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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,

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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 (Manlusoc *et al.* 2019).

The crude SPs from the red algae "*Corallina* sp. and *Pterocladia 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 *Pterocladia capillacea* (S.G. Gmelin) Bornet were freshly collected during summer season 2019 from Sidi Kirayr 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–H_2SO_4 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 % D_2O 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:

$$\text{Scavenging activity \%} = \left[1 - \frac{A_{sample} - A_{control}}{A_{control}}\right] x100$$

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).

Anti-inflammatory potential

Anti-inflammatory potential of different concentrations of both polysaccharides, in comparison to standard drug sodium diclofenac was *in vitro* estimated, using the method suggested by Rahman *et al*. (2015). The absorbance was measured using a UV visible spectrophotometer at 255 nm.

$$\text{Anti inflammatory activity \%} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] x100$$

Anticoagulant activity

Blood was collected by venous puncture from 3 individual healthy donors with no history of bleeding or thrombosis and carefully mixed with 3.2 % sodium citrate at a proportion of 9:1, and then the blood was centrifuged at 1000× g for 10 min at ambient temperature. After centrifugation, the supernatant was removed and stored in siliconized tubes that representing the citrated pool of plasma. Activated partial thromboplastin time (APTT) and prothrombin time (PT) of the plasma pool were mixed with different concentrations of the tested polysaccharides (25, 50, 75 and 100 µg/ml) as described by Hassan *et al*. (2009).

Antimicrobial activities

The antibacterial property of each isolated polysaccharide was determined by the standard disk diffusion technique (5 mm) (Kirby Bauer test) against six pathogenic bacterial species (*Bacillus subtilis* 6633, *Escherichia coli* 19404, *Enterococcus faecalis* 29212, *Klebsiella pneumonia* 13883, *Pseudomonas aeruginosa* 15442 and *Staphylococcus aureus* 25923) which were kindly taken from the Microbiology Laboratory at the National Institute of Oceanography and Fisheries "NIOF", Alexandria, Egypt. After incubation at 37 ºC overnight, the radius of the inhibition zone around each disc was measured in mm. Piperacillin (30 mg/ml) was used as control.

Four fungi species (*Aspergillus niger*, *Fusarium solani*, *Penicillium decumbens, and Rhizoctonia solani)* were obtained from the Microbiology Laboratory at NIOF, Alexandria, Egypt. About 1 mg of each polysaccharide was added to 100 ml of a modified Czapek Yeast Extract Agar (CYEA) medium then poured in sterile Petri dishes (9 cm). By using cork borer, pre–activated pathogenic fungi (5 mm diameter) were inoculated in the center of solidified plates, while the negative control (un–amended (CYEA) plates). The commercial antifungal miconazole was used as a positive control. Then all plates were incubated at 28 °C for a week. Miconazole is used. The inhibition ratio was calculated by using the following equation (APHA 1995):

$$\text{Inhibition growth} = \frac{\text{Diameter of fungal growth on control (mm) - Diameter on treatment plates (mm)}}{\text{Diameter of fungal growth on control plate (mm)}} x100$$

Antifouling activity

About 1 ml seawater was mixed with nutrient broth medium (20 ml) in 50 ml conical flask containing cover glass and incubated overnight at 28 ºC. The tested polysaccharides "200 µl" were added into the flask (as an antifouling agent), then stained with 0.4 % crystal violet solution for 10 minutes. The cover glass was washed by water, dried at room temperature and examined under the microscope (Kumaran *et al*. 2011). Another flask without polysaccharides was used as a control for comparison.

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 %) (Skriptsova & 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. mammillaris* (8.9 %) (Valiente *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 *Pterocladia capillacea* (SP2).

%	Protein	Sulfate content	Carbohydrate	Polysaccharides
SP1	0.004±0.0	15.023±0.97	42.73±2.51	36.57±1.06
SP2	0.001±0.0	31.248±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, ɣ (C–O–C), or ɣ (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 SP 1 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 ^{1}H 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) (Rupeärez *et al*. 2002).

Biological activities

Antioxidant activity

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

Table 2. DPPH scavenging activity/total antioxidant capacity (TAC) and anti-inflammatory activity of the extracted polysaccharides from *Corallina officinalis* (SP1) and *Pterocladia capillacea* (SP2).

