Journal of Applied Pharmaceutical Science Vol. 2 (10), pp. 01-010, October, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21001
ISSN 2231-3354

Polysaccharides of the red algae "*Pterocladia*" growing on the Lebanese coast: Isolation, structural features with antioxidant and anticoagulant activities

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ARTICLE INFO

Article history:
Received on: 25/09/2012
Revised on: 10/10/2012
Accepted on: 19/10/2012
Available online: 28/10/2012

Key words:
Antioxidant,
anticoagulant activities,
carrageenans,
NMR and Infra-Red
Spectroscopy, red algae,
sulfated galactans.

ABSTRACT

In addition to their high nutritional value, red algae are of great interest as a source of therapeutic substances. However, despite their abundance on the Lebanese coast red algae have never been studied before. Therefore, we attempted to study among the available biomass one of the species of red algae growing on the Lebanese coast "***Pterocladia***". We performed the extraction of trace elements, fatty acids, sulfated galactans and water-soluble polysaccharides of the phycocolloids family (carrageenans) whose physicochemical gelling or stabilizing effects attract many industrial sectors. The analysis of trace elements shows large amounts of potassium, calcium, silicon and iron. Moreover, Pterocladia appears to be rich in saturated fatty acids (69%) with palmitic acid as major fatty acid and 31% of monounsaturated fatty acids. The extraction yields were 2.7% for sulfated galactans and 11.5% for carrageenan. In order to elucidate their structures, H^1 NMR spectroscopy was performed as well as Infra-Red spectroscopy which allowed us to reveal their functional groups. A study of the antioxidant effect by the electrolysis method showed a greater effect for sulfated galactans. Furthermore, the study of the anticoagulant effect by APTT test, activated partial thromboplastin time, showed more pronounced anticoagulant power of sulfated galactans. The results of the present study confirmed the potential use of the red algae Pterocladia as a source of active known molecules.

INTRODUCTION

Seaweeds have been widely used by coastal populations for thousands of years owing to their high nutritional values (MacArtain *et al.,* 2007). They are found to be very rich sources of food, feed, medicines and energy. Moreover, marine algae are the only sources for industrially important phycocolloids like agar, carrageenan and alginate. They have a lot of applications as stabilizer, viscosifier, gelling and emulsifying agents (Delattre *et al.,* 2011). Apart from industrial uses, in recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products (Shanmugam *et al.,* 2000).

Thus, medical and pharmaceutical industries are also interested since marine plants have proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential (Pérez, 1997; Madhusudan *et al.,* 2011). Indeed, the complex polysaccharides from red algae especially sulfated galactans and carrageenans defined as polymers of galactose possess broad spectrum therapeutic properties. They are reported to exhibit blood anticoagulant (Farias *et al.,* 2000; Melo *et al.,* 2004; Cumashi *et al.,* 2007), immunomodulating, antitumor (Yuan *et al.,* 2006), antiviral (Trinchero *et al.*, 2009) and antioxidant activities (Yuan *et al.*, 2006; Costa *et al.,* 2010). In fact, red seaweed galactan sulfates are linear polysaccharides with alternating 3-linked β-D-galactopyranose units and 4-linked 3,6-anhydro-α -galactopyranose or α-galactopyranose units (Cosson *et*

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al., 1995), having different positions and degrees of sulfation. Othersubstituents, as methyl ethers, pyruvic acid ketals, and single stubs of β-D-xylopyranose and/or other monosaccharides are sometimes present (Estevez *et al.,* 2004). They have been divided in carrageenans, when the 4- linked residues are on the D-configuration (1,3-linked *β*-D-galactose and 1,4-linked *α*-D-galactose), and in agars, when these residues belong to the L-series (Estevez *et al.,* 2004; Delattre *et al.,* 2011). So in this context, the identification of natural and original polysaccharidic structures constitutes a new field of applications.

Red algae of the genus *Pterocladia* are used for industrial production of gelling galactans and are commonly distributed in the seas of Lebanon, Egypt, Brazil, Italy and other countries (Wassef *et al*., 2002; Silva *et al.,* 2010; Bottalico *et al.,* 2008). Previous researches have discussed the composition and the properties of polysaccharides from the red algae *Pterocladia capillacea*.

According to Whitfield et al (1999), the total amount of bromophenol from this species of red algae collected from eastern Australia was about 2590 ng / g. Their nutritional properties, their amino and fatty acid profiles were also identified (Wassef *et al*., 2002). On the other hand, the study of Guven et al (1982) shows that this species has anticoagulant activity and the active compound was identified as a polypeptide. As well, the study of Silva et al (2010) shows that lectin from the marine alga *Pterocladiella capillacea* has peripheral actions with both anti-inflammatory and antinociceptive properties and this by significantly reducing the migration of neutrophils, thereby inhibiting inflammatory mediators.

Nevertheless, despite their abundance on the Lebanese coast red seaweed have never been studied before. Therefore, we attempted to study among the available biomass one of the species of the red algae growing on the Lebanese coast "*Pterocladia*". We view that the potential of polysaccharides extracted from seaweed is antiviral, antioxidant and anticoagulant, which makes them agents of considerable interest; considering the immense biomedical prospects of sulfated polysaccharides, the profound and emerging functional properties published in recent times will be discussed here with experimental evidences. Thus, this paper describes the isolation, structural features, the antioxidant and anticoagulant activities of sulfated galactans and carrageenans present in the red seaweed *Pterocladia* grown on the Lebanese coast.

