RESEARCH ARTICLE OPEN ACCESS

A Study on the Antioxidant Potential of Chaetomorpha Antennina

[1]Ms. J. Jebamalar , [2]Dr. V. Judia Harriet Sumathy
PG & Research Department of Biotechnology, Women's Christian College ,Chennai – 600006

Abstract:

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites. They are characterized by a broad spectrum of biological activities with antiviral, antibacterial and antifungal activities which acts as potential bioactive compounds of interest for pharmaceutical applications. Seaweeds are commonly categorized into three groups such as Chlorophyceae (green seaweed) Phaephyceae (brown seaweeds) and Rodophyceae (red seaweeds) on the basis of the pigments. Sulfated polysaccharides (SP) from different sources have been studied in the light of their important pharmacological activities, such as anticoagulant, antioxidant, antiproliferative, antitumoral, anticomplementary, anti-inflammatory and antiviral properties. *Chaetomorpha* also known as Spaghetti algae or Green hair algae, is an excellent macro algae for refugiums. Each cell grows end to end, creating long, stiff strands. It grows in filamentous clumps. *Chaetomorpha* is a fast growing, hardy algae that is normally grown in refugium where it absorbs nitrate and phosphate out of water as it grows. Additionally this algae is a great habitat for microfauna. *Chaetomorpha sp.,* is able to chelate heavy metals (copper and zinc) in aqueous solutions. A heparin-like polysaccharide has been highlighted in the seaweed. Thus *Chaetomorpha* has the potential to be used as an antibacterial agent. The present study is aimed at screening the antioxidant potential of *Chaetomorpha antennina.*

Keywords **— Seaweeds, Pharmaceutical, Chaetomorpha, Green Algae and Antioxidant Potential**

INTRODUCTION

Green seaweeds have been repeatedly used as a natural material to extract bioactive compounds because of their widespread distribution and large biomass (Subathraa K and TV Poonguzhali, 2013). They are usually grown or collected for food consumption and especially known for their high nutritional value and health benefits. Marine green algae remain largely unexploited among the three divisions of macroalgae (i.e., Chlorophyta, Phaeophyta and Rhodophyta). Interest in utilizing seaweeds as natural resources has recently increased because of their many active ingredients, particularly those that may be used for medical purposes (Premalatha et. al., 2011).

Seaweeds are primitive non-flowering plants without true root, stem and leaves. They include one of the commercially important marine renewable source (Mitali Priyadharshini Pati et. al., 2016). Seaweeds have been used as food stuff in the Asia diet for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Fresh and dry seaweeds are extensively consumed by people especially in the coastal areas (Serhat Keser1 et. al., 2012). The edible seaweeds contain a significant amount of protein, vitamins and minerals, which are essential nutrition for human. Seaweed extracts are considered to be a rich source of

phenolic compounds. The large majority of these are terpenes, but fatty acids are also common with nitrogenous compounds (Massoumeh Farasat et. al., 2013).
The "Green algae" is the most diverse group of algae, it belong to the class Chlorophyceae with more than 7000 species growing in a variety of habitats. The green algae is a 'paraphyletic' group because it excludes the plantae (Sumathi. S and Krishnaveni. M, 2012). Like the plants, the green algae contain two forms of chlorophyll, which they use to capture light energy to fuel the manufacture of sugars, but unlike plants they are primarily aquatic. They are found both in fresh and salt water environment and some even live on land in very wet soils. Some of the most well-known are sea lettuce (S. Mahendran and S. Saravanan, 2013). Green algae are an important food source. They also contain beta carotene, which is used as food coloring and also for cancer prevention (Vadivukkarasi Sasikumar and Pavithra Kalaisezhiyen, 2014).

CLASSIFICATION - GREEN ALGAE

Binomial name : *Chaetomorpha antennina* (Kutzing), 1847
Kingdom : Plantae
Division : Chlorophyta
Class : Ulvophyceae
Order : Cladophorales
Family : Cladophoraceae
Genus : Chaetomorpha
Species : antennina

Chaetomorpha contains vitamin C and A. Some species are edible such as C.crassa, C.linum and C.brachygona. C.crassa is consumed as salad or dessert in far eastern countries due to its character of gelatinization (**Soad M. Mohy El-Din et al., 2015**). Chaetomorpha has possible applications in medicine, dietary supplements, cosmetics and food industries (**Chang, V.S.** and **Teo, S.S, 2016).** The aim of the present study is to identify the effects of Chaetomorpha antennina and its polysaccharides as a potential antioxidant agent. The extract would be characterized using FTIR and its Antioxidant activity using Phosphomolybdenum method .

