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Preparation of Crude Extracts From Sargassum Species Seaweed and their Antioxidant Potential

[1]Meenakshi S. Subaraman, [2*]Anitha Roy, [3]Pitchiah Sivaperumal, [4]Lakshmi T

[1]Saveetha Dental College And Hospitals, Saveetha Institute of Medical And Technical Sciences, Chennai 600077,Tamil Nadu, India
[2]Professor , Department of Pharmacology, Saveetha Dental College And Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai 600077,Tamil Nadu, India
[3]Assistant Professor, Marine Biomedical and Environmental Health Research Lab - BlueLab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-600077, Tamil Nadu, India
[4]Professor , Department of Pharmacology, Saveetha Dental College And Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai 600077,Tamil Nadu, India
[4]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamilnadu, India.

ABSTRACT

Introduction: Sargassum have been commonly known for their various biological activities including anti-inflammatory, anticancer, anti-herpes, anti-hyperlipidemic, anticoagulant, antioxidant, and antimicrobial activity. Antioxidants give positive effects on human health by protecting the human body against reactive oxygen species and the harmful effects caused by them. The aim of the present study was to evaluate the antioxidant potential of sargassum species.

Materials and Method: The seaweed sargassum was collected from Thondi coastal area, TamilNadu. The dried powdered seaweed samples were mixed with Ethanol. The extract was used to evaluate the total antioxidant activity, DPPH assay, lipid peroxidation.

Results: The antioxidant potential of *sargassum* species by DPPH assay showed a maximum scavenging activity of 84.67% at highest concentration (150µg/ml) while the standard ascorbic acid showed 98.6% at high concentration. The total antioxidant activity of *Sargassum* extract was analysed. Likewise the maximum lipid peroxide scavenging activity was seen at high concentration of (150µg/ml) with 95.86% while standard ascorbic acid which is compared showed 96.42% scavenging activity which is almost equal.

Conclusion: The present study concluded that crude extract of sargassum species has good antioxidant activity. The extract can be used in disease conditions such as cancers and heart diseases for its antioxidant property.

Corresponding Author: anitharoy@saveetha.com, Mobile No: 9840787458

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INTRODUCTION

Seaweeds are also known marine macroalgae and are classified according to their colour (pigmentation) into brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta) seaweeds. They are widely used as ingredients in cosmetics and fertilizers, food and in hydrocolloid production (e.g. agar and alginate). Some of the seaweeds have the capacity to remove heavy metals from the water and which can be potentially used in bioremediation and in biomonitoring of such pollutants. Seaweeds also have excellent survival strategies to withstand the many environmental stresses. The seaweeds have entered a new phase in genetic research following the first use of the expressed sequence tag (EST) approach to study seaweed genomics[(1)] a relatively inexpensive and quick approach for novel gene discoveries. The seaweed genetic model is easy to culture in vitro, is susceptible to genetic modification and testing, and has a wide geographical distribution. Because of the strong UV radiation in the tropical environment, the tropical macroalgae are expected to develop a very effective antioxidant defence system.[(2-3)] Previous studies have reported that UV radiation induces the promotion of antioxidant defence in macroalgae.

The genus Sargassum, a kind of brown algae, comprising 150 species, a tropical and sub-tropical brown seaweed in subtidal and intertidal areas.[(4)] The water, temperature, tidal levels, water movement and substrate types are the factors that influence the distribution and population structure of Sargassum species.[(5)]

KEYWORDS:
*A*antioxidant,
Assay,
DPPH assay,
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Indonesia is a tropical country that has two seasons, rainy and dry seasons. Sargassum have been commonly know for their various biological activities including anti-inflammatory ,anticancer, anti-herpes activity and anti-hyperlipidemic activity ,anticoagulant, antioxidant and antimicrobial.[6] Now many synthetic antioxidants such as butylated hydroxytoluene (BHT) and tertiary butyl hydroxy quinine (TBHQ) butylated hydroxyanisole (BHA), have proven markable effect through their free radicals scavenging activity either by acting as electron donors or hydrogen and thus protecting the body from degenerative diseases. But their safety and toxicity are considered to be of great concern for their broad spectrum usage. So, there is an increasing demand for alternative antioxidants which are nontoxic, safe and more active with low-cost production. Seaweeds are known to have reactive antioxidant molecules as well as secondary metabolites which act as a source of natural antioxidants. Similarly there are many studies done on different marine species[7-12] which led the researchers to investigate further on it.

