion scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide scavenging assay, reducing power assay. The results of the present study confirmed that *Sargassum wightii* may be the rich sources of phytoconstituents which can be isolated and further screened for various biological activities.

KEYWORDS: Phytochemicals, Antioxidant, *Sargassum wightii*.

1. INTRODUCTION

Seaweeds are primitive non-flowering plants without true root, stem and leaves. Seaweed has been used as food in the Asia diet for centuries as it contains carotenoids, dietary fibres,

proteins, essential faaty acids, vitamins and minerals. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals, which are essential nutrition for human.[1] Seaweeds have been considered as a potential source of marine medicinals including antimicrobial, cancer therapies, hypercholesterolemic and antihelminthic substances. Historically seaweeds provide essential economic, environmental aesthetic and cultural benefits to humanity.[2] Seaweeds or marine algae are potentilaly prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents.[3]

Sargassum, one of the marine macroalgal genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family *sargassaceae* and order *Fucales*. It is large, ecoomically important and ecologically dominant brown algae present in much of the tropical sea. It is found to be the most diverse genus among the Phaeophyta in India and is represented by 38 species. *Sargassum wightii* is one of the most important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species.[4] The inhibitory substances biosynthesized by the seaweeds were noted as early as in 1917.[5] The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds.[6]

The present study was undertaken to screen the bioactivity of brown seaweed *Sargassum wightii* collected from Mandapam coast of Tamilnadu, India and assess their antioxidant properties.

2. MATERIALS AND METHOD

2.1. Collection of samples

The samples of S.wightii were collected by hand picking from coastal area of Mandapam, Tamilnadu, India. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread

out room temperature in shade. Shade dried samples were grounded in to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

2.2. Preparation of Extracts

25 g of the powder of *Sargassum wightii* was transferred into three different 250 ml conical flask containing 100 ml of different solvents viz. Ethanol, Methanol and Water. The conical flask was shaked it well for 48 hours by free hand. After 2 days, the extracts were filtered using Whatmann filter paper No.1 and transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45ºC. The obtained extracts were stored at 4°c in air tight bottle until further use.

2.3. Preliminary phytochemical screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara, Trease and Evans and Harborne.[7] [8] [9]

2.4. Estimation of Total Phenol Content

The total phenolic concentration was measured using the Folin-Ciocalteau method of Kim et al.,[11] with slight modification.

Estimation of Total Flavonoid Content

The total flavanoid content was determined according to the method of Katasani.[10]

INVITRO ANTIOXIDANT ACTIVITY

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging ability of the seaweed extracts was monitored by a method of Shimada etal.[14]

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of the extracts was done based on the method described by Liu etal., (1997).[12]

2.4.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}- Ascorbate EDTA H_2O_2 system (Fenton reaction) according to the method of Yu et al., 2004.[15]

2.4.3. Nitric oxide radical scavenging assay

Nitric oxide radicals generated from Sodium nitroprusside solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Garrat method.[16]

Reducing Power assay

Reducing power of seaweed extract was determined by the standard method of Oyaizu.[13]

RESULTS

2.5. Phytochemical screening

Tannin, phlorotannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, carbohydtrates, proteins, polyphenols and glycosides showed the maximum presence in the ethanol and methanolic extract. In that, the polyphenol found in high concentrations. The phytochemical constituents such as tannin, saponin, flavonoids, steroids and other aromatic compounds are secondary metabolites of seaweed that serve as defense mechanism against predation by many other microorganisms, insects and other herbiviores. The present study carried out on the seaweed sample revealed the presence of medicinally active constituents.

Table 1. Phytochemical screening of different extract of *Sargassum wightii*

Secondary Metabolites	Ethanol	Methanol	Aqueous
Tannin	+	+	+
Phlobatannins	-	-	-
Saponin	+	+	+
Flavonoids	+	++	++
Steroids	+	+	+
Terpenoids	+	+	++
Triterpenoids	+	+	++
Alkaloids	+	+	-
Carbohydrate	++	+	++
Proten	+	+	+
Anthroquinone	-	-	-
Polyphenol	++	++	++
Glycoside	+	+	+

(+) Presence: (-) Absence (++) High concentrations.

2.6. Total phenolic content

Phenolic compounds are commonly found in plants, including seaweeds and have been reported to have a wide range of biological activities including antioxidant property. The total phenolic content of the crude extract was expressed as mg gallic acid equivalent (GAE) per gram of the extract.

