Qualitative Phytochemical Analysis and Antioxidant Studies of Marine algae *Sargassum natans* from Rameshwaram Tamil Nadu

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1. Introduction

Marine algae (Seaweeds) are a group of marine multicellular algae, plentiful in minerals, vitamins, and polysaccharides. They are considered as a potential source of bioactive substances such as proteins, lipids, and polyphenols possessing potent antibacterial, anticancer, antioxidant, antifungal, and antiviral properties (Sundaramurthy et al., 2016). Past few decades, marine algae have been used by food by humans and their extracts have bioactive compounds which have a demand in the pharmaceutical industry (Skulberg, 2000). Macroalgae were commercially important because it is an important source of food, fodder, fertilizer and medicines. Marine macroalgae, mainly including Chlorophyta, Rhodophyta and Phaeophyta, are important in marine ecosystems as they supply high trophic levels via herbivory or detrital food chain (Hurd, 2000). Marine algae (Seaweeds) are a group of marine multicellular algae which have minerals, vitamins, and polysaccharides. Marine algae occur in the saline water of the sea. These algae are either free-floating or are attached to a substratum with the help of an attachment disc. The largest and therefore most complex marine algae are called seaweeds/macroalgae. Seaweeds are generally multicellular marine algae that are large enough to be seen by the naked eye. They may generally grow up to 60 meters in length. These do not have well-defined cells or tissues like stoma, xylem or phloem; neither do they have roots, stems or leaves that are found in land plants. They have as a potential source of bioactive substances which possess antibacterial, anticancer, antioxidant, antifungal, and antiviral properties (Yuan and Walsh, 2006).

Evaluation of antioxidant phytochemicals constituents in macro-algae extracts has gained the interest of the researchers because of their important role in the prevention of diseases. The presence of antioxidant substances such as alkaloids, flavonoids, phenols, tannins, phlorotannin, terpenoids, pigments, glycosides, and steroids in algae was thought to act as a defense mechanism, protecting them against reactive oxygen species (ROS) resulting from harsh environmental conditions (Senguttuvan, 2014; De Alencar et al., 2016). Seaweeds

have been reported to contain secondary metabolites and related active metabolites, and have been extensively used in the drug and pharmaceutical industry (Eluvakkal et al., 2010). Recently, researches have proved that compounds originating from marine algae exhibit various biological activities (Wijesekara et al., 2011).Therefore, there is a new trend to isolate novel bioactive compounds and constituents from edible seaweeds (Li Yong-Xin et al., 2011).

Reactive oxygen species (ROS) refers to an array of metabolites derived from molecular oxygen (O_2). These cellular renegades damage DNA, proteins, and lipids, altering biochemical compounds and corroding cell membrane. Such molecular may plays a major role in the development of various diseases such as cancer, atherosclerosis, and respiratory ailments. Several antioxidant and detoxification enzymes in algae are involved in scavenging of free radicals and also in biotransformation of toxic metabolites and xenobiotic.

Sargassum natans is an invasive species and large biomass was found in the coastal area of India, Srilanka and the Maldives. *Sargassum* provides ecological roles as food and habitat for many aquatic organisms it was under-utilized and has several bioactive compounds. It has a potential for producing alginate, biofuels, fucoidan and other pharmaceutical products. Last few years researchers used *Sargassum natans* for alginate production. It also has several polyphenols compound which has antimicrobial activity. Phytochemical analysis of seaweeds can help the manufacturers for identification and selection of raw materials for drug production. Hence the present evaluate the phytochemical and antioxidant activity in *Sargassum natans*

2. Materials and Methods

2.1 Sample Collection

The algal samples (*Sargassum natans*) were collected from the seashore of Rameshwaram 9.2876° N latitude, 79.3129° E longitude Tamil Nadu. The sample was collected from the intertidal zone and washed thoroughly with seawater for removing the cohered sand particles. The samples were placed aseptically in the plastic bottle contains 200ml of freshwater and transported to the laboratory. The collected sample was subjected to washing by double distilled water for the removal of dirt

2.2 Preparation of Extracts

Crude Sample extract was prepared by Soxhlet extraction method. About 20gm of powdered sample material was uniformly packed into a thimble and extracted with 250ml of different solvents methanol and ethyl acetate separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºCtill all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future use.

2.3 Phytochemical Screening

Preliminary phytochemical analysis was carried out for methanol and ethyl acetate extracts of *Sargassum natans*as per standard methods described by Brain and Turner 1975 and Evans 1996.

2.3.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) **Wagner's test**: Filtrates were treated with wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) H_2SO_4 test: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

2.3.3 Detection of Steroids

Liebermann- Burchard test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

2.3.4 Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

2.3.5 Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

2.3.6 Detection of Phenols

a) **Ferric chloride test**: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

2.3.7 Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.3.8 Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

2.3.9 Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Pottassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

2.3.10 Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.4 Antioxidant activity

2.4.1 DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of Molyneux, 2004. To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound.

