Characterization and biological properties of sulfated polysaccharides of Corallina officinalis and Pterocladia capillacea

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ABSTRACT
Red seaweed possess various sulfated polysaccharides (SPs) that could potentially be exploited as bioactive agents for medical and industrial applications. Crude polysaccharides from the red algae Corallina officinalis (SP1) and Pterocladia capillacea (SP2) were extracted and characterized according to their chemical content and their antioxidant, anti-inflammatory, anticoagulant, antibacterial, antifungal, and antifouling activities. The isolated polysaccharides contained low levels of protein and high levels of carbohydrate and sulfate. The extracted SPs were characterized by Fourier–transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectral data and revealed that SP1 is composed of carrageenan, while SP2 is composed of polysaccharides containing sulfated galactans plus κ– and ι–carrageenan. Both isolated SPs exhibited all the tested biological activities but those of SP2 were superior. These results reflect the beneficial effects that red algal polysaccharides have as a natural renewable bio–product and that there is a significant relationship between polysaccharide structure, sulfate content and their biological properties. Further studies should be undertaken on the fractionation and characterization of polysaccharides extracted from species of red seaweed in addition to experiments to verify the efficiency of the extracted SPs for food and medical uses in vivo.

Keywords: Anticoagulant, antifouling, antimicrobial, antioxidant, polysaccharides, red seaweed

Introduction

Macroalgae are one of the largest biomass producers in the marine ecosystem and have many bioactive metabolites with valuable applications in the nutritional and pharmaceutical industry (Ismail et al. 2016; Tanna & Mishra 2019). Also, they are renewable, easily cultivated, non–toxic and without side effects (Ismail & El-Sheekh 2017).

Among the different bioactive compounds, sulfated polysaccharides (SPs) represent the main biochemical structure relevant to the algal taxonomic position. Diversity in the chemical composition of algal polysaccharides varied according to phylum, species, different habitats and harvest time (Li et al. 2008). Whereas, SPs are complex and heterogeneous anionic macromolecules and present at high concentrations up to 4 – 76% of macroalgal dry weight (Paniagua-Michel et al. 2014). The macroalgae cell wall is characterized by a high amount of polysaccharides that are majorly substituted by sulfate, which are not present in terrestrial plants (Mourao 2007).

In developing countries, many reports are investigated for extraction of algal polysaccharides due to their biological activities e.g. antiviral, antibacterial, antifungal,
antioxidant, antitumor, immune-stimulatory, anti-inflammatory, gastrointestinal, regenerative, anti-diabetes and nanomedicine, anticoagulant/antithrombotic and antifouling applications (Dai-Hung & Se-Kwon 2013; Tanna & Mishra 2019). The sulfate content of algal polysaccharides determines the biological potency especially their anticoagulant and antioxidant activities (Zhang et al. 2003). Many studies confirmed the safety of algal polysaccharides for using in various economical applications (Silva et al. 2011; Benattouche et al. 2017).

Red seaweed dietary fibers are mostly composed of sulfated polysaccharides galactans (a polymer of galactose), e.g. agar or carrageenan (Fonseca et al. 2008; Cunha & Grenha 2016). Carrageenans are used mainly in the nutrition manufacture due to their gelling, suspension, thickening or water–holding properties (Norziah et al. 2006). The structures of polysaccharides and their sulfate contents markedly varied between species (Amorim et al. 2011). This variation has gained the scientist attention as this contributes to the various facets of their pharmacological ability (Manlusco et al. 2019).

