

Purification, Characterization and Antioxidant Activity of Green Seaweed *Codium* sp

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Abstract In the present study the *Codium* sp. polysaccharide was extracted and partially purified in DEAE cellulose. The polysaccharide was estimated for carbohydrate, sulfate content and uronic acid. The elemental analysis of polysaccharides was analyzed for carbon, hydrogen, nitrogen and sulfur. The antibacterial activity of polysaccharide showed maximum 19 mm of inhibition zone against *Bacillus cereus* and 12 mm of inhibition zone against *Xanthomonas* sp. Free radical scavenging activity of polysaccharide from *Codium* sp. was assayed for total antioxidant capacity, reducing power, hydrogen peroxide scavenging activity, DPPH, ABTS, hydroxyl scavenging assay, superoxide anion radical scavenging and nitric oxide. The green seaweed *Codium* sp. polysaccharide showed rich sources of antibacterial and antioxidant activity.

Keywords *Codium* sp. Polysaccharide, DEAE Cellulose, Antibacterial Activity, *in vitro* Antioxidant

1. Introduction

Seaweeds act as potential bioactive compounds for pharmaceutical applications [1]. Most of these bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides [2].

The major polysaccharides of green algae are more heterogeneous in sugar compositions, three main groups are glucurono xylo rhamnans, glucurono xylo rhamno galactans and xylo arabino galactans. Red algae are galactans commercially known as agar and carrageenan and those of brown algae are fucans, including fucoidan, ascophyllan, sargassan and glucurono xylo fucan [3].

Polysaccharides comprise a complex group of macromolecules with a wide range of important biological properties. Marine algae are the most important source of non-animal polysaccharide. Polysaccharides from algae

possess important pharmacological activities such as anticoagulant [4], antioxidant [5], anti-inflammatory, antiviral [6], antibacterial [7], antiproliferative [8], antitumoral [9], anticomplementary [10] and antiadhesive activities [11]. In recent years, algal polysaccharides especially that extracted from phaeophyta are generally known as fucoidans, as they are rich in the sugar, fucose. They have been demonstrated to play an important role as free radical scavengers and antioxidants for the prevention of oxidative damage in living organisms [12].

The algal polysaccharides play an important role as free-radical scavengers *in vitro* and antioxidants for the prevention of oxidative damage in living organisms. The present study was designed to study the partial purification, antibacterial and antioxidant activities of polysaccharides of *Codium* sp. [13].

2. Materials and Methods

2.1. Extraction and Partial Purification

Polysaccharide was extracted from the green seaweed *Codium* sp. [14]. The crude polysaccharide was further purified by DEAE-cellulose column chromatography [15].

2.2. Estimation of Total Carbohydrates

The carbohydrates content in polysaccharide was estimated by phenol sulphuric acid method [16].

2.3. Determination of Sulfate Content

Sulfate content in polysaccharides was determined by the barium chloride gelatin method [17].

2.4. Determination of Uronic Acid Content

The carbazole reaction which is the most satisfactory method for estimating uronic acid in polysaccharide was employed for quantification [18].

2.5. Analysis of Elements

The percentage content of carbon, hydrogen, nitrogen and sulfur of polysaccharides from *Codium* sp. was estimated by using the operating mode of PE 2400 series II CHNS/O analyzer EA1112 (CE Instrument, Italy).

2.6. Antibacterial Assay

To study the antibacterial activity of the polysaccharides, nine human clinical pathogens were selected for the present study, namely *Klebsiella* sp. *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia* sp. *Citrobacter freundii* and *Clostridium* sp. The three plant pathogens namely, *Erwinia* sp. *Xanthomonas citri* and *Xanthomonas* sp. Antibacterial activity of polysaccharides from *Codium* sp. was determined against nine clinical pathogens and two plant pathogens using paper disk assay method [19].

2.7. Antioxidant Activity of Polysaccharide

The antioxidant activity of *Codium* sp. polysaccharides in total antioxidant activity [20], Reducing power [21], Hydrogen peroxide assay [22], DPPH [23], ABTS [24], Hydroxyl radical scavenging activity [25], Superoxide anion scavenging activity [26] and Nitric oxide radicals [27].

