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Research Article

COMPARISON OF POLYSACCHARIDE CHARACTERIZATION OF *CHAETOMORPHA ANTENNINA* AND *CERATOPHYLLUM SUBMERSUM*

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ABSTRACT

Green seaweeds have been repeatedly used as a natural material to extract bioactive compounds because of their widespread distribution and large biomass. 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. Seaweeds are also 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. Polysaccharides have the potential to be used as drugs and drug intermediates. They can be used as bio-absorbers, nutrient resources, for protection of cellular material, cold agent, lowering blood pressure and as an anti- oxidant. Polysaccharides are thus significant in the field of medicine, food and health care. 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. The present study is aimed at extracting and comparing the Polysaccharides from *Chaetomorpha antennina* and *Ceratophyllum submersum*.

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INTRODUCTION

Seaweeds are classified into different groups according to their pigments e.g., green algae, red algae and brown algae.

Green Algae

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 [1]. 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 [2]. Green algae are an important food source. They also contain beta carotene, which is used as food coloring and also for cancer prevention [3].

Red Algae

Red algae or Rhodophyta is one of the oldest groups of eukaryotic algae. Red algae are red in color because of the presence of the pigment phycoerythrin; this pigment reflects red light and absorbs blue light [4]. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at greater depths. It has high level of protein and vitamin. Red algae are also commercially important. Carrageenan is a gel used to stabilize man made products such as ice creams, paste, facial creams etc [5].

Brown Algae

Brown algae is the largest and most complex type of algae. Brown algae contain chlorophyll a and c and a pigment called fucoxanthin, which gives the color. Fucoxanthin is not found in other algae or plants. Brown algae include a number of edible seaweeds. All brown algae contain alginic acid (alginate) in their cell walls, which is extracted commercially and used as an industrial thickening agent in food and for other uses. The

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polysaccharide is a major component of brown algae and is not found in land plants. Alginic acid can also be used in aquaculture [6].

Classification - Green Algae

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

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 [6]. *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 possible significant applications in medicine, dietary supplements, cosmetics and food industries [7].

Aquatic Weeds

Binomial name : *Ceratophyllum submersum*
Kingdom : Plantae
Clade : Angiosperms
Order : Ceratophyllales
Family : Ceratophyllaceae
Genus : Ceratophyllum
Species : submersum

Ceratophyllum submersum, is commonly known as coontails or soft hornwort. It is a submerged, free-floating aquatic plant. *Ceratophyllum* is a cosmopolitan genus of flowering plants commonly found in ponds, marshes and quiet streams in tropical and in temperate regions. It is the only genus in the family Ceratophyllaceae. *Ceratophyllum sp*., extracts has anti-diarrheal, antipyretic and carminative effects. Traditionally *Ceratophyllum* is used to cure ulcer, diarrhea and wound and also has novel kidney protective action. In Ayurveda, this is used as a herb in burning sensation, thirst, erysipelas and blood troubles because of its cooling properties. It is also useful in the management of dysuria [8 - 10]. The present study is aimed at extracting, characterizing and comparing the Polysaccharides of *Chaetomorpha antennina* and *Ceratophyllum submersum*.

MATERIALS AND METHODOLOGY

Collection, Processing and Extraction of Seaweeds

Chaetomorpha antennina and *Ceratophyllum submersum* were collected from the shores of Royapuram fishing harbour (N4beach) in Chennai. The samples were manually collected; epiphytes and debris were removed by washing in running tap water and washed again with distilled water. The samples were then allowed to shade dry for 7 days at room temperature and were finely powdered using an electric blender. The materials required for the extraction process are *Chaetomorpha antennina* and *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.