MATERIALS AND METHODS

Isolation and structural analysis of the polysaccharides from the red algae *Pterocladia*

Extraction of Sulfated Galactans

All The chemicals were purchased from Sigma Aldrich-Lebanon. The marine red algae *Pterocladia* was collected at Rawché beach, Lebanon, separated from other species and sun-dried. The extraction was carried out by the method of Farias *et al*. (2000). The dried tissue (25 g) was cut in small pieces, suspended in 250 ml of 0.1 M sodium acetate buffer (pH 6.0) containing 510 mg of papain (Sigma-Aldrich, Switzerland), 5 mM EDTA and 5 mM cysteine, and incubated at 60˚C for 24h. The incubation mixture was then filtered and the supernatant saved. The residue was washed with 138 ml of distilled water, filtered again, and the two supernatants were combined. Sulfated polysaccharides in solution were precipitated with 16 ml of 10% cetylpyridinium chloride solution. After standing at room temperature for 24h, the mixture was centrifuged at 2560 x g, for 20 min, at 5˚C. The sulfated polysaccharides in the pellet were washed with 610 ml of 0.05% cetylpyridinium chloride solution, dissolved with 172 ml of a 2 M NaCl, ethanol (100:15, v/v) solution, and precipitated with 305 ml of absolute ethanol. After 24 h at 4˚C, the precipitate was collected by centrifugation (2560 x g for 20 min at 5˚C), washed twice with 305 ml of 80% ethanol, and once with the same volume of absolute ethanol. The final precipitate was dried at room temperature overnight and 660 mg (dry weight) of crude polysaccharide were obtained after these procedures.

Extraction and purification of carrageenans

After washing with water to remove all possible impurities such as salt, sand, shells... samples were grinded to optimize the contact between the samples and solvents at various subsequent operations. They were then submitted to depigmentation: algae were treated with acetone overnight stirring, decant and filter to extract the hydrophobic pigments (chlorophylls and carotenoids), and with 80% ethanol by heating to reflux for 1 hour, filter then treat them with absolute ethanol to extract the hydrophilic pigments. View that carrageenan compounds are very soluble in water, this property is used for their extraction. 20g of algae pretreated were heated in 200 ml of water at a slightly alkaline pH (8-9) (0.5M $NaHCO_3$ solution) in a water bath at 90°C for 3 h. This is the pH where carrageenans are assumed to be stable. Then the mix was filtered in order to remove insoluble residues (cellulose), a viscous solution containing carrageenans was obtained and submitted to purification. This latter step is based on the ability of carrageenan to form a precipitate in the presence of excess alcohol or in a KCl solution. Therefore, a double volume of alcohol was added to the solution of carrageenan by stirring with a glass rod allowing the formation of a whitish filament carrageenan insoluble in alcohol. The carrageenan was washed with ethanol and was dried at room temperature for 24 h, then pulverized, reduced to powder in a mortar and finally sieved. This process allowed us to obtain 2.3 g of carrageenan powder.

^{1}H NMR spectroscopy

About 3 mg of each sample (carrageenan or sulfated galactans) were dissolved in 0.5 ml of 99% D_2O. All ^{1}H NMR spectra were recorded at 27˚ C on an "Ultrashield 300 Bruker" spectrometer operating at a frequency of 300 MHz with an acquisition time of 5.29 s , a pulse duration of 11 µs. All chemical shifts were expressed in ppm and reported relative to an internal tetra-methyl silane reference.

Infrared spectroscopy

The infrared spectra were recorded on a "JASCO FT-IR 6300" spectrometer for a range of frequencies between 400 and 4000 cm^{-1}. The resolution was 4 cm^{-1}. All samples were analyzed as KBr pellet.

Determination of fatty acids from *Pterocladia*

The following test was performed in the institute of Monocrystals, Kharkov, Ukraine. Approximately 1.2 g of dry algae *Pterocladia* previously grounded into particles of 0.5 mm was extracted with methanol-chloroform in portions of 10 ml three times for 3 hours. The mix was filtered through the paper filter into a 10 ml flask. 1g of anhydrous sodium sulfate was added to the extract obtained, which was evaporated at 60˚C in the nitrogen stream until dryness (a residue of 40mg). 1 ml of diethyl ester, 5 ml of methanol and 0.2 ml of acetyl chloride were added to the residue and the flask was filled with nitrogen, and then it was boiled with the reflux condenser on the glycerin bath for 45 min at 70˚C. The solution obtained was evaporated in the nitrogen stream to a volume of 0.3 ml. Then 2 ml of cyclohexane were added and stirred for 1 min. After the complete stratification of the layers, the upper cyclohexane layer was used as a test sample. It was filtered through a filter with 0.2 g of sodium sulfate. The resulting solution was subjected to analysis by gas chromatography Shimadzu GC-14B, FID chromatography under the following conditions: capillary column (60m x 0.32mm HP-23; 0.25µm), the column temperature was held at 175˚C for 2 min, and then raised to 225˚C with a rate of 3˚C / min, injector and detector temperatures were 240˚C and 250˚C respectively, the carrier gas flow rate (nitrogen) was 1.0 ml/min, split ratio was 1:60. The content of each fatty acid was calculated by the internal regulation method (Kanaan *et al*., 2005).

Determination of trace elements from *Pterocladia*

Following tests were conducted in the Institute of Monocrystals, Kharkov, Ukrain. 2 g of *Pterocladia* powder were placed in a capillary tube of fluorized polymers in order to disperse them under pressure and microwaves. After adding 5 ml of HNO_3 (70%) the capillary was firmly closed and placed in a steam room for 20 min, under a pressure not exceeding 120 psi. After cooling and filtration, the substance was recovered in a 50 ml tube filled with water, thereby obtaining liquid number 1. 1ml was taken from liquid 1 and put it in a 100 ml tube filled with water up to 100 ml obtaining therefore liquid 2 (Kanaan *et al*., 2005).