Percentage of inhibition was calculated from the equation

[(Absorbance of control - Absorbance of test)/ Absorbance of control] × 100.

IC_{50} value was calculated using Graph pad prism 5.0.

2.4.2 Reducing power assay

The sample together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700 nm against extract

concentration. Ascorbic acid was used as standard. Higher absorbance indicates higher reducing power (Oyaizu, 1986).

2.5 Results and Discussion

Marine organisms are potentially fertile sources of extremely bioactive secondary metabolites that might symbolize functional leads in the development of novel pharmaceutical compounds (Iwamoto et al., 2001). Marine algae produce a diverse array of compounds and chemicals that facilitate their survival and metabolism in extremely harsh and competitive environments. Research into the natural and unique bioactive compounds produced as a result of their biosynthesis of secondary metabolites has generated a renewed interest in the pharmaceutical industry. Their biodiversity and bio-distribution made them unique in chemical composition and mineral content. Hence, they are a promising source of various therapeutic bioactive substances for the treatment of various diseases including cancer.

2.5.1 Phytochemical analysis

To detect the presence of following biomolecules by standard qualitative phytochemical procedures (Chandran et al., 2014; Indumathi et al., 2014). Seaweed contributes to its efficacy as nutraceutical and traditional medicine based on the presence of their chemical components. Some factors like climatic condition, season, species, subspecies, harvest and the method used for extraction of compounds will devastate the chemical compositions of the extract (Roy and Anantharaman, 2017). Seaweeds are primitive non- flowering plants without roots, stems, and leaves. They contain different vitamins, minerals, trace elements, proteins and bioactive substances (Dharmesh et al., 2014). In our present study, the qualitative phytochemical analysis of methanol, ethyl acetate extracts of *Sargassum natans* revealed that methanol extract had better activity. Steroids, phenol and carbohydrates were present in all the two extracts. Alkaloids, tannins, oils and resins were present only in the methanol extract. Terpenoids, anthraquinones, flavaniods and tannins were absent in all extracts of *Sargassum natans.* From the study, it was observed that the algae *Sargassum natans* possesses medicinally important phytochemicals such as alkaloids and steroids.

Table: Qualitative phytochemical analysis of *Sargassum natans*

Phytochemicals	Observations	Extracts	
		Methanol	Ethyl Acetate
Alkaloids Mayer's test Wagner's test	Cream colour Reddish brown solution/ precipitate	+ +	- -
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	- -	- -
Steroids Liebermann-Burchard test	Violet to blue or Green colour formation	+	+
Terpenoids Salkowski test	Reddish brown precipitate	-	-
Arthroquinone Borntrager's test	Pink colour	-	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+ +	+ +
Saponin	Stable persistent	-	-
Tannin	Brownish green / Blue black	+	-
Carbohydrates	Yellow / brownish / blue / green colour	+	+
Oils & Resins	Filter paper method	+	-

Figure: Qualitative phytochemical analysis of *Sargassum natans* in Methanol extract

Figure: Qualitative phytochemical analysis of *Sargassum natans* in Ethyl acetate extract

2.5.2 Antioxidant Activity

Marine algae nutritional fibres execute a diverse array of functions such as antioxidant, anticoagulant, anti-mutagenic, antitumor etc., (Dhargalkar and Neelam, 2005). There are reports that marine algae were also a rich source of antioxidant compounds (Kuda et al., 2002). Antioxidant activity was determined by free radical scavenging (DPPH) and inhibition of lipid peroxidation in Sargassum dentifolium, Jania corniculata &

Laurencia papillosa. Dichloromethane extract of each algal species established better antioxidant activities than the ethanol extract (Shanab, 2007). Quite a few studies have done for the antioxidant activity of natural products of freshwater and marine algae (Fujimoto and Kaneda, 1984; Matsukawa et al., 1997; Lim et al., 2002; Xue et al., 2004).

The antioxidant activity of *Sargassum natans* methanol extract was studied using the reducing power assay and DPPH assay. In all four assays, the % inhibition of the extracts was found to be directly proportional to the concentration of the extract. IC50 was highest in the reducing power assay indicating that the methanol extract had significant reducing power. *Sargassum natans* extract also showed a significant dose dependent reduction of DPPH radicals in the DPPH assay.