3. Results

Total carbohydrate was found to be $63.21 \pm 0.25\%$, Sulfate content $9.18 \pm 0.54\%$ and uronic acid $3.45 \pm 0.74\%$ for *Codium* sp. shown in Fig. 1.

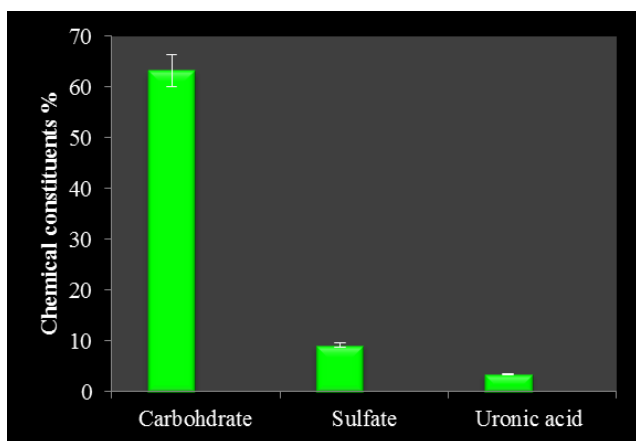


Figure 1. Chemical constituents of polysaccharides from *Codium* sp.

3.1. Elemental Analysis Seaweed Polysaccharide

The chemical composition of *Codium* sp. polysaccharides was analyzed. The percentage of elemental carbon (27.63%), hydrogen (6.87%), nitrogen (1.08%) and sulfur content (3.54%).

3.2. Antibacterial Activity of Polysaccharides against Pathogens

The *Codium* sp. polysaccharides showed maximum of 19 mm of inhibition zone against *Bacillus cereus* and maximum of 10 mm of inhibiting zone against *Pseudomonas aeruginosa*. The *Codium* sp. polysaccharides showed maximum of 15 mm of inhibition zone against *Erwinia* sp. and maximum of 12 mm of inhibiting zone against *Xanthomonas* sp. of plant pathogens.

3.3. Antioxidant Activity of Polysaccharide

The *in vitro* antioxidant activity of *Codium* sp. polysaccharide was determined by total antioxidant activity ($85.53 \pm 0.25\%$), reducing power [$(0.248 \pm 0.45\%) - (1.579 \pm 0.32\%)$], hydrogen peroxide scavenging assay ($79.34 \pm 0.18\%$), DPPH radical scavenging assay ($71.18 \pm 0.54\%$), ABTS inhibition assay ($69.74 \pm 0.49\%$), hydroxyl scavenging assay ($70.44 \pm 0.33\%$), superoxide anion radical scavenging assay ($66.43 \pm 0.27\%$) and nitric oxide scavenging assay ($65.74 \pm 0.18\%$).

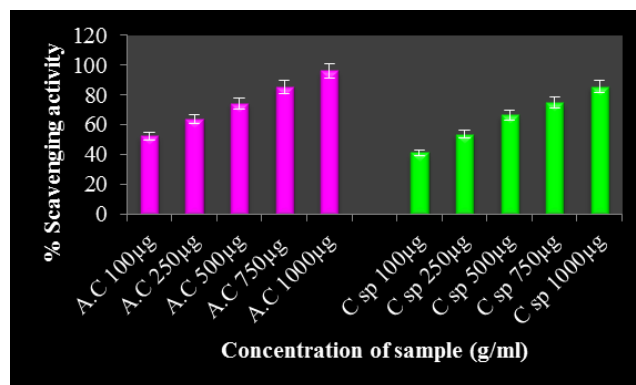


Figure 2. Total Antioxidant Activity of polysaccharides from *Codium* sp. compared with standard Ascorbic acid (A.C)