In order to determine macro-elements percentages, a Thermo Jarrel Ash atomic absorption spectrometer was used.

Liquid 1 was used to determine the percentage of Fe, Zn, Cu, Ni, Mn, Al and Se.

Liquid 2 was used to determine the percentage of Ca, Mg, K and Na.

Conditions: Liquid flow speed: 1.85 ml / 1mn 2 sec

The flow speed of added Argon (Ar): 1 l / min

The flow speed of initial Argon (Ar): 14 l / min

Evaluation of biological activities

Antioxidant activity

20 ml of tyrode solution (NaCl 137.0 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.5 mM, NaH_2PO_4 0.4 mM, $NaHCO_3$ 12.0 mM) was introduced in a basin provided with two platinum electrodes maintained at a distance of 2 cm from each other, and connected by electric wires to an electrical stimulator delivering a current of 10 mA controlled by a digital multimeter. A cascade of free radicals will be as a result generated by electrolysis in the tyrode solution (Chahine *et al*., 1998).

A constant current of 10 mA generated by the stimulator was applied during 5 min in 20 ml of physiological solution (tyrode) to generate free radical and their derivatives. We carried out the electrolysis of the tyrode solution in the absence of polysaccharides isolated (sulfated galactans or carrageenan) used as control and in the presence of different concentrations of these extracts (0.15, 0.3, 0.6 g/l). At each minute of electrolysis a volume of 1 ml of electrolyzed tyrode was added to 2 ml N,N di-ethyl-p-phenylenedialanine (DPD) (25 mg/ml). Then the absorbance was determined by spectrophotometer at 515 nm.

Anticoagulant activity

Activated partial thromboplastin time (APTT) assays were carried out by the method of Anderson *et al.* (1976). Normal human platelet-poor plasma (100 µl) was incubated with 10 µl of a solution of polysaccharide (0.05, 0.5, 2.5, 5.0 µg) at 37˚C for 1 min. Then 100 µl of APTT reagent (Human Gesellschaft, Germany) were added and incubated at 37˚C. After 2 min of incubation 100 µl of 0.25 M CaCl2 were added to the mixtures and the clotting time was recorded on a coagulometer (Thrombotimer "Behnk elektronik").

RESULTS AND DISCUSSION

Polysaccharides content of Pterocladia

In enzymatic digestion using papain, the total yield of sulfated galactans extracted from 25g of red algae *Pterocladia* collected at the Lebanese coast was 2.7%. According to the literature and using the same extraction method, the content of sulfated galactans isolated from *Botryocladia occidentalis* collected at Brazilian coast was about 4% (Farias *et al*., 2000) and Pereira et al (2005) obtained a yield of 2.6% from the red alga *Gelidium crinale*. The differences in the sulfated galactans compositions of red algae are probably due to the fact that the specimens were collected at different sites, at different times and were different species (Marsham *et al.*, 2007) and that the latter species seems to be richer in sulfated galactans.

Moreover, the content of carrageenan isolated from *Pterocladia* treated in distilled water at 90˚C was about 11.5%. According to Mouradi et al (2006), the content of carrageenan isolated from *Hypnea musciformis* treated at 80˚C was 14.55% and 7.33%. at 100˚C.

Thus, it appears that the yield of carrageenan at 90°C was satisfactory bearing in mind that the yield and quality of

carrageenan depend on environmental factors and species (Mtolera *et al*., 2004; Marsham *et al*., 2007).

Determination of fatty acids from Pterocladia

The fatty acids detected in the algal sample were analyzed from the spectrum, their concentrations were determined and shown in Table 1. We noticed that the red algae *Pterocladia* was composed of 69% of saturated fatty acids, 31% of monounsaturated fatty acids and did not contain polyunsaturated fatty acids (Summarized in Table 1) The major fatty acid was palmitic acid (53.749%) followed by the unsaturated vaccenic acid (14.485%). The study of MacArtain et al (2007) showed that *Porphyra* and *Palmaria spp* contain respectively 64.95 and 60.48% saturated fatty acids, 18.91 and 10.67% monounsaturated fatty acids and 16.1 and 28.86% polyunsaturated fatty acids.

On the one hand, according to Norziah et al (2000) *Changgi Gracilaria* appeared to be rich in unsaturated fatty acids (74%) especially omega 3 fatty acids (eicosapentaenoic acid 20:5 ω3 (33.1%), docosahexaenoic acid 22:6 ω3 (12.9%) that didn't appear in *Pterocladia*.

Its content in oleic acid 21.9% was higher than that present in our case 9.835%. Like *Pterocladia* but with a lower rate, palmitic acid was the principal fatty acid (22%). On the other hand, *Pterocladia capillacea* collected at the Egyptian coast contained high levels of polyunsaturated fatty acids (11%): arachidonic acid omega-6 (10%) and low omega-3 eicosapentaenoic acid (1%). The most abundant fatty acids were oleic acid (55%) and palmitic acid (31%) (Wassef *et al*., 2002). This indicates that the composition of algae differs by place of collection and geographic area (Marsham *et al*., 2007).

Determination of trace elements from Pterocladia

The elements were detected in the algal sample and their concentrations were determined and summarized in table 2.

Pterocladia appeared rich in K, Ca, Si, Mg, Na, P, Fe and Zn. Each element is essential for the proper functioning of the body.