2.7. Total flavonoid content

The total flavonoid content was measured as Quercetin equivalent (QE) g of extract. The total flavonoid of the dried seaweed extract was found to be 209.41 mgQE/g of extract.

2.8. INVITRO ANTIOXIDANT ACTIVITY

2.8.1. DPPH activity

The effect of seaweed extract and standard on DPPH radical was compared and shown in Fig.1. The scavenging effect increases with the concentration of standard and sample. At 80 µg/ml concentration of *S.wightii* possessed 86.37±6.04% scavenging activity on DPPH. DPPH results are often interprets as the "inhibition concentration" or IC_{50} value, which is defined as the concentration of the substrate that causes 50% loss of the DPPH activity. The IC_{50} value of DPPH radical scavenging activity was comparable with that of the reference standard, ascorbic acid IC_{50} = 35.03 µg/ml and the extract of *S.wightii* exhibited a significant dose dependent inhibition of DPPH activity with an IC_{50} value of 47.18 µg/ml.

Table 2- % of DPPH Radical scavenging activity of *Sargassum wightii*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Sea weeds	20.91±1.46	38.19±2.67	67.28±4.70	86.37±6.04	47.18
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	35.03

Values were expressed as Mean ± SD for triplicates.

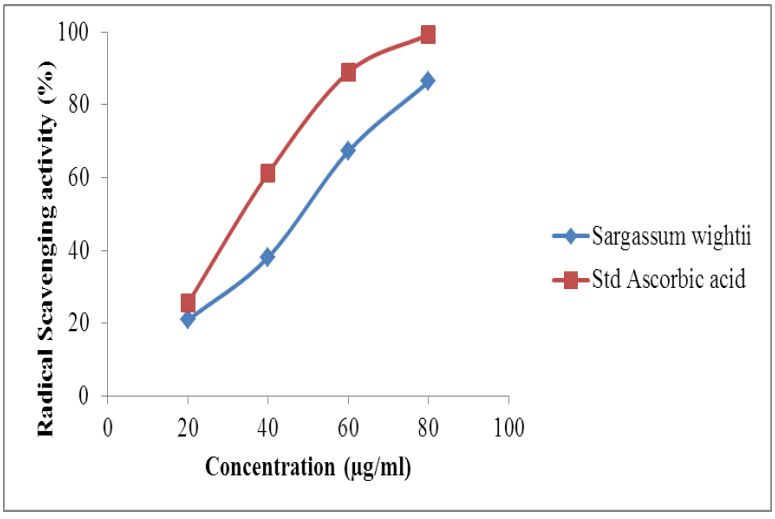

Fig.1 – DPPH radical scavenging activity of *S.wightii*

2.8.2. Superoxide anion scavenging activity

The seaweed extract was subjected to be superoxide scavenging assay and the results were shown in Figure.2. It indicates S.wightii (80 µg/ml) exhibited the maximum superoxide scavenging activity of (88.75±6.21), which shows that higher scavenging activity.

Table 3- % of Superoxide radical scavenging activity of *Sargassum wightii*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Sea weeds	27.85±1.94	58.21±4.07	71.25±4.98	88.75±6.21	38.26
Standard (Ascorbic acid)	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.62

Values were expressed as Mean ± SD for triplicates

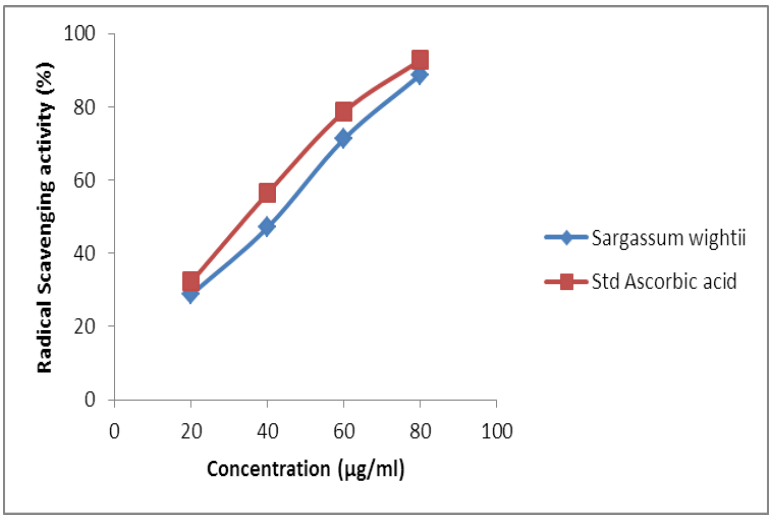

Fig.2 – Superoxide radical scavenging activity of *S.wightii*

2.8.3. Hydroxyl radical scavenging activity

The scavenging effect of OH was investigated using the Fenton reaction and the results shown as % of inhibition rate in Fig.3. *Sargassum wightii* exhibited the inhibition of about 86.71±6.13, but this is lower than the standard Ascorbic acid (80µg/ml) (92.75±7.20) %.