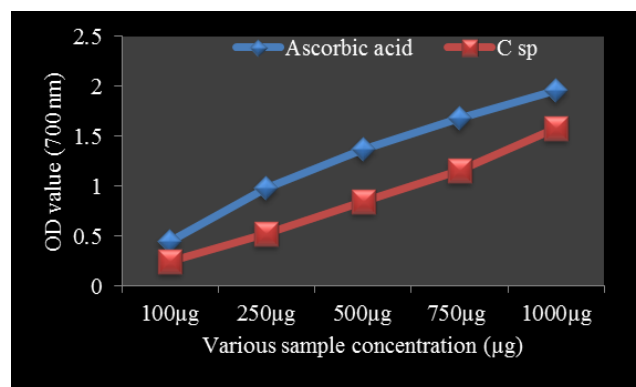


Figure 3. Reducing power of polysaccharides from *Codium* sp. compared with standard Ascorbic acid (A.C)

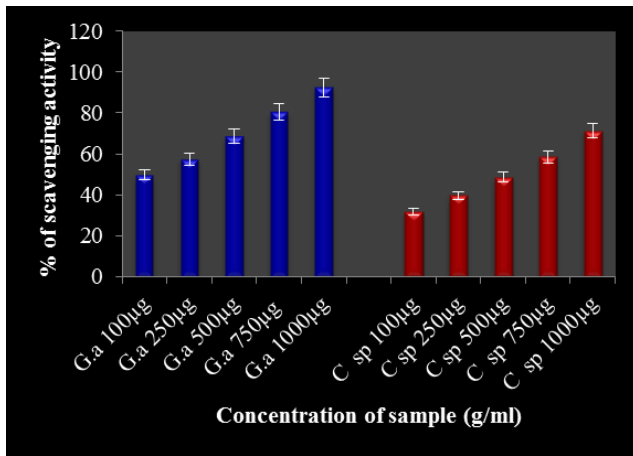


Figure 4. Hydrogen peroxide scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA)

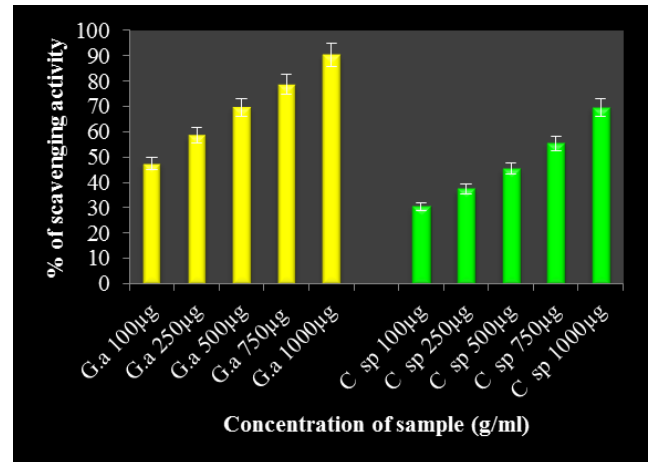


Figure 7. Hydroxyl scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA)

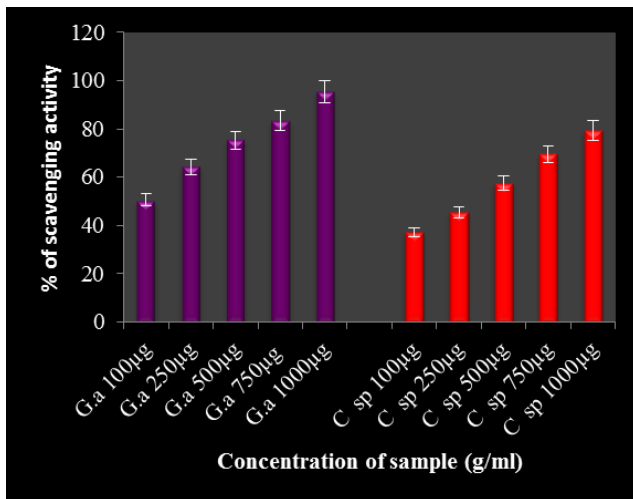


Figure 5. DPPH scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA)