Therefore, it seems possible to use the algae to reduce high blood pressure due to its high concentration in K (2220mg/100g). Its high content of Fe (110mg/100g) allows it to be used as food for people with syndrome of iron deficiency taking into account that the daily intake of Fe is 10mg per day for humans. The Ca and Si play a role in bone formation and connective tissue. Zn is an essential cofactor for certain enzymes. Detection of phosphorus can indicate the level of water pollution due to the long-term use of phosphate fertilizers. The seaweed is one of the best bio-accumulators of various elements; it could be used to study the environment where it grows (Bino Devi *et al*., 2011). The relatively low content of Pb, Hg, Cd and As, even negligible, indicates that this alga is cultivated in an unpolluted environment.

As the red algae have not been studied previously in Lebanon, our results will be compared with various species of red algae collected from different countries. Thus, *Pterocladia* collected at the Lebanese coast appeared to be richer in calcium (740 mg/100g), Iron (110 mg/100g), Zinc (74 mg/100g) and copper (3.7 mg/100g) than *Changgi Gracilaria* collected from Malaysia beach containing 651 mg/100g of Ca, 95.6 mg/100 g of Fe, 13.8 mg/100g of Zn and 0.8 mg/100 g of Cu (Norziah *et al*., 2000). According to MacArtain *et al.,* 2007, the mineral composition of the three species of red algae collected from Asia: *Porphyra umbilicalis*, *Palmaria palmata* and *Chondrus crispus* comparing to *Pterocladia* is presented in Figure 1 below. The results showed that *Pterocladia* is richer in Ca, K, Cu, Fe and Zn that these three species of red algae. While *Chondrus crispus* is the richest in Mg and Na. Hence *Pterocladia* appears to be useful in the food industry as a source of highly nutritious ingredients.

Table. 1: Types of fatty acids contained in the red algae *Pterocladia* and their percentage concentration

Peak #	Compound	Common name	Retention Time	Area	Height	Concentration (%)
1	C14:0	Myristic acid	7.594	19186	3655	6.982
2	**C16:0**	**Palmitic acid**	12.436	147703	29990	**53.749**
3	C16:1 n9	Palmitoleic acid	12.953	18336	4098	6.672
4	C18:0	Stearic acid	16.641	22744	4810	8.276
5	C18:1 n9	oleic acid	16.984	27027	5390	9.835
6	C18:1 n11	vaccenic acid	17.123	39806	8903	14.485
total				274802	56846	

Table. 2: Trace elements content of red algae *Pterocladia* and their concentrations (mg of trace element/100 g of algae)

Number	Element	Concentration mg of trace element /100g of algae (mg/100g)
1	Fe	110
2	**Si**	**590**
3	P	125
4	Al	59
5	Mn	11
6	Mg	225
7	Pb	0.07
8	Ni	1.5
9	Mo	<0.02
10	**Ca**	**740**
11	Cu	3.7
12	Zn	74
13	Na	150
14	**K**	**2220**
15	Sr	2.2

Co <0.03, Cd <0.01, As <0.01, Hg < 0.01

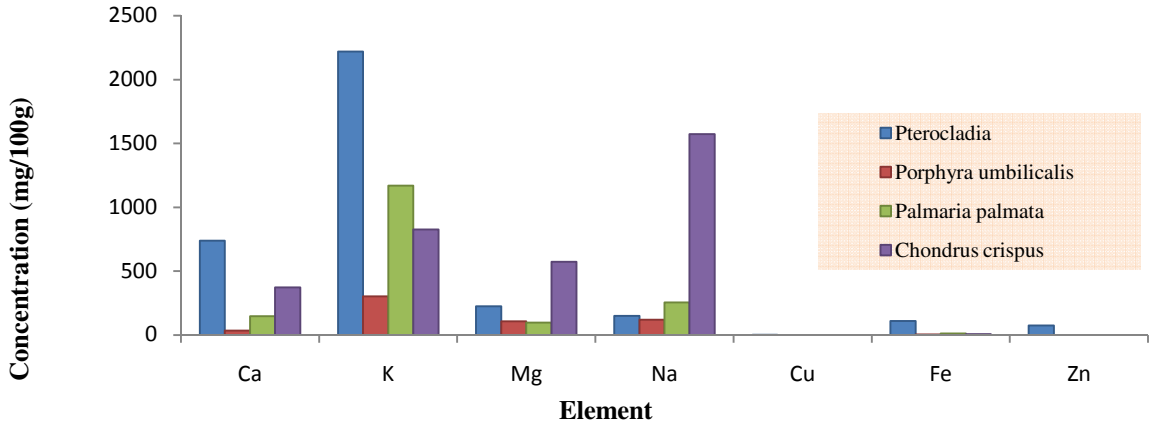

Fig. 1: Comparison of the mineral composition of ***Pterocladia*** with that of three species of red algae collected from the Asian coast (Porphyra umbilicalis, Palmaria palmate, Chondrus crispus). Concentration was expressed as mg of element / 100 g of alga.