Purification of Polysaccharides

Column chromatography and Dialysis

The materials required for the Column Chromatography and Dialysis are Crude polysaccharides, DEAE Cellulose column (3×45cm), Sodium chloride (0-3M), Dialysis bag and Distilled water. 50mg of crude polysaccharides was dissolved in 10ml of distilled water. It was applied to a DEAE cellulose column pre equilibrated with water and eluted in NaCl gradient (0-3M) until no carbohydrate was detected. Each fraction was assayed for carbohydrate content by phenol sulphuric acid method. The carbohydrate-positive fractions were pooled together and dialyzed (MWCO 14,000) for 24 hours against distilled water.

Chemical analysis

Estimation of Carbohydrates (Phenol-Sulphuric Acid-* Dubois *et al.,)

The materials required for the Estimation of Carbohydrates by Phenol-Sulphuric acid method are Polysaccharides, 5% Phenol, 96% Sulphuric acid, 2.5N Hydrochloric acid, Sodium carbonate, Glucose (standard), Stock- 100mg glucose dissolved in 100 ml of distilled water and Standard- 10ml of stock made upto 100ml. 100mg of the sample was weighed into the boiling tubes. They were hydrolysed by keeping in boiling water bath for 3 hrs with 5ml of 2.5N Hydrochloric acid and was cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased. It was made to a volume of 100ml and was centrifuged. The standard in ranging concentration (0.2ml-1ml) was pipette into a series of test tubes.0.2ml of the extract was pipette in 2 separate test tubes. The volume was made up to 1ml in each tube with distilled water. 1ml of phenol solution was added to each tube followed by 5ml of 96% Sulphuric acid and was shaken well. After 10 minutes the contents in the tubes were shaken and were placed in a water bath at 25°-30° C for 20 minutes. A blank with 1 ml of distilled water was set. The

colour was read at 490nm and the amount of total carbohydrates was calculated using the standard graph.

Estimation of Protein (Lowry's Method)

Protein was estimated using the Lowry's Method. The materials required are

- Fiolin-ciocalteau reagent
- Reagent A- 20% sodium carbonate in 0.1N Sodium hydroxide
- Reagent B- 0.5% copper sulphate in 1% potassium sodium tartarate
- Reagent C- Alkaline copper solution (50ml of A and 1ml of B reagents).
- Stock solution- 50mg of Bovine serum albumin dissolved in distilled water and made upto 50ml in standard flask.
- Standard solution- 10ml of the stock solution was diluted to 50ml with distilled water in standard flask. 1.0ml of this solution contains200µg of protein
- Polysaccharides

The standards in ranging concentration (0.2ml-1ml) were transferred into a series of test tubes. 0.2ml of sample extract was also transferred into two other test tubes. The volume was made upto 1.0ml in all the test tubes. 5ml alkaline copper solution was added to each tube including the blank. It was mixed well and was allowed to stand for10mins. 0.5ml of Fiolin's-ciocalteau reagent was added**.** It was mixed well and was incubated at room temperature in the dark for 30 minutes till Blue colour developed. The Absorbance was read at 620nm.

RESULTS AND DISCUSSION

Collection, Processing and Extraction of Seaweeds

10gms of the dried Green algae and aquatic plant were extracted separately and was placed 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 (Figures 1 & 2).

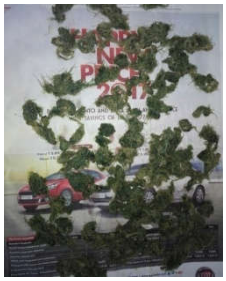


Figure 1 *Chaetomorpha antennina*

Figure 2 *Ceratophyllum submersum*

Extraction of Crude Polysaccharides* (Silva *et al)

The total polysaccharides extract were dried under vacuum. Extracted polysaccharides were re-suspended in distilled water and were used for further analysis (**Figures 3& 4**).