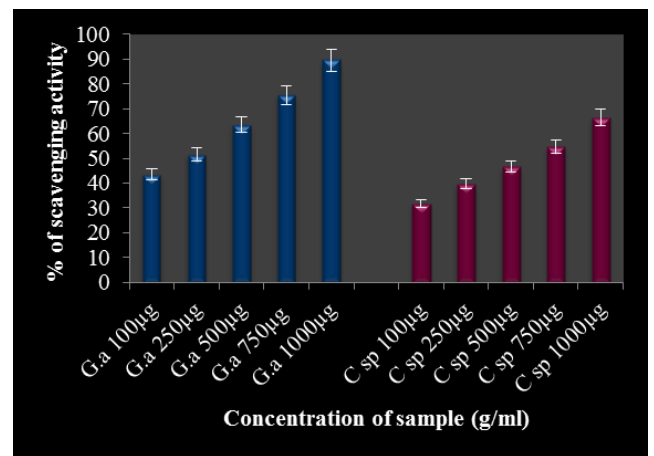


Figure 8. Superoxide anion radical scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA)

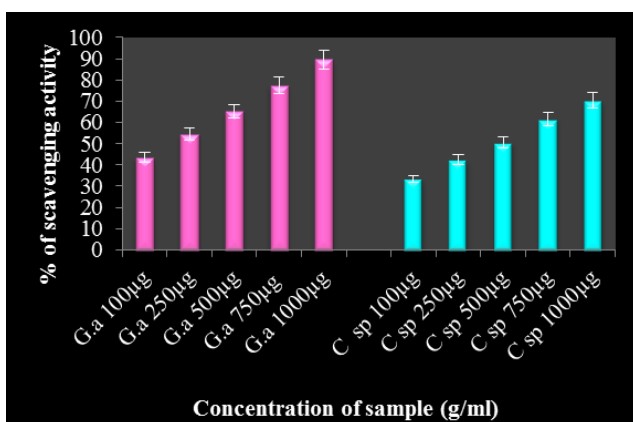


Figure 6. ABTS scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA.)

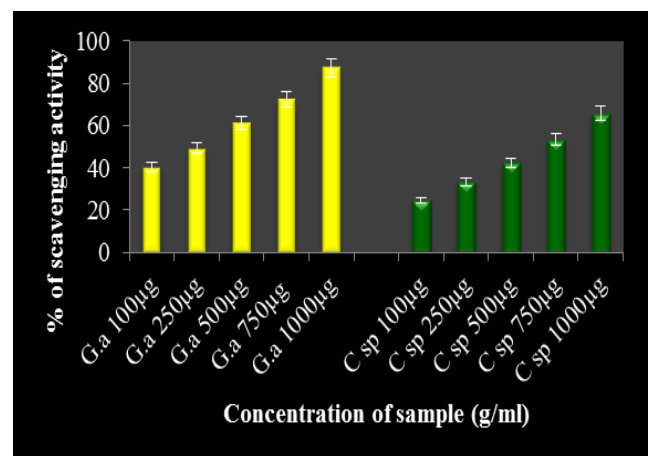


Figure 9. Nitric oxide scavenging assay of polysaccharides from *Codium* sp. compared with standard Gallic acid (GA)

4. Discussion

In present study the total carbohydrate present in the crude extracts of *Codium* sp. was found to be maximum in phenol sulphuric acid method (Dubois *et al.*, 1956) $63.21 \pm 0.25\%$. The sulfate content was found to be $9.18 \pm 0.54\%$ for *Codium* sp. The uronic acid was found to be $3.45 \pm 0.74\%$ for *Codium* sp. Similarly, Mahendran and Saravanan [15] reported total carbohydrate content (47.43%), sulfate content (12.86%) and uronic acid content (4.9%) from the polysaccharide from green seaweed *C. racemosa* which supports the present study.

In the present study, the chemical composition of *Codium* sp. polysaccharides were carbon (27.63%), hydrogen (6.87%), nitrogen (1.08%) and sulfur (3.54%). Similarly, Vaseela [28] reported the sulfated polysaccharides from *Sargassum tenerrimum* in high percentage of carbon 24.44% for *S. tenerrimum*, hydrogen 4.34% for *S. tenerrimum*, nitrogen and 0.13% for *S. tenerrimum*, sulfur 1.80% for *S. tenerrimum*.