MATERIALS AND METHODOLOGY

COLLECTION, PROCESSING AND EXTRACTION OF SEAWEEDS

Ceratophylum sp., aquatic plant was collected from a pond in Ajax (Thiruvottiyur) in Chennai. The sample was manually collected; epiphytes and the debris was removed by washing in running tap water and washed again with distilled water. The sample was then allowed to shade dry for 7 days at room temperature and was made into a fine powder using an electric blender. The materials required for the extraction of Seaweeds are Aquatic plant (Ceratophyllum submersum), Solvent (Methanol) 500ml and Conical flask (500 ml). 10gms of the dried Green algae and aquatic plant were extracted separately in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis.

EXTRACTION OF CRUDE POLYSACCHARIDES (Silva *et al)*

The materials required for the extraction of Crude Polysaccharides are Dried powdered sample, Acetone, 0.25M Sodium chloride (Nacl), Sodium hydroxide (NaOH), Trypsin, Filter paper or cheese cloth and Centrifuge tubes. 10g of powder sample was incubated overnight with acetone to

remove lipid and pigments. The residue was then dissolved in 5 volumes of 0.25M NaCl, and the pH was monitored periodically and adjusted to 8 using NaOH. 10mg of trypsin was added to the content for proteolysis and incubated for 24hours. After incubation, the content was filtered through cheese cloth or filter paper. The filtrate was precipitated using ice cold acetone under gentle agitation at 4°C. The precipitate formed was centrifuged at 10,000rpm for 20 minutes.The total polysaccharide extract was dried under vacuum. Extracted polysaccharide was re-suspended in distilled water and was used for further analysis.

FREE RADICAL SCAVENGING ACTIVITY

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY (Phosphomolybdenum method by Pireto *et al.*)

The materials required for Free Radical Scavenging Activity are 28mM Sodium phosphate, 4mM Ammonium molybdate, 0.6M Sulphuric acid, Sample extracts and Polysaccharides. 1ml of the extract was mixed with 1ml of the standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695nm against a reagent blank. The Total Antioxidant Capacity was expressed as milligram of Ascorbic Acid Equivalence (AAE) per gram of extract.

DETERMINATION OF REDUCING POWER ASSAY (Oyaizu *et al.*)

A reductant is not necessarily an antioxidant but an antioxidant is commonly a reductant. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. The materials required for Determination of Reducing Power Assay are 1% Potassium ferricyanide, 0.2M Sodium phosphate buffer, 10% Trichloroacetic acid, 0.1% Ferric chloride, Samples extract and Polysaccharides.The reaction mixture contained 1ml of various concentrations of extracts (2-10 mg/ml), 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The mixture was incubated at 50°C for 30 minutes and the reaction was terminated by the addition of 2.5 ml of 10%TCA, followed by centrifugation at 3000rpm for 10 minutes. 2.5 ml of the upper layer was mixed with 2.5 ml of the deionized water and 0.5 ml of the 0.1% Ferric chloride. The absorbance was measured at 700 nm against blank. The reducing power ability of the sample is determined by the increase in absorbance of sample. BHT was used as standard for comparison.

HYDROGEN PEROXIDE SCAVENGING ASSAY (Ruch *et.al*)

Hydrogen peroxide is a weak oxidizing agent and can inactive a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical amd this ,may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The materials required for Hydrogen Peroxide Scavenging Assay are 43mMHydrogen peroxide, 1M Phosphate buffer, Ascorbic acid, Weed extracts and Polysaccharides. A solution of hydrogen peroxide(43mM) is prepared in phosphate Buffer (1M pH 7.4). Different concentration of sample was added to a hydrogen peroxide solution(0.6 ml , 43 mM). Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without

hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined using the Formula

Percentage scavenging (H2 O2) = ((Ao-A1) / Ao) x 100

RESULTS AND DISCUSSION

COLLECTION OF SAMPLE

Green algae *Chaetomorpha antennina* was collected from shore of Royapuram beach **(Figure 1)**.