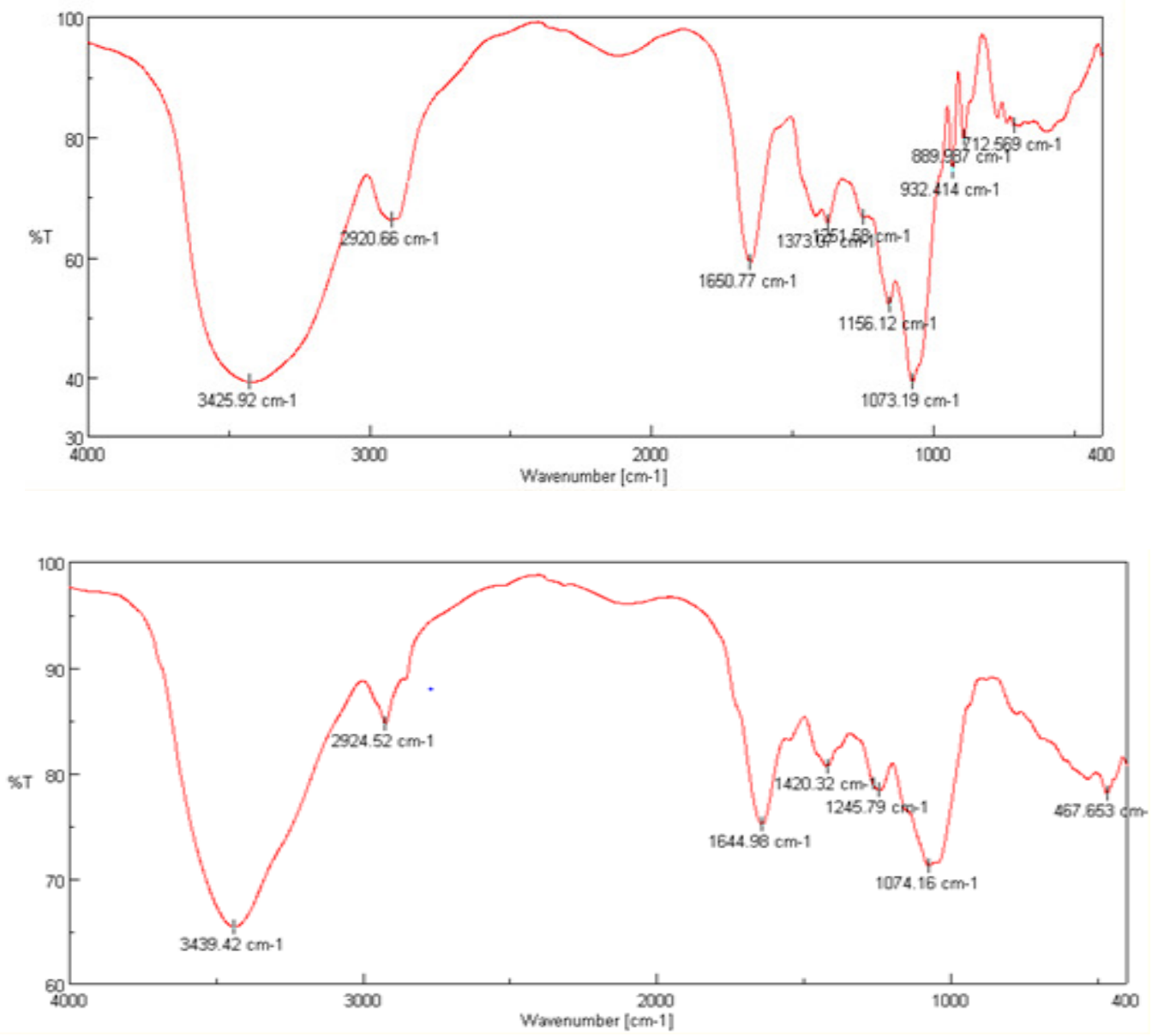

Fig. 2: Infrared spectrum of carrageenan(A) and sulfated galactans *(B)* isolated from *Pterocladia*

Infrared analysis

The carrageenan spectrum below (Fig. 2 A) shows a strong absorption band at 3425.92 cm^{-1} due to the stretching vibration of υ (O-H), a band at 2920.66 cm^{-1} due to the vibration υ (C-H) and a band at 1650.77 cm^{-1}assigned to an asymmetric stretching vibration of O-C-O (Chopin *et al*., 1999). The absorption bands at 1373.07 and 1251.58 cm^{-1} correspond to the asymmetric stretching vibration of the ester sulphate (Chopin *et al*., 1999). The weak signal at 125.58 cm^{-1} is due to the elimination of the sulfate group leading to the formation of 3,6-anhydrogalactose residue. The band located at 1156.12 cm^{-1} may be due to the stretching vibration of sulfate esters, υ (C-O-C) or υ (C-C). And one located at 1073.19 cm^{-1} is due to the symmetric stretching vibration of sulfate group SO^{-} (Chopin *et al*., 1999). The absorption band at 932.414 cm^{-1} is characteristic of the 3,6-anhydrogalactose residue, that at 712.569 cm^{-1} appears to be characteristic of C4 galactose sulfate (COSO4) and the absorption band located at 889.987 cm^{-1} corresponds to the vibration δ (C-H) glucoside. But the absence of the absorption band at 805 cm^{-1} characteristic of the 3,6-anhydrogalactose-2-sulfate residue indicates that this type of carrageenan does not match iota-carrageenan. In addition, this spectrum does not show the two absorption bands characteristic of the λ carrageenan: a strong band at 830 cm^{-1} corresponding to the galactose-2-sulfate and a band at 820 cm^{-1} corresponding to the galactose 6-sulfate (Rochas *et al*., 1986). Thus the various peaks attributed above indicate that the studied carrageenan is probably of Kappa type.

The IR spectrum of sulfated galactans (Fig. 2 B) shows the same bands as those observed with carrageenan. But the characteristic absorption bands of the vibrations of the sulfate groups (at 1420.32, 1245.79 and 1074.16 cm^{-1}) are of greater intensity than those observed with carrageenan. In addition, the absence of the band at 930 cm-1 characteristic of the 3,6-anhydrogalactose residue show that this polysaccharide has been esterified with numerous sulfate functions.