In the present study, the antibacterial properties of *Codium* sp. polysaccharides against nine human and three plant pathogenic strains using tetracycline as a standard. The *Codium* sp. sulfated polysaccharides showed maximum of 19 mm of inhibition zone against *Bacillus cereus* and maximum of 10 mm of inhibiting zone against *Pseudomonas aeruginosa*. The *Codium* sp. sulfated polysaccharides showed maximum of 15 mm of inhibition zone against *Erwinia* sp. and maximum of 12 mm of inhibiting zone against *Xanthomonas* sp. of plant pathogens. Rodrigo *et al.* [29] reported that the crude sulfated polysaccharides from *G. ornata* was tested on the growth of bacteria *B. subtilis*, *S. aureus*, *E. aerogens*, *E. coli*, *P. aeruginosa*, *S. choleraesuis* and *S. typhi*.

In the present study, the total antioxidant capacity of polysaccharides from *Codium* sp. was found to be $85.53 \pm 0.25\%$. Costa *et al.* [30] reported that total antioxidant activity of total polysaccharides from the macroalgae *Dictyota cervicornis*, *Dictyopteris delicatula*, *Dictyota menstrualis*, *Dictyota mertensii*, *Sargassum filipendula*, *Spatoglossum schroederi*, *Gracilaria caudata*, *Caulerpa cupressoides*, *Caulerpa prolifera*, *Caulerpa sertularioides* and *Codium isthmocladum*.

The reducing properties are generally associated with the presence of reductions. Reductions were reported to be terminators of free radical chain reactions by donating a hydrogen atom. In most cases, irrespective of the stage in the oxidative chain, in which the antioxidant action is assessed, most non-enzymatic antioxidant activity is mediated by redox reactions [31]. In the present study, the sulfated polysaccharides polysaccharides from *Codium* sp. [$(0.248 \pm 0.45\%) - (1.579 \pm 0.32\%)$] and were compared with the standard ascorbic acid [$(0.447 \pm 0.16\%) - (1.956 \pm 0.24\%)$].

In the present study, the hydrogen peroxide scavenging activity of polysaccharides of *Codium* sp. is $79.34 \pm 0.18\%$. Collen and Pedersen [32] reported that *C. taxifolia* when epiphytized by *Lophocladia lallemandii* showed

increased lipid peroxidation which could be related to the increased H_2O_2 production to compete against *Lophocladia* rather than a marker of oxidative damage.

In the present study, the DPPH radical scavenging assay for the *Codium* sp. is $71.18 \pm 0.54\%$. Zubia *et al.* [33] reported that phenolic compounds of algal extracts of *Halimeda tuna*, *C. cupressoides* and *C. paspaloides* also exhibited relatively high DPPH radical scavenging activities were 6.17 ± 0.10 , 6.35 ± 0.15 and $7.36 \pm 0.16 \text{ mg ml}^{-1}$ respectively.

In the present study, the ABTS inhibition assay for the polysaccharides from *Codium* sp. is $69.74 \pm 0.49\%$. Mahendran and Saravanan [15] reported that the ABTS assay from polysaccharide of *C. racemosa* ($73.32 \pm 1.27\%$). In the present study, the hydroxyl scavenging assay *Codium* sp. is $69.74 \pm 0.49\%$. The metal complexes thus formed cannot further react with H_2O_2 to give a hydroxyl radical [34].

In the present study, the superoxide anion radical scavenging assay for the *Codium* sp. is $66.43 \pm 0.27\%$. Mahendran and Saravanan [15] reported that the superoxide anion radical scavenging activity of polysaccharide from *C. racemosa* was found to be ($66.17 \pm 0.77 \%$). In the present study, the nitric oxide scavenging assay for the *Codium* sp. is $65.74 \pm 0.18\%$. Mahendran and Saravanan [15] reported that the nitric oxide scavenging activity of polysaccharide of *C. racemosa* was found to be ($40.64 \pm 1.82\%$).

5. Conclusions

The present study concludes the opportunity to project the importance of marine green algae possessing antibacterial and antioxidant value since India has rich in biodiversity of marine algae. The sulfated polysaccharide extracted from *Codium* sp. showed higher antibacterial and antioxidant activity. Hence the future research and development of India should pay more attention for the development of drug systems from marine algae especially green algae to save the life of mankind.

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