Figure 1 : Chaetomorpha antennina

EXTRACTION OF SEAWEED

The aquatic weed (*Chaetomorpha antennina)* was washed with water, the calcareous, stones and epiphytes were removed by manual setting and intensively washed with tap water and then again with distilled water. The aquatic weeds were shade dried for 7-10 days. The bioactive compounds from seaweeds were obtained by using the solvent extraction **(Figure 2)**. Methanol was taken in a conical flask and the weeds were weighed using an electronic weighing balance and added to the solvents in the ratio of 1:10 and left for 2-3 days and the extract was filtered using a muslin cloth.

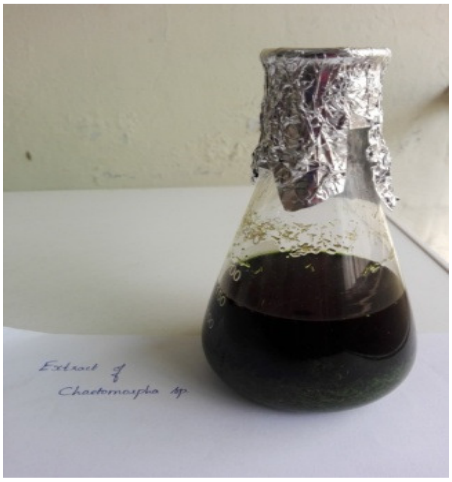


Figure 2 : Chaetomorpha Extract

EXTRACTION OF CRUDE POLYSACCHARIDES

Extraction resulted by yielding 0.5g of green solid crude polysaccharides from 10g of Chaetomorpha antennina (Figures 3 & 4).