^{1}H N M R analysis reveals β unit

The ^{1}H NMR analysis has become the tool most commonly used for characterization of carrageenans. The ^{1}H one dimensional spectra of carrageenan and sulfated galactans from *Pterocladia* are shown in Fig 3 A and B. These spectra reveal a triplet centered approximately at 1.05 ppm corresponding to a proton methylene CH2 and a quadruplet at 3.55 ppm. This signal may be due either to the presence of O-CH3 bond or is assigned to the β unit (Farias *et al.,* 2000). But these spectra do not show signals between 4.4 and 5.4 ppm relative to the region of anomeric sugars (Praiboon *et al.,* 2006) nor the signal at 5.2 ppm corresponding to the unit α (Farias *et al.,* 2000) . This seems to be related to the temperature applied during the analysis and which should be set at around 65°C and even higher. In fact, temperature affects positively the viscosity reducing the height of the peaks and shifting the solvent resonance (Claudio *et al.*, 2008). Hence, a higher resolution requires high temperature.

Antioxidant action of sulfated galactans and carrageenans from the red algae Pterocladia

We further investigate the antioxidant effects of the polysaccharides derived from the red algae *Pterocladia* by the electrolysis method. The electrolysis of the Tyrode solution generates between the cathode and the anode oxidizing species (free chlorine, hypochlorous acid, ROS). The latter react instantly with DPD reagent to reveal a red color whose intensity varies with the amount of ROS released as a function of time.

The graphs below (Fig 4) illustrate the antioxidant effect of carrageenan and sulfated galactans isolated from the red algae *Pterocladia* against oxygenated free radicals generated by electrolysis of a physiological medium. The present study showed a greater effect for sulfated galactans. Moreover, in the absence of these active compounds (control curve), the concentration of ROS increased gradually with time until reaching a maximum absorbance of 0.45 corresponding to a bright red color in the 5th minute of electrolysis (Fig 4). While for the same concentration of the initial solution used (3 g / l), we notice that as far as the concentration of the extracts (sulfated galactans or carrageenan) increases ranging from 0.15 g / l to 0.6 g / l, the formation of ROS during the electrolysis is gradually attenuated comparing to the control curve (Fig 4), thus illustrating the power of these extracts to scavenge free radicals therefore their antioxidant effects. As well, we observed a decrease in the intensity of the color of the solution (the red color appeared less deep than in the absence of the extract) and a subsequent decrease in the absorbance over time compared to the control curves.

Furthermore, this study showed more pronounced antioxidant power of sulfated galactans which makes it depend on the composition and the degree of sulfation of the polysaccharide. Indeed, three fractions of sulfated polysaccharides isolated from *Porphyra haitanesis* which differ in the content of sulphates show that the fraction richest in sulphate has the strongest antioxidant effect (Zhang *et al*., 2003). Another study by Souza et al (2007) also showed a positive correlation between sulfate content and antioxidant activity which confirms our results. As well, the study of Yuan et al (2006) indicates that the chemical modification (oversulfated) of carrageenan oligosaccharides can enhance their antioxidant activity in vitro. We concluded that the richer the compound is in sulfate the higher is its antioxidant activity.

In order to confirm the greater effect of sulfated galactans, we calculated the percentage of inhibition of oxygenated free radicals by carrageenan and sulfated galactans (Fig. 5). At the 5th minute of electrolysis and at the highest concentration 0.6 g / l, the maximum absorbance for sulfated galactans and carrageenan was respectively 0.13 and 0.24 (Fig 4). For the control curve and in the absence of these two compounds the absorbance was 0.45 (Fig 4) which indicates 100% of inhibition. Thus, we obtain as the ability of these active ingredients to inhibit ROS 71% and 47 % for sulfated galactans and carrageenans respectively (Fig. 5) indicating that the galactans rich in sulfates exhibit more pronounced antioxidant activity than carrageenan.