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).

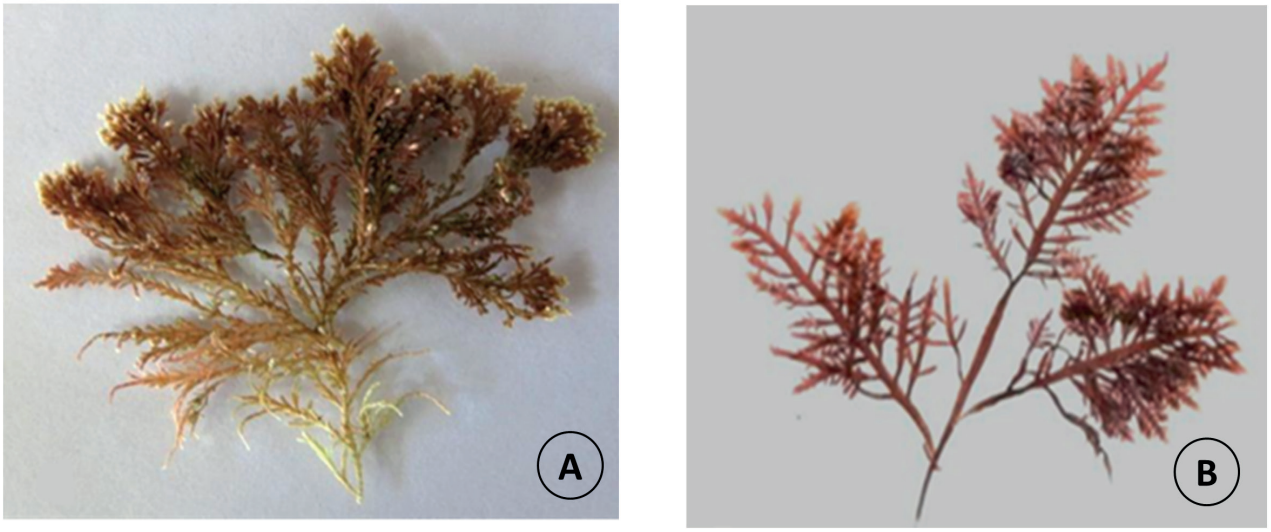

Figure 1. Photo of **(A)** *Corallina officinalis* Linnaeus and **(B)** *Pterocladia capillacea* (S.G.Gmelin) Bornet.