The crude SPs from the red algae “Corallina sp. and Pterocladiad capillacea” have different biological activities such as antimicrobial, antioxidant and anticoagulant properties (Sebaaly et al. 2012; 2014; Abou Zeid et al. 2014). The SPs from C. officinalis have shown its relevance as natural antioxidants in many economical applications (Benattouche et al. 2017). The high antioxidant activity of Pt. capillacea may be attributed to galactose and mannose sugars in the polysaccharide chain besides its high content of phenolic compounds (Fleita et al. 2015). In addition, Pt. capillacea polysaccharide fraction revealed anticoagulant activity by different anticoagulant analyses (Abou Zeid et al. 2014). However, there are few systematically studied reports on the biological abilities of polysaccharides from Egyptian seaweed. Hence, this research aims to characterize the crude polysaccharides extracted from the tested seaweed “C. officinalis and Pt. capillacea” as well as screens their antioxidant, anti–inflammatory, anticoagulant, antimicrobial, and antifouling efficiencies.

Materials and methods

Collection and identification of the selected red algae

The red seaweed Corallina officinalis Linnaeus and Pterocladiad capillacea (S.G. Gmelin) Bornet were freshly collected during summer season 2019 from Sidi Kirayar coast, Mediterranean sea, Egypt (Longitude 29°65’ to 29°85’ E and Latitude 31°3’ to 31°9’ N), and then were washed with distal water to remove epiphytes and debris. On the same day of collection, some of the seaweed samples were prepared as herbarium and other complete thalli were preserved in 5 % formalin in seawater for taxonomical identification according to Aleem (1993); Jha et al. (2009); Kanaan & Belous (2016). The names of the species were used according to Guiry & Guiry (2019). The other part was air–dried at room temperature on absorbent paper. The dried algae samples were crushed to a fine powder and stock up at –20 °C.

Extraction and chemical analysis of the tested crude polysaccharides

Macroalgal polysaccharides were extracted by Imbs et al. (2009) methods. Total sugars were measured by the phenol–H2SO4 reaction using D–glucose as a standard (Dubois 1956). Polysaccharides sulfate contents were estimated turbidimetrically (Hach 2100A) after acid hydrolysis of the polysaccharides (HCl 6 mol/L, 100 °C, 4 h) as indicated by the gelatin–barium method (Lloyd et al. 1961), sodium sulfate was used as standard. The contaminant protein content was estimated by Bradford assay (1976), using bovine serum albumin as standard.

Characterization of the extracted polysaccharides

Fourier transform infrared spectra were recorded on a DRS–800 spectrometer (FTIR). Data were collected in the range of 4000 – 400 cm–1 at a resolution of 4 cm–1. The two extracted SPs were prepared for measurement in the form of KBr pellets. Also, the extracted sulfated polysaccharides (2 – 3 mg) were dissolved in 0.5 ml of 99 % D2O and analyzed using Nuclear Magnetic Resonance spectra (NMR) (JEOL ECA 500), at the Central Labs, Mansoura University, Egypt, with a frequency of 300 MHZ, an acquisition time of 5.29 s and duration of impulse of 11 μs at room temperature.

Biological activities of the extracted polysaccharides

Antioxidant activity

The ability of both isolated polysaccharides methanolic extract to scavenge DPPH free radical was estimated according to Ye et al. (2009) method. Briefly, a 0.1 mM of methanolic DPPH solution was prepared, to give the initial absorbance value of 0.993 at 517 nm. The different concentration of samples (in 0.1 ml) of each sample (with appropriate dilution if necessary) was added to 3.0 ml of ethanolic DPPH solution. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. The percentage of DPPH scavenging activity which was scavenged was calculated using the following formula:

\[
\text{Scavenging activity} \% = \left( 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

About 1 ml of methanolic extract of both tested polysaccharides was mixed with 3 ml of TAC reagent solution. The tubes were capped and incubated at 95 °C for 90 min. After cooling, the absorbance of each sample was measured at 695 nm and ascorbic acid was used as standard (Prieto et al. 1999).
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Results and discussion

The used alga is classified as Phylum: Rhodophyta; Order (1) Corallinales; Family: Corallinaceae; Genus (2): Corallina officinalis (Fig. 1A).