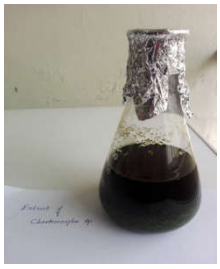


Figure 3 *Chaetomorpha* extract

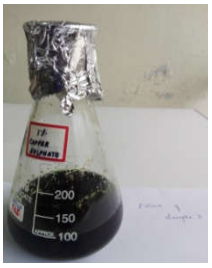


Figure 4 *Ceratophyllum* extract

Extraction of Crude Polysaccharides

Extraction resulted by yielding 0.5g of green solid crude polysaccharides from 10g of *Chaetomorpha antennina* and 0.4g of brownish green crude polysaccharides from 10g of *Ceratophyllum submersum* **(Figures 5 & 6).**

Figure 5 Polysaccharides after centrifuge

Figure 6 Dry crude polysaccharides

Column Chromatography and Dialysis

A few grams of crude polysaccharides were dissolved in 10ml of distilled water. From that 3ml of diluted samples were added to DEAE- Cellulose Column and were eluted with different gradients of NaCl (0-3M). Different fractions which contain polysaccharides were separated based on their ionic character at different molarity. 50ml of partially purified polysaccharides from *Chaetomorpha antennina* and 35ml of partially purified polysaccharides *Ceratophyllum submersum* were collected. The partially purified polysaccharides were subjected to dialysis. 26ml of purified polysaccharide from *Chaetomorpha antennina* and 17ml of purified polysaccharide from *Ceratophyllum submersum* were obtained (Figures 7 - 10).

Figure 7 Column Chromatography of *Chaetomorpha antennina*

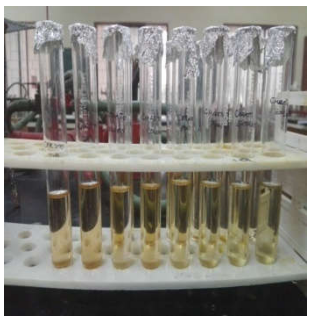


Figure 8 Partially purified Polysaccharides of *Chaetomorpha antennina*

Figure 9 Column Chromatography of *Ceratophyllum submersum*

Figure 10 Partially purified Polysaccharides of *Ceratophyllum submersum*

Chemical analysis

Estimation of Carbohydrates
Glucose (Standard)

By phenol sulphuric acid method, 64mg/ml of carbohydrates in *Chaetomorpha antennina* and 68mg/ml of carbohydrates in *Ceratophyllum submersum* were estimated. Chemical composition of the purified polysaccharide from *Chaetomorpha antennina* and *Ceratophyllum submersum* were determined as carbohydrate content (Tables 1-3 and Graphs 1 & 2).

Table 1 Total Carbohydrate Content of *C. antennina*

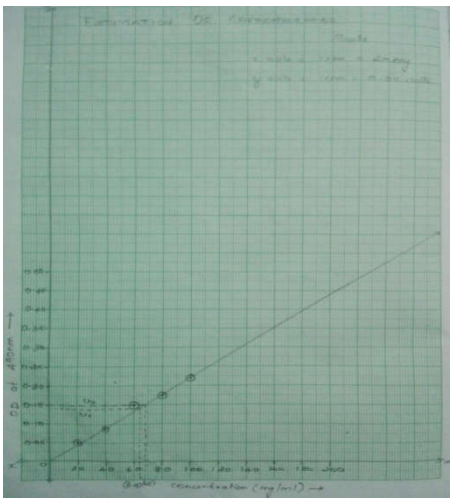
Sample	O.D (490nm)	Concentration of Glucose (mg/ml)
C.antennina	0.14	64

Table 2 Total Carbohydrate Content of *Ceratophyllum submersum*

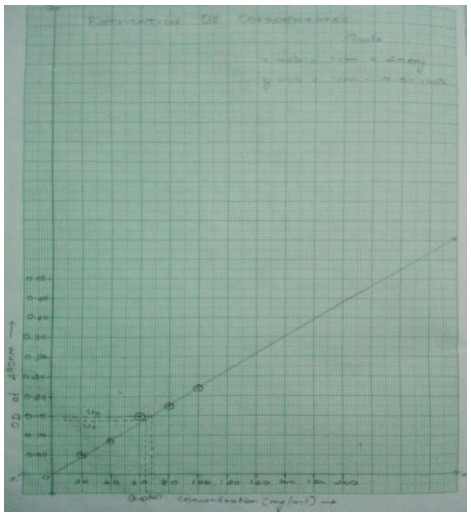
Sample	O.D (490nm)	Concentration of Glucose (mg/ml)
C.submersum	0.15	68