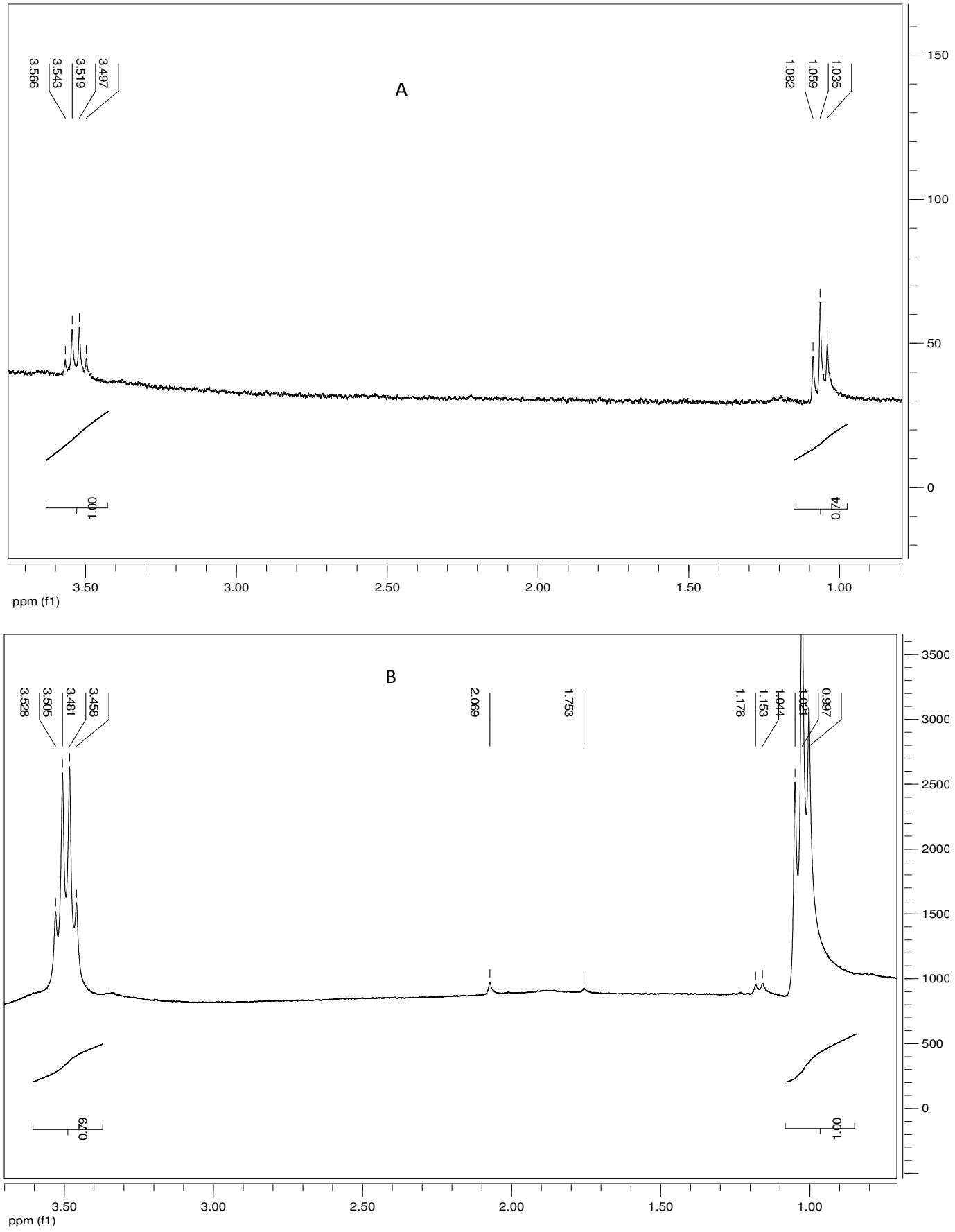

Fig 3: [1] H NMR spectrum of carrageenan (A) and sulfated galactans (B) isolated from *Pterocladia*

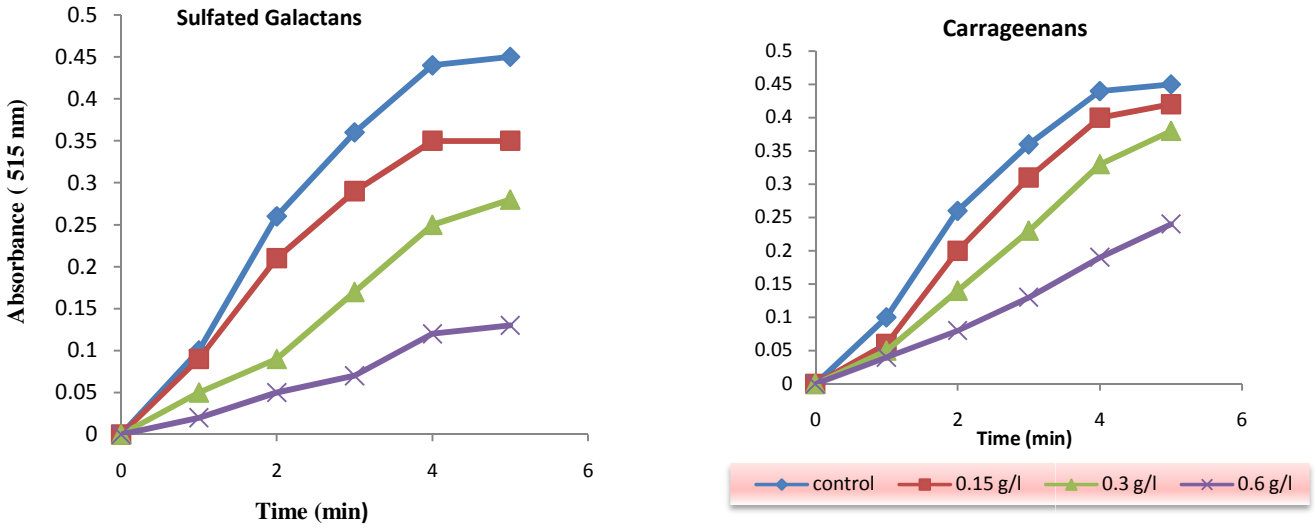

Fig 4: Antioxidant effect of sulfated Galactans and carrageenans isolated from *Pterocladia.* Different concentrations of these extracts (0.15, 0.3 and 0.6 g/l) were added to the physiological solution after 5 min of electrolysis. (control = physiological solution alone).

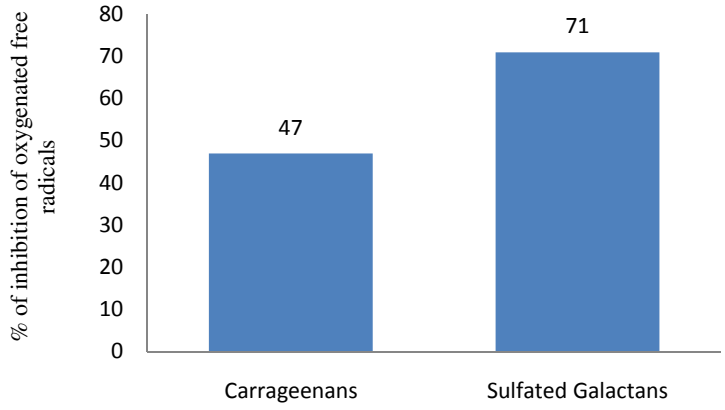

Fig 5: Inhibition of the oxygenated free radicals by carrageenans and sulfated galactans

Table. 3: Anticoagulant properties of sulfated polysaccharides derived from red algae *Pterocladia.*

Polysaccharides	Quantity (µg)	APTT (s)	Ratio
Carrageenan	0.05	38.0	1.27
	0.5	39.5	1.32
	2.5	39.9	**1.33**
	5	42.1	**1.40**
Sulfated Galactans	0.05	37.6	1.25
	0.5	38.7	1.29
	2.5	43.7	**1.46**
	5	61.0	**2.03**

APTT was measured in second by a coagulometer. Ratio was calculated by the formula : Ratio = APTT measured / APTT control = APTT measured / 30 .