Antioxidants give positive effects on human health by protecting the human body against reactive oxygen species, the harmful effects caused by them are damaging macromolecules such as DNA, membrane lipids, and proteins which can lead to the development of several diseases, such as heart diseases, neurodegenerative and inflammatory.[13-14] Antioxidant compounds play an essential role against various diseases such as cardiovascular disorders, atherosclerosis, chronic inflammation, and cancer and aging processes. Recently, there is an increased interest in the development of antioxidants from natural sources of marine flora and fauna.[15] Marine algae are very rich in biologically active compounds and are well recognized for their pharmacological and therapeutic application.[16] Many studies have been conducted exploring the anti-inflammatory (17,18), anti-cancer (19,20) and anti -diabetic (21–25) activity of plant extracts. Our team has extensive knowledge and research experience that has translated into high quality publications (26–30) (31)The aim of the present study was to evaluate the antioxidant potential of sargassum species.

MATERIALS AND METHOD

Study Settings: Marine Biomedical and Environmental Health Research Lab - Blue Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, India.

Sample collection and preparation

The Seaweed Sargassum sp., was collected from Thondi coastal area, Tamilnadu. The sample was washed thoroughly with tap water then shade dried on table tissue paper for 4 weeks and turned into a fine powder.

Preparation of extraction: 10g of dried powdered seaweed samples were mixed with 100ml of methanol/Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passed through whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadow aluminum container at 4ºC for further analysis.

Total antioxidant activity: Total antioxidant activity of the crude Seaweed extract was determined by following method: 0.3 ml of sample was prepared in different concentrations (0.5 – 3mg/ml) with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95¤c for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

DPPH Assay: The antioxidant potential of seaweed crude extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120µM) in methanol and incubated in darkness at 37°c for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

$$\text{Percentage of Inhibition (I \%)} = \frac{\text{(A blank - A sample)}}{\text{A blank}} \times 100$$

Where, A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample. Ascorbic acid was used as positive control (Kamala et al., 2015) and all the tests were carried out in triplicate.

Lipid peroxidation

Lipid peroxidation of the seaweed extract was determined with 2ml of 20% trichloroacetic acid and 2ml of 0.67% thiobarbituric acid solutions containing (0.5-3mg/ml) crude mangrove extract. This mixture was kept in a water bath at 100¤C for 10 min and after cooling to room temperature, it was centrifuged at 3000 rpm for 20 min to form a red complex, measured at 532nm. Percentage of scavenging of lipid peroxidation was calculated as above. Ascorbic acid was used as standard.

RESULTS

The antioxidant potential(scavenging activity) of *sargassum* species by DPPH assay, lipid peroxide scavenging activity and total antioxidant activity was compared with the standard Ascorbic acid and represented in tables and bar charts. At low concentrations (25µg/ml) the crude extract showed 13.62 % scavenging activity while the ascorbic acid which is used as standard showed 37.3% scavenging activity. With increase in concentration of crude extract of sargassum species, it showed a tendency to increase the scavenging activity. The maximum scavenging activity was seen at high concentration of (150µg/ml) with 84.67% while standard ascorbic acid which is compared showed 98.6% scavenging activity. Likewise the maximum lipid peroxide scavenging activity was seen at high concentration of (150µg/ml) with 95.86% while standard ascorbic acid which is compared showed 96.42% scavenging activity which is almost equal. These results showed that increase in concentration of extract, increased the scavenging activity and total antioxidant activity of the extract was equivalent to standard ascorbic acid.

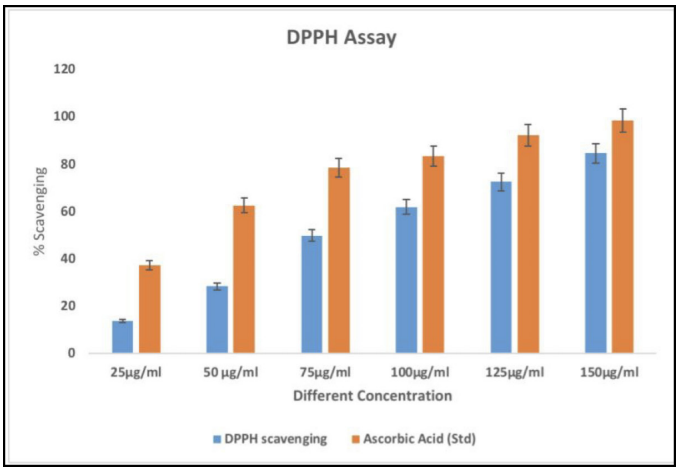

Fig. 1: Represents the graphical representation of the antioxidant activity of sargassum species using DPPH assay. X-axis represents the different concentrations of sargassum extract and ascorbic acid and Y- axis represents the percentage of scavenging. Blue colour represents the DPPH scavenging and orange color represents the activity of Ascorbic acid. The values were done in triplicate (n= 3) with mean ± SE. There was an increase in the activity with an increase in concentration .