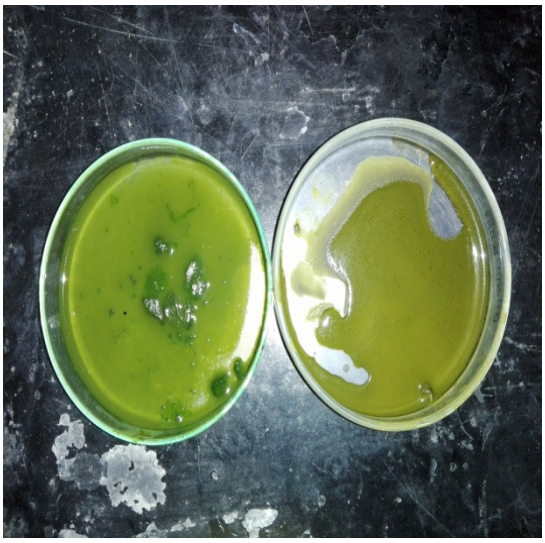


Figure 3 : Polysaccharides after centrifuge

Figure 4 : Dry crude polysaccharides

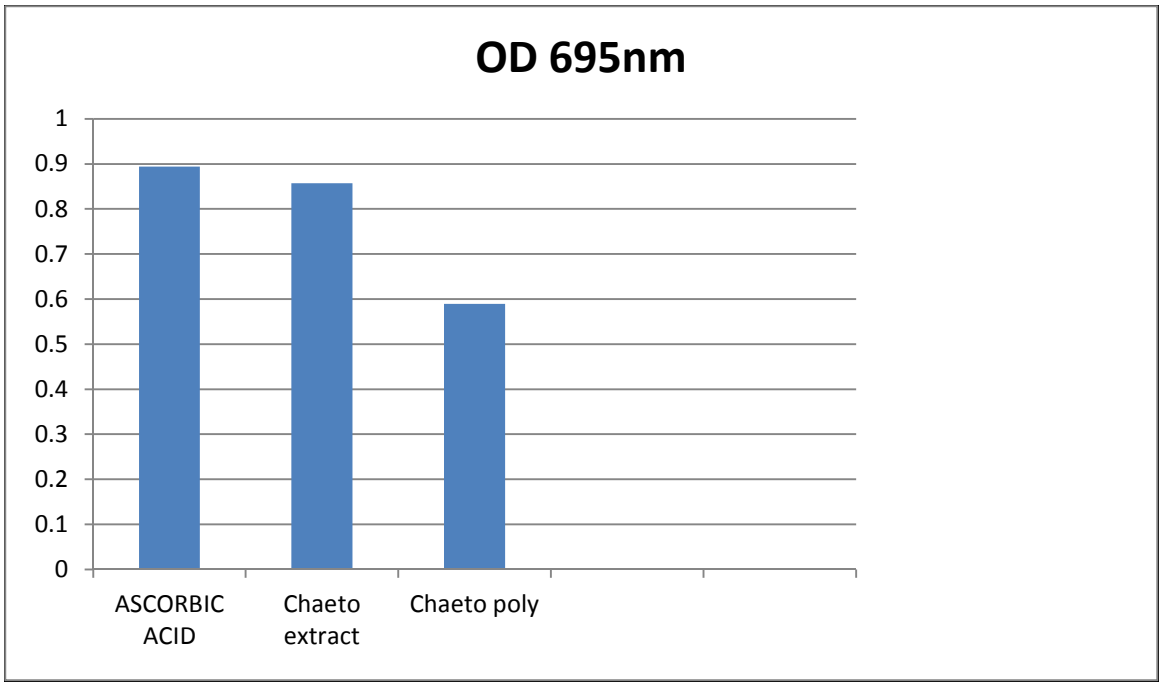
FREE RADICAL SCAVENGING ACTIVITY

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY

The total antioxidant activity of the extract and the polysaccharides were measured spectrophotometrically through Phosphomolybdenum method. The higher the absorbance, the stronger is the antioxidant activity. The total antioxidant activity was found to be higher in Chaetomorpha extract. The total antioxidant capacity is expressed as milligram of Ascorbic Acid Equivalence (AEE) per gram of extract (Table 1 & Graph 1).

S.NO	SAMPLE	O.D. (695nm)
1	Ascorbic acid (Standard)	0.894
2	*Chaetomorpha* extract	0.857
3	*Chaetomorpha* polysaccharides	0.789

Table 1 : Total Antioxidant Activity



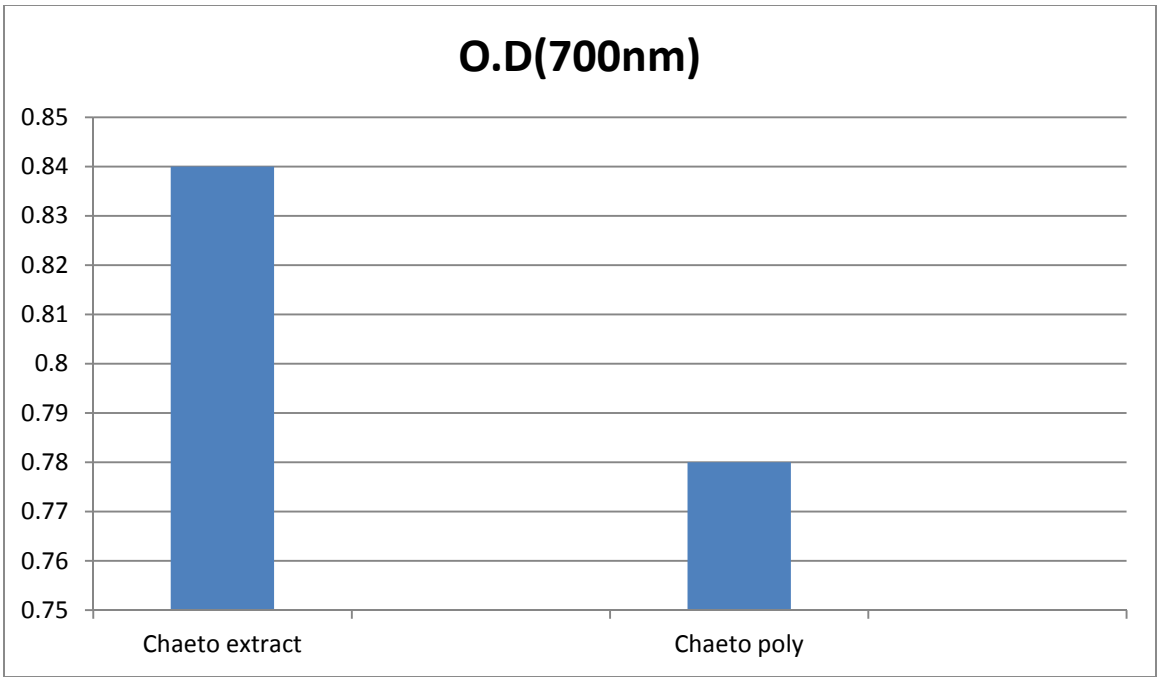
Graph 1 : Total Antioxidant Capacity of Aquatic Weed Extraction and its Polysaccharides

DETERMINATION OF REDUCING POWER

In reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be monitored by measuring the formation of Prussian blue at 700nm. A higher absorbance indicated the higher reducing power. The activity of Chaetomorpha extract was higher (Table 2). The reducing power capacity of Chaetomorpha polysaccharide was higher (Graph 2).