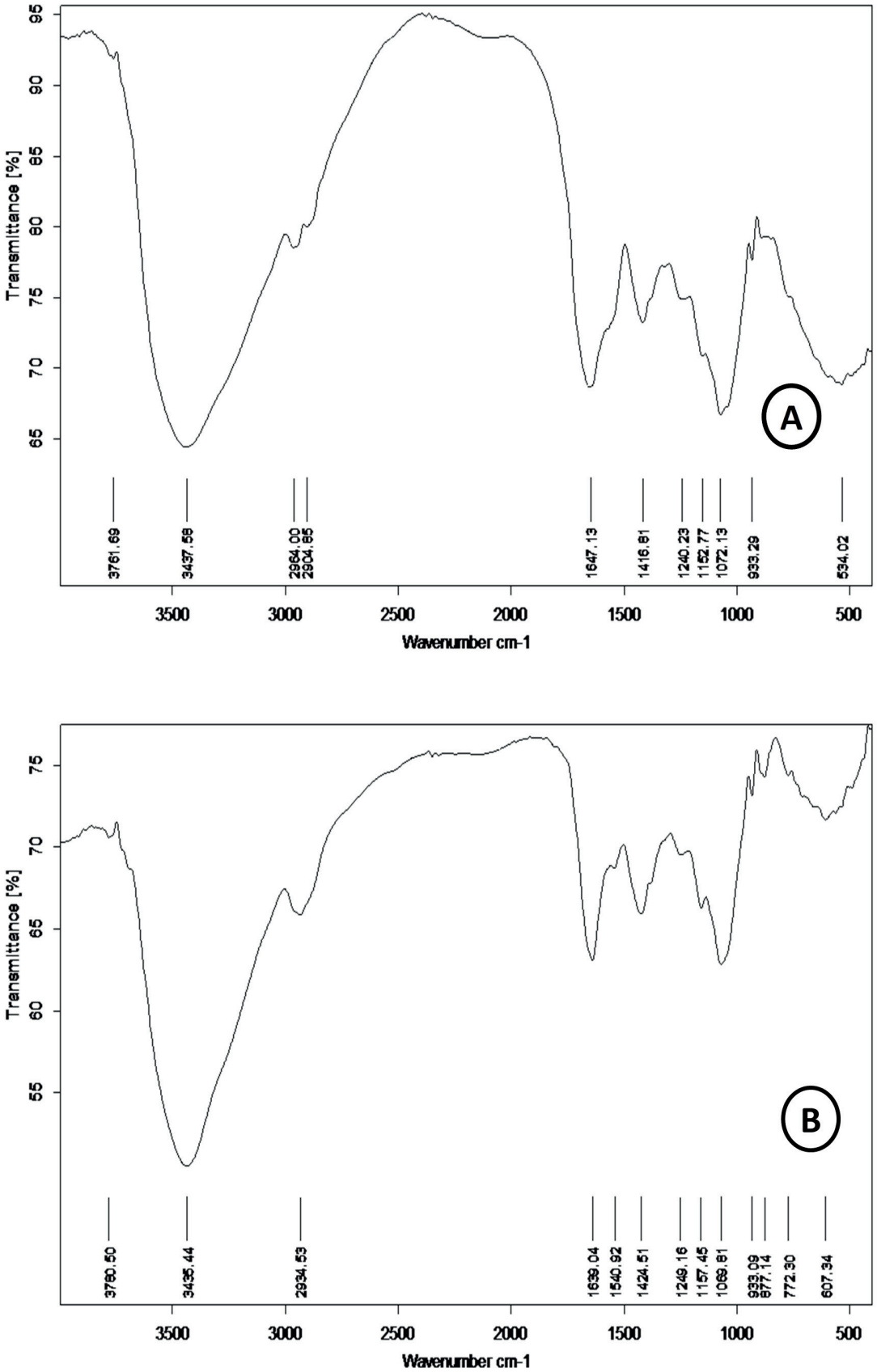

Figure 2. Infrared spectra (KBr, cm^{-1}) of crude polysaccharides isolated from **(A)** *C. officinalis* (SP1) and **(B)** *Pt. capillacea* (SP2).

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 TNFα) (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