Order (2): Ceramiales; Family: Rhodomelaceae; Genus: Pterocladia capillacea (Fig. 1 B).

Polysaccharides chemical characterization

The yield of the polysaccharide extracted from C. officinalis and Pt. capillacea, obtained from aqueous extraction, under heating represented 36.57 and 42.19 % of the seaweed dry weight, respectively (Tab. 1). The estimated yield for both seaweed was similar that obtained for G. gracilis (36.8 – 46.6 %) (Skrivpova & Nabivailo 2009). The sulfate content of the tested polysaccharides was 3.2 and 1.5 % corresponding to C. officinalis and Pt. capillacea. These ratios were higher than those obtained for G. birdiae 1.0 % (Barros et al. 2013) but they were lower than determined from G. domingensis (7.6 %) and G. mamlilarius (8.9 %) (Valente et al. 1992). The results in (Tab. 1) showed the tested seaweed contained high levels of polysaccharides (48.73 – 61.75 %). On the other hand, no protein was detected in the isolated polysaccharides.

Table 1. Chemical composition (%) of the crude polysaccharides isolated from Corallina officinalis (SP1) and Pterocladiad capillacea (SP2).

|      | Protein | Sulfate content | Carbohydrate | Polysaccharides |
|------|---------|----------------|--------------|-----------------|
| SP1  | 0.004±0.0 | 15.02±0.97 | 42.73±2.51 | 36.57±1.06 |
| SP2  | 0.001±0.0 | 31.24±1.21 | 59.91±2.19 | 42.19±2.12 |

Data are mean of three replicates (±SD).

Molecular structure

The molecular structures of the polysaccharides are shown by using FTIR and NMR techniques.

FTIR analyses show the most functional groups and similarities between compounds (Fig. 2). Broad bands are assigned at 3437–3435 cm⁻¹ for SP1 (Fig. 2A) and SP2 (Fig. 2B) that are interpreted as being due to the stretching vibration of O–H (Sekkal & Legrand 1993). The small band
at 2934 cm⁻¹ may be related to the C–H stretching vibration. The signals at 1647 cm⁻¹ for SP1 and 1639 cm⁻¹ for SP2 correlated to the carboxyl group of uronic acid (Silva et al. 2005).

The regions at 1416.81 cm⁻¹ (SP1) and 1424.51 cm⁻¹ (SP2) may be assigned to the C–OH bending vibration with the contribution of carboxyl group O–C–O (Mathlouthi et al. 2001). FTIR of the SP1 and SP2 showed absorption at 1240 and 1249 cm⁻¹ corresponding to S–O stretching vibration and suggesting the presence of ester sulfate. The weak bands at 1152.77 and 1157.45 cm⁻¹ are due to the stretching vibration of sulfate esters, γ(C–O–C), or γ(C–C).

The FTIR of SP2 polysaccharides had a small signal at 770 cm⁻¹ which is characteristic of the agarocolloids of the red seaweed compound (Fonseca et al. 2008).

The band around 1072.13 for SP1 and 1069.81 cm⁻¹ for SP2 was equivalent to the skeleton of galactans and stretching vibration of sulfate group SO (Chopin et al. 1999). Whereas FT–IR spectrum of SP2 showed a weak peak located at 877.14 cm⁻¹ correspond to a specific agar band (Souza et al. 2012). The spectrum appeared the band at 933.29 cm⁻¹ (SP1) and 933.09 cm⁻¹ (SP2) has been assigned to 3,6–anhydrobridge which is common in κ- and ι–carrageenan not in λ–carrageenan (Silva et al. 2010). The FTIR of SP2 polysaccharides had a small signal at 770 cm⁻¹ which characteristic bands of agarocolloids of the red seaweed compound (Fonseca et al. 2008). These compounds are mainly galactans consisting entirely of galactose or modified galactose units.