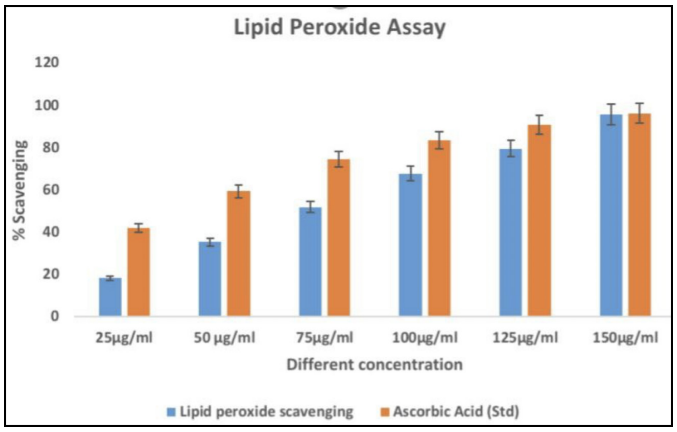

Fig. 2: Represents the graphical representation of antioxidant activity of sargassum species using Lipid peroxide Assay. X- axis represents different concentrations of sargassum extract and Y- axis represents the percentage of scavenging. Blue colour represents the lipid peroxide scavenging and orange color represents the activity of Ascorbic acid. The values were done in triplicate (n= 3) with mean ± SE. There was an increase in the activity with an increase in concentration .

Table 1: Represents the total antioxidant activity of Sargassum species equivalent to ascorbic acid as standard. TAA as a total antioxidant activity and AAE as ascorbic acid equivalent.

Concentration of extract (µg/ml) with TAA	*AAE*
25	32.85±1.27
50	56.52±1.3
75	76.48±0.8
100	97.54±1.22
125	119.47±1.4
150	134.59±1.6

DISCUSSION

The biologically active species with antioxidant potential in the crude sargassum extract results in a scavenging of the stable 2,2-diphenyl-1-picryl hydrazyl radical. The results from this study reported that there is an increase in scavenging activity. For comparison Ascorbic acid was used as standard because it has high antioxidant potential. At low concentrations (25µg/ml) the crude extract showed 13.62 % scavenging activity while the ascorbic acid which is used as standard showed 37.3% scavenging activity. With increase in concentration of crude extract of sargassum species, it showed a tendency to increase the scavenging activity . The maximum scavenging activity was seen at high concentration of (150µg/ml) with 84.67% while standard ascorbic acid which is compared showed 98.6% scavenging activity. From this study, the crude extract showed a promising result. (Fig 1) which was supported by the findings of the previous study (32) that DPPH radical scavenging was moreover the same with seaweed species (brown seaweed) and their results are so promising.

From figure 2, the results depicted that there is an increase in lipid peroxide scavenging activity, For comparison Ascorbic acid was used as standard due to its good lipid peroxide scavenging activity. At low concentrations (25µg/ml) the crude extract showed 18.29% scavenging activity while the ascorbic acid which is used as standard showed 41.95% scavenging activity. With increase in concentration of crude extract of sargassum species, it showed a tendency to increase the scavenging activity.The maximum scavenging activity was seen at high concentration of (150µg/ml) with 95.86% while standard ascorbic acid which is compared showed 96.42% scavenging activity which is almost equal. From this study, the crude extract showed a promising result.

The total antioxidant activity of *Sargassum* extract was analysed. Like above activity for comparison Ascorbic acid was used as standard because of its high antioxidant activity. The analysis was done using various (25µg/ml to 150µg/ml) and their activity was observed. The minimum concentration of 25µg/ml *Sargassum* extract was 32.85±1.27 concentration of ascorbic acid.(table1) Similar antioxidant activity was assessed by the previous study [(33)] where the results reported that extract possesses good antioxidant activity only in acute application while in chronic application the activity is very poor due to the elimination process. In this study Trolox was used as standard. Many plants were evaluated for antioxidant activity and other activities or after isolation of the active components or preparing nanoparticle with it.[34-35]

Our team has extensive research expertise and has cooperated with several writers on a variety of issues over the last decade.[17, 36-51] The limitation of the study is that it was done to analyse only the antioxidant activity of one species of seaweed and only the antioxidant potential of the sargassum species was evaluated. In future, further studies can be done in purified extract and other activities like anti cancer, anti inflammatory and anti diabetic can be analysed.

CONCLUSION

The present study concluded that crude extract of sargassum species has good antioxidant activity. So the extract can be used in treating disease conditions such as cancers and heart diseases for its antioxidant property. Further studies can be done to find the other bioactive properties of the species.

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