Table 3 Results for Glucose Standard Curve

S.NO	Concentration of Glucose (mg/ml)	O.D (490nm)
1	20	0.05
2	40	0.09
3	60	0.15
4	80	0.18
5	100	0.22



Graph 1 Chemical Analysis for Carbohydrates of *Chaetomorpha antennina*



Graph 2 Chemical Analysis for Carbohydrates of *Ceratophyllum submersum*

Estimation of Proteins

Bovine Serum Albumin- BSA (Standard)

By Lowry's method, 4mg/ml of protein content was estimated both in *C.antennina* and *Ceratophyllum submersum* (Tables 4 - 6). In the chemical composition of purified polysaccharides only a small concentration of protein were present.

Table 4 Total Protein Content of *C.antennina*

SAMPLE	O.D (620nm)	Concentration of protein (mg/ml)
C.antennina	0.03	4

Table 5 Total Protein Content of *Ceratophyllum submersum*

Sample	O.D (620nm)	Concentration of protein (mg/ml)
Ceratophyllum submersum	0.03	4

Table 6 Results for Bovine Serum Albumin Standard Curve

S.No	Concentration Of BSA (mg/ml)	O.D (620nm)
1	20	0.10
2	40	0.27
3	60	0.43
4	80	0.56
5	100	0.70

FT-IR Spectrum for Chaetomorpha Crude Extract

The FTIR spectrum for the *Chaetomorpha* extract was analysed (**Figure 11**). The absorbance band were in the region of 3437cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharides and that at 2923cm^{-1} corresponds to a weak C-H bonds. The intense peak at 1636cm^{-1}were equivalent to that of galactans. The region at 1415cm^{-1} indicates the carboxylic acid. The peaks around 1324cm-1 are the skeleton of galactans. The most important band were found at 1253^{-1}cm which indicated sulphatic groups (S=O). The most important band was found at 1028.06cm^{-1}.The band at 825cm^{-1} shows the mannuronic units.

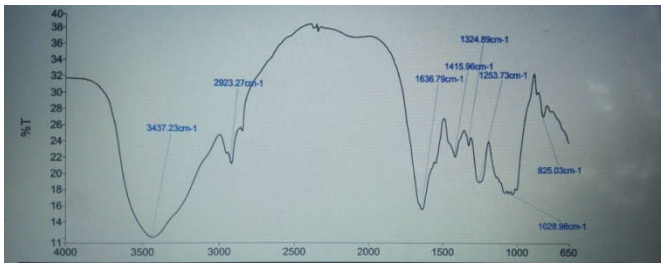


Figure 11 FT-IR Image of *Chaetomorpha* Crude Extract

FT-IR Spectrum for Ceratophyllum crude extract

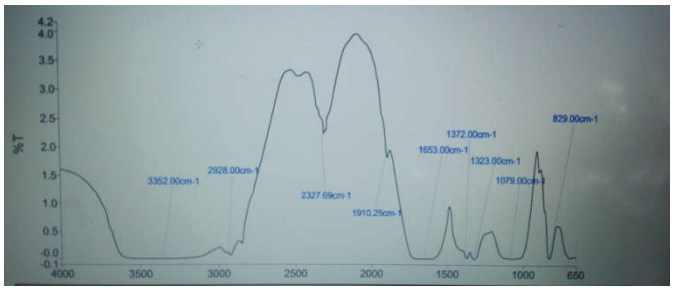


Figure 12 FT-IR image of *Ceratophyllum* Crude Extract

The FTIR spectrum for the *Ceratophyllum* extract was analysed (**Figure 12**). The absorbance band in the region of 3352cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharides and that at 2928cm^{-1} corresponds to a weak C-H bonds. The region at 2327cm^{-1}are equivalent to the alkyl group. The range at 1910cm^{-1} indicates the carbonyl group. The peak around 1653cm^{-1} are the C=H bonds. The band found at 1372cm^{-1} indicated carboxylic acid. The band at 1323cm^{-1} shows the galactan units. The most important band in the region 1079cm^{-1} was indicated as carbohydrates.