The ¹H NMR analysis is considered to be the most common technique used for seaweed polysaccharides characterization (Valiente et al. 1992). The NMR spectrum of SP1 was similar to the SP2 (Fig. 3A–B). The ¹H NMR spectrum of the isolated polysaccharides contained intense signals between the ranges (0.5 – 4.5 ppm) (Fig. 3). The signal at δ 4.43 ppm was assigned to H–1 of β–d–galactose linked to α–l–galactose–6–sulfate (Barros et al. 2013). The signal at 4.61 ppm from Pt. capillacea (Fig. 3B) corresponded to a 3–linked d–galactopyranosyl residue (Ale et al. 2011).

The signals at δ 5.198 & δ 5.39 ppm attributed to H–1 of the l–galactose residue linked to a pyruvated d–galactose residue and anomeric hydrogen of 6–O–sulfate–l–galactopyranose at NMR spectrum of SP1, respectively (Mazumder et al. 2002). This spectrum also revealed a β–glycosidic configuration at δ 4.8 ppm from SP1 (Fig. 3A) (Rupeárez et al. 2002).

**Biological activities**

**Antioxidant activity**

Table 2 clarifies significant DPPH inhibitory potency and TAC of the isolated crude polysaccharides.

| Samples     | DPPH %      | TAC     | Anti–inflammatory activity (%) |
|-------------|-------------|---------|--------------------------------|
| SP1         | 11.97±1.83  | 2.85±0.95| 2.41±0.72                      |
| SP2         | 53.82±3.03  | 6.08±1.03| 30.59±2.02                     |

Data are mean of three replicates (± SD).

The DPPH free–radical scavenging efficiency demonstrated that the isolated crude polysaccharides had a moderate impact on preventing the formation of these radicals. These results are in agreement with those of Souza et al. (2012) who detected that the aqueous extracted SP from Gracilaria birdiae exhibited moderate antioxidant potency as estimated by DPPH scavenging effect. Two SP fractions “galactose and xylose” from C. officinalis had considerable antioxidant capacities (Yang et al. 2011). The SPs from C. officinalis showed a high antioxidant property (Costa et al. 2010).

**Figure 1.** Photo of (A) Corallina officinalis Linnaeus and (B) Pterocladiad capillacea (S.G. Gmelin) Bornet.

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Figure 2. Infrared spectra (KBr, cm⁻¹) of crude polysaccharides isolated from (A) *C. officinalis* (SP1) and (B) *Pt. capillacea* (SP2).
Table 2 shows that the TAC of both isolated SPs varied by algal species and their sulfate concentrations. The antioxidant efficiency of SP2 appeared higher TAC than SP1; this might be related to its sulfate concentration. Whereas there is a positive correlation between the antioxidant ability of algal polysaccharides and their sulfate content (Zhong et al. 2019).

**Anti-inflammatory activity**

The SP2 exhibited a higher capacity for anti-inflammatory than SP1 (Tab. 2). Besides, the extracted polysaccharides had the highest anti-inflammatory activity compared to standard drug 'Diclofenac'. As documented by many studies, the anti-inflammatory ability of the isolated polysaccharides extracted from Gelidium pacificum (Cui et al. 2019); Hypnea musciformis (Brito et al. 2013). Gracillaria verrucosa had anti-inflammatory potency by their inhibitory impacts on the pro-inflammatory mediator’s production (NO, IL–6, and TNFa) (Dang et al. 2008).

**Anticoagulant activity**

The anticoagulant activity of two SP depended on algal species, their structure and concentrations. In agreement with Shanmugam & Mody (2000) who demonstrated the

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**Figure 3.** $^1$H NMR 1H NMR spectra (300 MHZ, deuterated solvent used) of crude polysaccharides isolated from (A) C. officinalis (SP1) and (B) Pt. capillacea (SP2).
anticoagulant potency of algal polysaccharides is attributed to their composition, sulfate content and molecular weight. Also, they may be related to the similarity between heparin and SPs from marine algae, while red seaweed SPs had 4.8 times more activity than heparin (Güven et al. 2019).