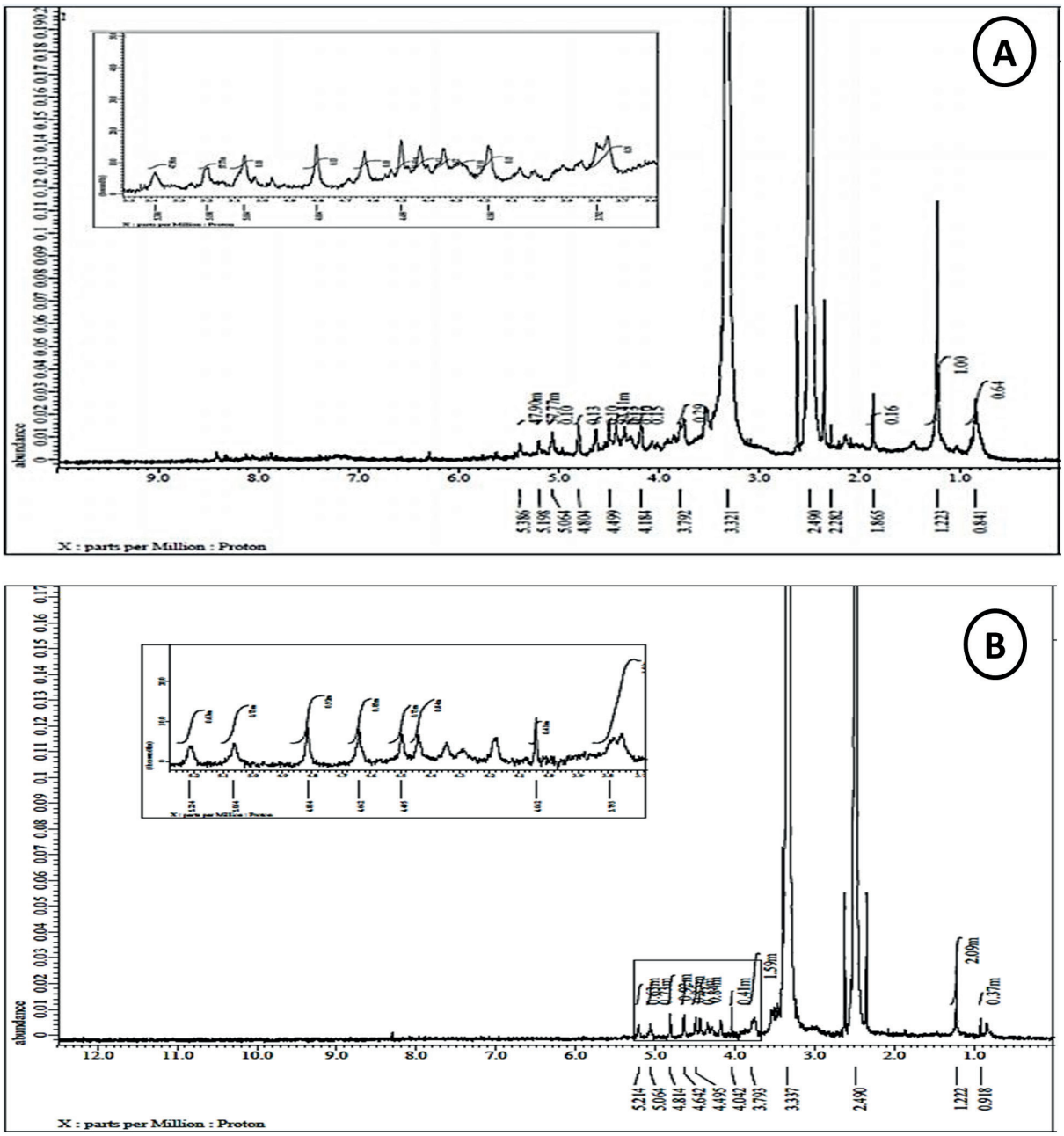

Figure 3. [1]H NMR 1H NMR spectra (300 MHZ, deuterated solvent used) of crude polysaccharides isolated from **(A)** *C. officinalis* (SP1) and **(B)** *Pt. capillacea* (SP2).

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 $-O-SO_3H$ 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)	APTT (s)				PT (s)	
	SP1	%	SP2	%	SP1	SP2
25	37±0	1.23±0	40±0	1.33±0	13±1	14±0
50	39±0	1.30±0	43±0	1.43±0	15±0	16±1
75	42±1	1.40±0.03	49±1	1.63±0.03	19±0	20±1
100	46±1	1.53±0.03	51±1	1.7±0.03	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".

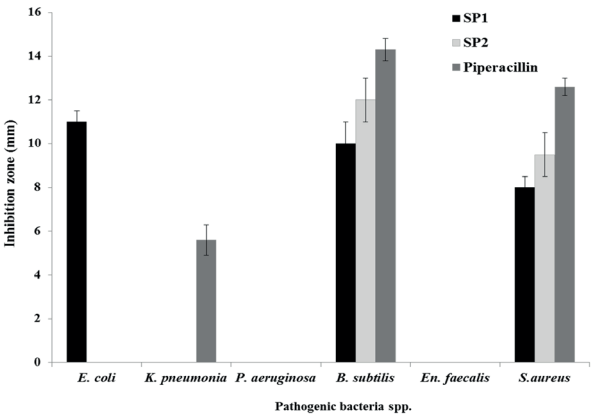

Figure 4. The antibacterial activity of SP1 and SP2 (mm) (The data are given as means ± SD).