FTIR Spectrum for Polysaccharide of Chaetomorpha

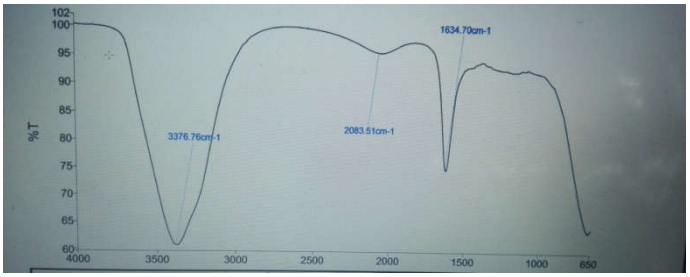


Figure 13 FT-IR Image for *Chaetomorpha* Polysaccharide

The FTIR spectrum for the polysaccharide was analysed (Figure 13). The intense band at the region of 3376cm^{-1} indicated the hydroxyl group. The vibration at the region of 2083cm^{-1} shows the alkenes groups (C=C). The narrow steep range at 1634cm^{-1} represents the galactans.

FTIR Spectrum for Polysaccharide of Ceratophyllum

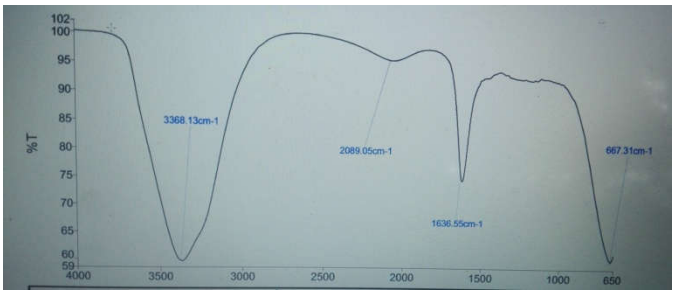


Figure 14 FT-IR Image for *Ceratophyllum* polysaccharide

The FTIR spectrum for the polysaccharide of *Ceratophyllum* was analysed (Figure 14). The maximum absorbance at the region of 3368cm^{-1} was indicated as hydroxyl group stretching vibration of polysaccharides. The mild vibration at region of 2089cm^{-1} represents alkenes (C=C). The band at the region of 1636cm^{-1} indicates carboxylate O-CO bonds. The intense peak at the region of 667cm^{-1} represents the sulphate ester. The polysaccharide samples show a maximum absorption peak at 2900cm$^{-1.}$ Many intense peaks represents C=O, C-H, carboxylic bond, mannuronic unit, galactans and OH bonds which are evident to show there is presence of carbohydrates.

CONCLUSION

Carbohydrates, also called saccharides are molecules composed of carbon, hydrogen and oxygen. They are the most abundant biomolecules and essential components of many natural products and have attracted the attention of researchers because of their numerous human health benefits. Among carbohydrates the polysaccharides represent some of the most abundant bioactive substances. Many seaweeds are good resources of carbohydrates with diverse applications due to their bi-functional properties. Polysaccharides have numerous pharmaceutical activities such as antioxidative, anitibacterial, antiviral, immuno-stimulatory, anticoagulant and anticancer effects. Moreover, these polysaccharides have many general beneficial effects for human health, and have therefore been developed into potential cosmeceuticals and nutraceuticals. The present study thus compares the Polysaccharides of both the species and paves way for future research owing to its multi-faceted biological significance.

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