Sulfated galactans from red seaweed have being associated to anticoagulant, fibrinolytic and platelet aggregation activities (Pereira et al. 2002).

The activated partial thromboplastin time “APTT” and prothrombin time “PT” analysis are common tests that characterize blood coagula ion, while APTT estimates the influence of compounds under intrinsic and common coagulation pathways (Silva et al. 2005). On the base of the standard range of clotting time APTT (28–38 s) which may vary depending on the reagents used and the laboratory. Both tested polysaccharides showed anticoagulant potency, while SP2 had more anticoagulant ability (40–51 Sec.) than SP1 (37– 46 Sec.). The increases in anticoagulant activity were contributed to the increases in polysaccharide and sulfate concentrations. This variation may be due to the polysaccharides types, structure, content and position of sulfate group (Suwan et al. 2009). The polysaccharides such as agar, galactan, carrageenan, porphyran from red seaweed contained –O–SO 3 H group which played a critical role in blood clotting inhibition (Güven et al. 2019). Moreover, carrageenans extended the clotting time via inactivation of thrombin and antithrombin III (Kindness et al. 1979). In this connection, Sebaaly et al. (2014) detected the carrageenans more pronounced anticoagulant effect than galactan isolated from the same species Corallina. On the other hand, galactan from Pt. capillacea was higher APTT than carrageenan (Sebaaly et al. 2012).

According to the APTT / APTT control ratio, the compounds that have a ratio greater than 1.2 acts as a reactive anticoagulant agent (Karaki et al. 2013) so all tested polysaccharides (1.23 – 1.7 %) are recommended as safe anticoagulant compounds.

PT test estimates the influence under extrinsic and common coagulation pathways (Silva et al. 2005). SP2 had more PT activity than SP1 which increases with the increase in the polysaccharides concentrations (Tab. 3).

**Table 3. Anticoagulant properties of SP1 and SP2**

| Sample (μg/ml) | SP1   | SP2   | SP1 | SP2 |
|---------------|-------|-------|-----|-----|
| 25            | 12.3±0 | 1.33±0 | 13±1 | 14±0 |
| 50            | 13.0±0 | 1.43±0 | 15±0 | 16±1 |
| 75            | 14±1  | 1.63±0 | 19±0 | 20±1 |
| 100           | 15±1  | 1.7±0  | 20±1 | 23±1 |

Data are mean of three replicates (± SD). Ratio was calculated by the formula: Ratio = APTT measured / APTT control “30 second”.

Results of our study showed that SP1 and SP2 had an anticoagulant impact that prolonging the PT and APTT. The prolongation of PT indicates that the extrinsic pathway of coagulation was inhibited, whereas the prolongation of APTT suggests the inhibition of the intrinsic and/or common pathway (Liu et al. 2018).

There is a significant relationship between anticoagulant potency (APTT and PT) of the SPs and their sulfate content. Carrageenan with a high level of sulfate content displayed an anticoagulant efficiency higher than that of low sulfate content (Shanmugam & Mody 2000).

**Antimicrobial activities**

The marine algal polysaccharides have antimicrobial potency against different pathogenic microbe (Jun et al. 2018). Antibacterial potency of both SPs toward three Gram-positive and three Gram-negative strains are illustrated in Figure 4. There are significant variations in antibacterial potency of the isolated SPs, which may be related to the used pathogenic bacteria species and polysaccharides structure and seaweed species. Both SPs had no impact on the growth of all bacterial Gram-negative, except E. coli growth was inhibited by SP1 “11±0.5 mm”. Both SPs showed antibacterial toward gram-positive bacteria “Bacillus cereus “10±1.8 mm; 12±1.2 mm, respectively” and Staphylococcus aureus “8±1.3 mm; 9.5±0.7 mm, respectively”.