Table 4- % of Hydroxyl radical scavenging activity of *Sargassum wightii*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Sea weeds	28.75± 2.01	47.08±3.29	71.25±4.98	86.71±6.13	41.25
Standard (Ascorbic acid)	32.21±2.51	56.45±4.40	78.65±6.13	92.75±7.20	35.14

Values were expressed as Mean ± SD for triplicates

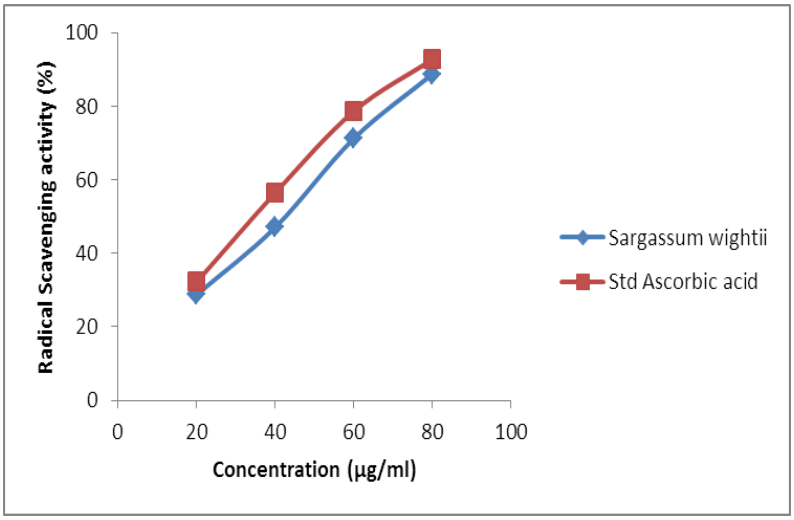

Fig.3 – Hydroxyl radical scavenging activity

2.8.4. Nitric oxide scavenging activity

NO release suppression may be attributed to a direct NO scavenging effect on the seaweed extraxt decreased the amount of nitrite generated from the decomposition of sodium nitroprusside invitro as shown in Fig.4. The results show that *S.wightii* had scavenging activity of (92.38±6.46) % lower than the standard ascorbic acid (96.45±7.52)%.

Table 5- % of Nitric oxide radical scavenging activity of *Sea Sargassum wightii*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Sea weeds	24.28±1.69	53.33±3.73	69.52±4.86	92.38±6.46	41.05
Standard (Ascorbic acid)	26.21±2.04	59.62±4.65	84.23±6.56	96.45±7.52	46.63

Values were expressed as Mean ± SD for triplicates

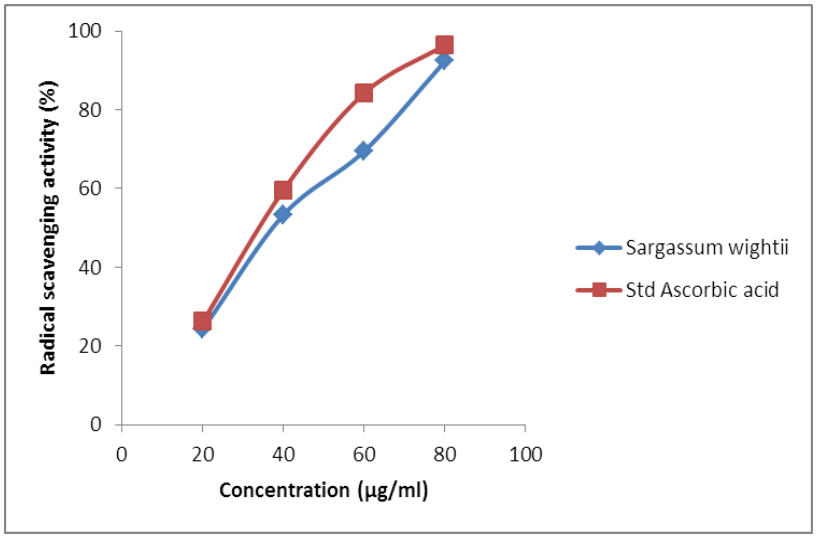

Fig.4 – Nitric oxide scavenging activity

2.8.5. Reducing power assay

The reducing power of *Sargassum wightii* was compared with the standard ascorbic acid. The reducing power increases with the increasing concentration. The reducing power of the sample was shown in Fig.5 and it was found to be in the following order: Ascorbic acid [(0.31±0.03) – (0.94±0.08)], *S.wightii* [(0.26±0.01) – (0.89±0.06)].