Anticoagulant effect of Sulfated galactans and carrageenans from Pterocladia

According to the literature, anticoagulant and antithrombotic activities are among the most widely studied properties of sulfated polysaccharides. Sulfated polysaccharides either extracted from marine brown (Cumashi *et al*., 2007) and red algae (Farias *et al*., 2000), or obtained by chemical sulfation of natural polysaccharides (Alban *et al.*, 1995) have been described as anticoagulants. Therefore, it seems interesting to evaluate the anticoagulant activity of the polysaccharides isolated from *Pterocladia*. The APTT assay (summarized in Table 3) indicates that crude *Pterocladia* polysaccharides have anticoagulant action.

After having measured the clotting time, the ratio was then calculated by the formula: ratio = APTT/ Control = APTT/ 30; knowing that the normal clotting time APTT is between 28-38 s, more precisely 30s was taken as control. A significant anticoagulant effect was observed when the ratio was above 1.2. Thus, the results indicate that both carrageenans and sulfated galactans exhibit anticoagulant action at the lowest dose 0.05 µg (Summarized in Table 3). Moreover, the data in Table 3 indicate that an increase in the quantity of polysaccharides from 0.05 µg to 5 µg induces an increase in the APTT. Thereby the ratio increases and the anticoagulant effect appears to be more pronounced.

We also noticed that sulfated galactans exhibit more powerful anticoagulant activity than carrageenans. For example, for the same dose of 2.5 µg the ratio is 1.46 and 1.33 for galactans and carrageenan respectively. This ratio rises dramatically in the case of sulfated galactans to reach 2.03 for a dose of 5 µg while that of carrageenan increases to 1.40 illustrating an anticoagulant power of galactans more pronounced (Table 3). This is justified by the fact that the sulfate content is important for the anticoagulant action (Farias *et al*., 2000; Shanmugam *et al*., 2000) and the interaction with antithrombin depends on the distribution of sulfate groups (position), the monosaccharide composition and the molecular weight of the compound (Melo *et al.*, 2004) thereby the bulky structure of sulfated galactans is involved in its anticoagulant activity. Confirming this, K, λ, i-carrageenan of high molecular weight and having a high content of sulfates exhibit an anticoagulant activity higher than that of low sulfate content and low molecular weight (Shanmugam *et al.,* 2000).

On the other hand, the sulfated galactans extracted from *Botryocladia occidentalis* exhibit an APTT (130 IU / mg) compared with standard polysaccharides: heparin (193 IU / mg) and lambda carrageenan (77 IU / mg) (Farias *et al.,* 2000). These results also showed greater effect of sulfated galactans in comparison with carrageenan. These same results are shown in the study of Melo et al (2004). A study of Silva et al (2010) shows that APTT for Kappa and iota carrageenan (100 µg) was respectively 240 and 132 s and lambda carrageenan (20 µg) was the most potentially anticoagulant 240s. Our study may lead to such results if we continue to increase the dose and this can give us an idea about the type of carrageenan obtained.

CONCLUSION

Red algae are a major resource for economic and industrial development. In addition to their high nutritional value, they take great interest as a source of therapeutic substances. In summary, our results indicate that the red alga *Pterocladia* growing on the Lebanese coast appears to be useful in the food industry as a source of highly nutritious ingredients. On the other hand, this species contains polysaccharide-type compounds such as sulfated galactans and carrageenans that exhibit a broad spectrum of biological activity. Indeed, these algal polysaccharides have potent anticoagulant and antioxidant activities. Moreover, the study of the antioxidant effect by the electrolysis method showed a greater effect for sulfated galactans. Furthermore, the study of the anticoagulant effect by APTT test, activated partial thromboplastin clotting assays, showed more pronounced anticoagulant power of sulfated galactans. A plausible reason for the greater antioxidant and anticoagulant effects of the sulfated galactans is its high sulfate groups compared with carrageenan.

Other surveys may be conducted in the future on this type of algae covering the extraction of the active ingredients, the possibility of formulating drugs from these products and the study of other therapeutic activities particularly antiviral or anti-tumor activity. New compounds with obvious practical applications may be found. Thus we can also test the carrageenan as lubricant to facilitate movements of the joints due to its ability to form a gel. Finally the sulfated galactans and carrageenans isolated from *Pterocladia* are natural candidate molecules for testing in experimental thrombosis.

ACKNOWLEDGEMENTS

We are grateful to the head of the National Council for Scientific research in Lebanon and to the Rector of the Lebanese University, Dean of the Doctoral School of Science and Technology, for their financial support to the research program.

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How to cite this article:

C. Sebaaly, N. Karaki, N. Chahine A. Evidente A. yassine, J. Habib and H. Kanaan. Polysaccharides of the red algae "*Pterocladia*" growing on the Lebanese coast: Isolation, structural features with antioxidant and anticoagulant activities . J App Pharm Sci. 2012; 2 (10): 001-010.