![Figure 4](image)

**Figure 4.** The antibacterial activity of SP1 and SP2 (mm) (The data are given as means ± SD).

Our results agreement with sulfated polysaccharides “galactans” extracted from Corallina sp. which had an inhibitory effect on Gram-positive strains (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus epidermidis* CIP 444). Besides, carrageenan from the same Corallina sp. suppressed the growth of *Staphylococcus epidermidis* (Sebaaly et al. 2014). Abou Zeid et al. (2014) found the hot and cold water–extracted polysaccharides from *Pt. capillacea* hindered *B. cereus* and *S. aureus* growth in disc diffusion assays. The antibacterial mechanism may be correlated to the hypothesis of the presence of glycoprotein–receptors in the polysaccharides cell–surface that recognizing and binding to the charged compounds presents in the bacterial cell–surface, cytoplasmic membrane, and DNA or/and the
repulsion between the sulfated groups and bacterial cell wall (Rostand & Esko 1997).

Algal polysaccharides enhance plant defense responses and protection by activating salicylic acid, jasmonic acid, and/or ethylene signaling pathways at a systemic level against various pathogenic fungi (Vera et al. 2011). The antifungal mechanisms of carrageenans from Chondracathus teedei are depended on alterations of the cell walls of A. fumigatus and A. infectoria (Soares et al. 2016).

The antifungal ability of the isolated sulfated polysaccharides against four pathogenic fungi is cleared in Figure 5. These variations ranged from 30 % to 100 % according to algal species and pathogenic fungi species. Generally, the average of antifungal inhibition of SP2 exhibited the maximum value “74 %” comparing with SP1 “35 %” and miconazole “65 %” toward all the tested pathogenic species.

Algal polysaccharides enhance plant defense responses and protection by activating salicylic acid, jasmonic acid and/or ethylene signaling pathways at a systemic level against different pathogenic fungi (Vera et al. 2011). The antifungal mechanisms of carrageenans from Chondracathus teedei depending on alterations of the cell walls of A. fumigatus and A. infectoria (Soares et al. 2016).

Antifouling activity

Marine fouling is the main problem faced by mankind in its oceanic activities. Seaweed and their extracts are natural, renewable and safe antifouling agents for epibiosis inhibition, in addition to corals, ascidians, and many invertebrates species (Da-Gama & Pereira 1995). Figure 6 explains the antagonistic effect of SP1 (Fig. 6A) and SP2 (Fig. 6B) on biofilm formation compared with the control (biofilm formed without the addition of the polysaccharides) (Fig. 6C). This demonstrated the potential antifouling effect of

**Figure 5.** The antifungal inhibition ratio % of SP1 and SP2 (The data are given as means ± SD).

**Figure 6.** Photographs illustrating the antifouling effect induced by crude polysaccharides isolated from C. officinalis (SP1) and Pt. capillacea (SP2) on marine bacterial biofilm.
both extracted polysaccharide which decreased the bacterial density due to their antibacterial activity. In this context, Carvalho et al. (2016) observed the antifouling potency of Pt. capillacea against bacterial quorum sensing (QS). Pérez et al. (2016) detected the antifouling activity of the aqueous extracts of 30 marine algal species against 35 isolates of marine bacteria in vitro.

**Conclusion**

The results of this study indicate that the crude polysaccharides from *Pterocladia capillacea* have promising antioxidant, anticoagulant, antibacterial and antifungal capabilities that require more investigation to be integrated into nutritional and/or medical uses. Moreover, they can be used as a natural antifouling agent against the bacterial biofilm which is the base layer of the fouling process. The main extracted polysaccharides with various biological activities were identified as κ- and τ-carrageenan in SP2. The present results serve as a starting point for further studies on the isolation, purification, and molecular identification of polysaccharide compounds, which could contribute to the production of innovative natural bioactive compounds in the field of medical and anti-fouling materials required on a large scale.

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