Table 6- % of Reducing power assay of *Sargassum wightii*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)
Sea weeds	0.26±0.01	0.52±0.36	0.71±.04	0.89±0.06
Standard (Ascorbic acid)	0.31± 0.03	0.68 ± 0.05	0.86± 0.07	0.94 ± 0.08

Values were expressed as Mean ± SD (Optical density) for triplicates

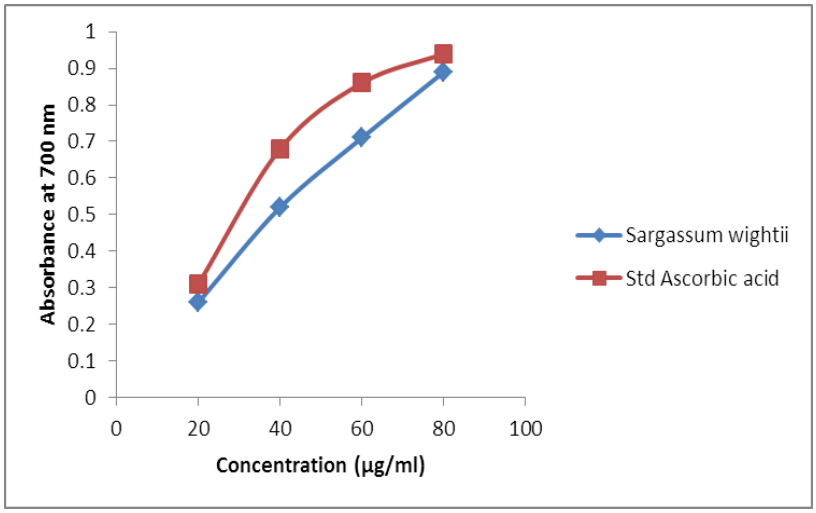

Fig.5 – Reducing power scavenging activity

3. DISCUSSION

Seaweeds are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites which are of great medicinal value and have been extensively used in pharmaceutical industries. The results of the phytochemical analysis of aqueous, ethanolic and methanolic extracts of *S.wightii* revealed the presence of a good number of secondary metabolites which was similar to the result obtained by Babu *et al.*,[17] Thus, marine algae are the richest source of known and novel bioactive compounds.[18]

Superoxide and hydroxyl radicals are the two most effective representative free radicals. In cellular oxidation reactions, superoxide radical is normally formed first and its effects can be

magnified because it produces other kinds of cell damaging free radicals and oxidizing agents.[19]

A number of studies focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers.[20][21] [22] Earlier reports revealed that seaweed extracts, especially polyphenols, have antioxidant activity.[23][24][25]

NO radicals play an important role in inducing inflammatory response and their toxicity multiplies only when tey react with oxygen radicals to form peroxynitrite, which damages biomolecules like proteins, lipids and nucleic acids.[26] Nitric oxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. Seaweed extracts were shown to inhibit nitrite formation by competing with oxygen to react with nitric oxide directly. *S.wightii* ehibited 92.38% at 80µg/ml which was comparable to the standard, which exhibited 96.45% inhibition.

The reducing ability of a compound generally depends on the presence of reductone, which has exhibited antioxidative potential by breaking the free radical chain by donating hydrogen ion.[27] For the measurement of reductive ability of the seaweed extract. We investigated Fe^{3+} to Fe^{2+} tansformation in the presence of an ethanolic environment. *S.wightii* showed increasing absorbance with the control Fig.5. The reducing capacity of is a significant indicator of its potential antioxidant activity.

CONCLUSION

The results obtained from the study clearly indicate that methanolic extract of *S.wightii* has a powerful antioxidant activity inder invitro condition. The most probable reason for their potential antioxidant activity might be phytochemical constituents. The significance of antioxidant activity from *S.wightii* will be further characterized and they will be evaluated for their bioavailability in future.

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