S.NO	SAMPLE	O.D (700nm)
1	*Chaetomorpha* extract	0.841
2	*Chaetomorpha* polysaccharide	0.780

Table 2 : Reducing Power Capacity



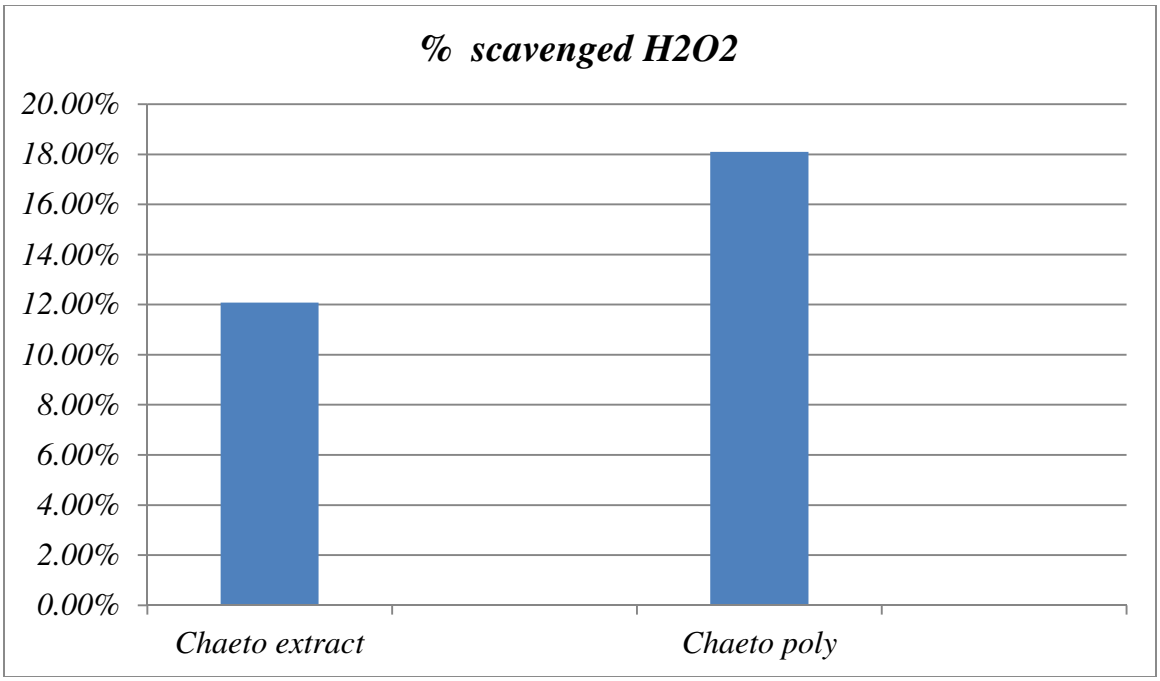
Graph 2 : Reducing Power Capacity of the Aquatic Weeds Extract and its Polysaccharides

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The reactive oxygen species (ROS) such as superoxide anion (O_2^-), Hydrogen peroxide, hydroxyl radical, single oxygen and peroxynitrite are known to cause oxidative damage, contributing to the development of chronic diseases such as cancer, heart disease and cerebrovascular disease (Table 3). The highest scavenging activity was found in *Chaetomorpha* polysaccahrides (Graph 3).

S.NO	SAMPLE	O.D (230nm)	% SCAVENGED (H_2O_2)
1	Ascorbic acid (Standard)	0.993	-
2	*Chaetomorpha* extract	0.873	12.08%
3	*Chaetomorpha* polysaccharide	0.813	18.1%

Table 3 : Hydrogen scavenging activity



Graph 3 : Hydrogen Peroxide Scavenging Activity

CONCLUSION

Many disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, cancer etc., may be the result of increased concentrations of free radicals in an organism. Reactive oxygen species (ROS) and nitrogen (RNS) species, are the most frequent pro-oxidants originating either from normal metabolism or induced by UV radiation and different pollutants. Harmful effects of disturbed antioxidant- prooxidant balance can be largely prevented by intake of antioxidant substances. Antioxidants have already been found in seaweeds. Due to their natural origin, the antioxidants obtained from seaweeds are of greater benefit in comparison to synthetic ones. The use of natural antioxidants from seaweeds does not induce side effects, while synthetic antioxidants are found to have genotoxic effect. Hence the present study paves way for the implication of these seaweeds in Pharmaceutical Industries.

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