Table: DPPH Assay for Methanol extract

Concentration	%IC_{50}	IC_{50}
50	36.24	153.01
250	44.30	
500	51.68	
750	60.40	
1000	66.44	

Figure: DPPH Assay for Methanol extract

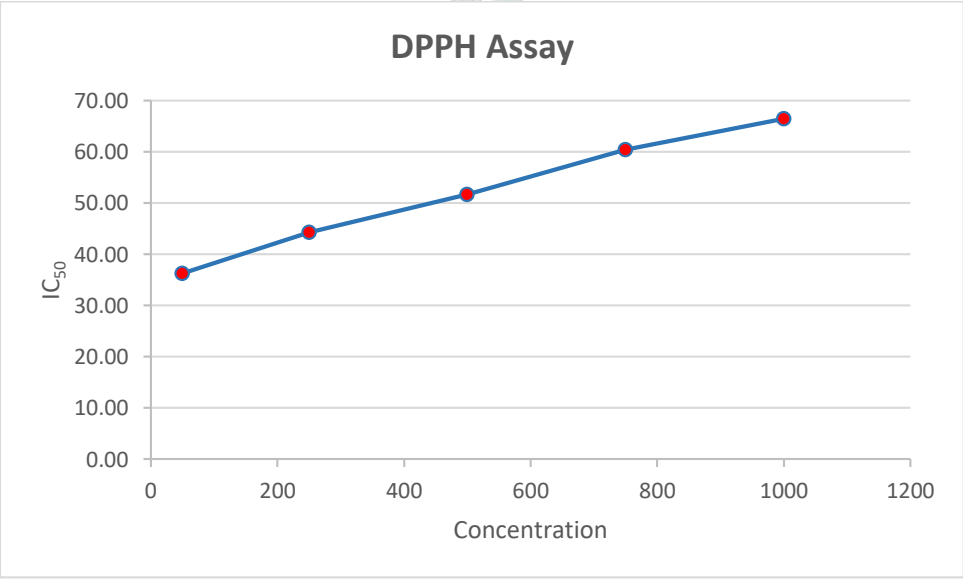
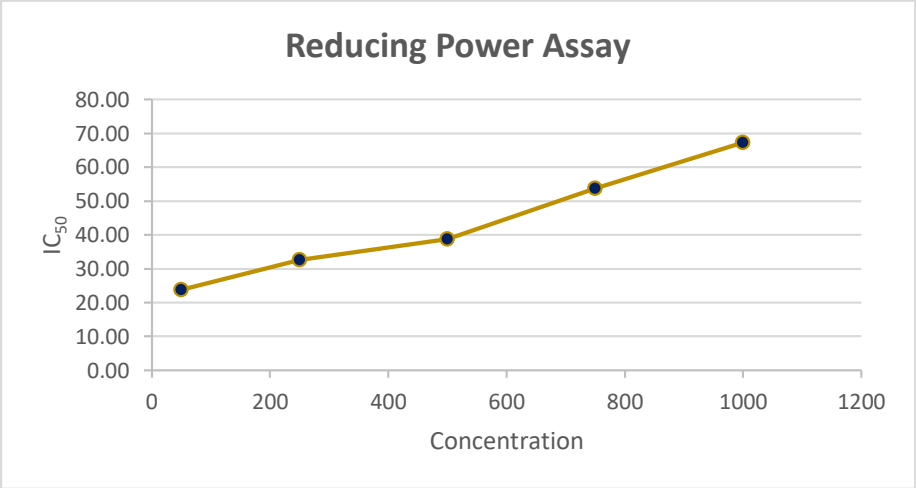


Table: Reducing Power Assay for Methanol extract

Concentration	%IC_{50}	IC_{50}
50	23.81	178.76
250	32.65	
500	38.78	
750	53.74	
1000	67.35	

Figure: Reducing Power Assay for Methanol extract



Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from marine algae and used in medicine and pharmacy (Dhargalkar and Neelam, 2005). Marine algae nutritional fibers execute a diverse array of functions such as antioxidant, anticoagulant, antimutagenic, antitumor *etc*., (Kuda et al., 2002). There are reports that marine algae were also a rich source of antioxidant compounds (Kannan et al., 2014). Dhee et al., 2014 shows the similar result in the free radical scavenging (DPPH) in the methanolic extract. Likewise reducing power assay shows the better result which was similar to S.lomentaria reducing assay by Kuda et al., 2005.

CONCLUSION

Marine algae are used as raw materials for many industrial productions. Algae are consumed as food and feed in all over world. In the present study, the phytochemical screening of the algae *Sargassum natans* was done in methanol and ethyl acetate extracts. The analysis revealed that metabolites with higher medicinal

activities such as alkaloids, phenols and tannins and steroids were present. Terpenoids, flavonoids and anthraquinones, were absent in the extracts. The methanol extracts also showed significant antioxidant potential in the reducing power, and DPPH assays. Thus, the algae *Sargassum natans* can be a significant source of important compounds which can be used in formulation of drugs by the pharmaceutical industries.

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