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

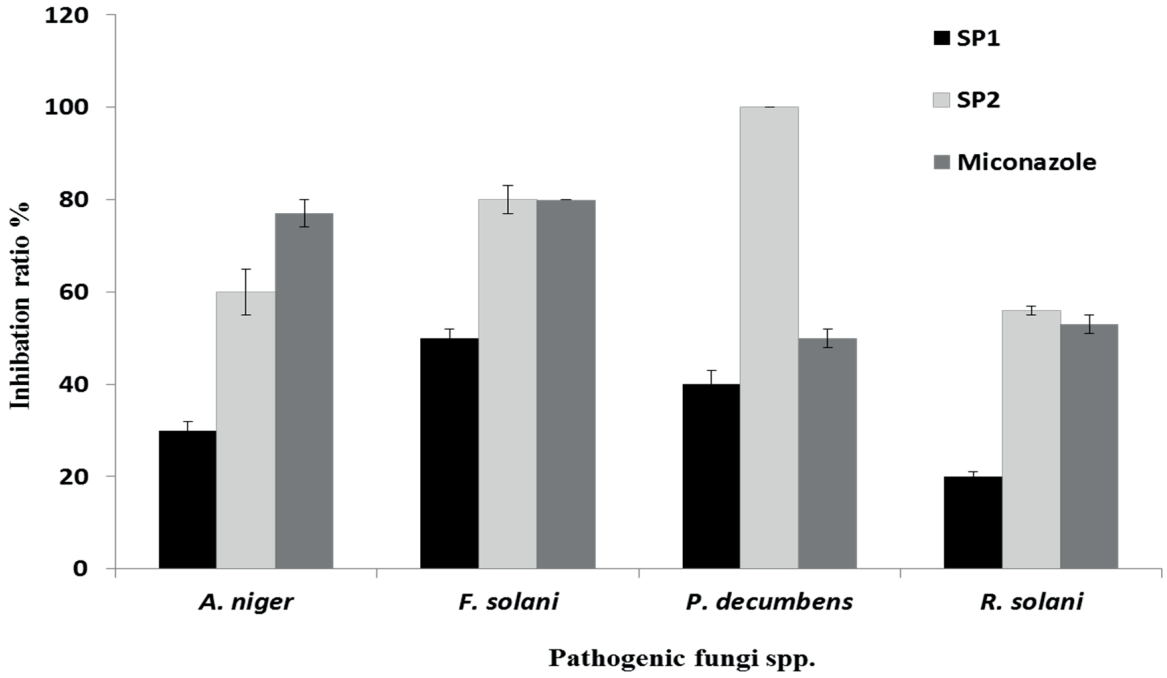

Figure 5. The antifungal inhibition ratio % of SP1 and SP2 (The data are given as means ± SD).

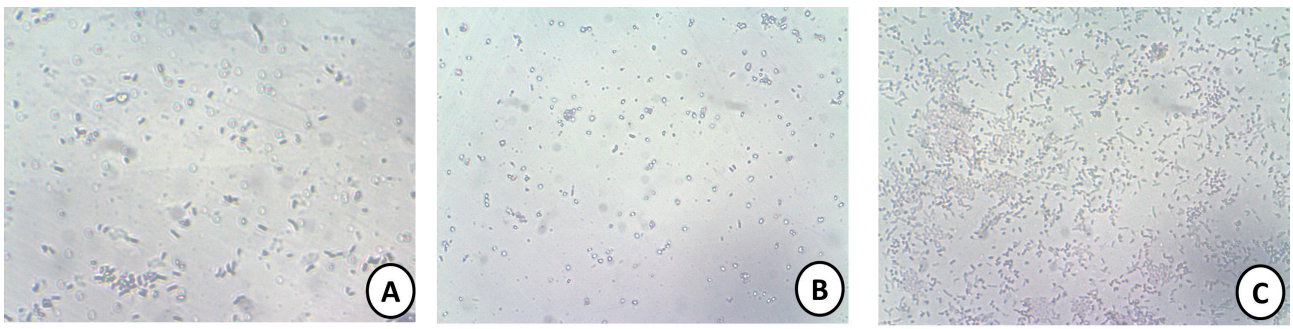

Figure 6. Photographs illustrating the antifouling effect induced by crude polysaccharides isolated from *C. officinalis* (SP1) and *Pt. capillacea* (SP2) on marine bacterial biofilm.

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 ι-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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