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The antioxidant and anticoagulant activities of polysaccharides isolated from the brown algae *Dictyopteris polypodioides* growing on the Lebanese coast

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ABSTRACT

In this study, we attempt to isolate the polysaccharides from the brown seaweed *Dictyopteris polypodioides* (Dp) growing on the Lebanese coast. The percentages of the main polysaccharide alginic acid were 4.6% in May and 6.25% in July. The extracted yields of Fucoidan, Laminaran and Mannuronan (FLM) and Mannuronan (M) alone were respectively 0.75% and 0.38% in May and 1.15% and 0.67% in July. We performed the infrared spectroscopy in order to reveal the functional groups of alginate and mannuronan. Moreover, a non-destructive technique ^{1}H NMR was used to determine the structure of alginate and the distribution of guluronic (G) and mannuronic acid (M) in the chain, allowing us therefore to calculate the ratio M/G. The latter was 0.96 showing a strong ability of alginate to capture heavy metals. The analysis of trace elements showed great amounts of K, Si, Na and Mg in both seasons. Moreover, *Dictyopteris* appears to be rich in fatty acids (10 items) with palmitic and oleic acids as main fatty acids. Furthermore, the anticoagulant activity of polysaccharides was investigated by activated partial thromboplastin time (APTT) clotting assay and the antioxidant activity was studied by electrolysis method. It has been shown that the FLM and M fractions of *Dictyopteris polypodioides* have a significant antioxidant and anticoagulant activities. Further investigations are imperative in order to develop new molecules based on these polysaccharides to be used as drugs or for healthcare in general.

INTRODUCTION

Marine algae are a natural resource representing many interests for the medical, therapeutic and nutritional fields. However, the potential value of polysaccharides has been poorly studied in Lebanon. In industrialized countries, a large number of research teams are investigating ways of isolating polysaccharides, known to be very important in the medicinal and pharmaceutical fields such as antioxidants (Wang *et al*., 2009), anticoagulants (Ushakova *et al*., 2008), anti-inflammatory drugs (Cardozo *et al*., 2010), anti-diabetes induced by alloxan (Huang *et al*., 2005), as well as biomolecules that block anxiety in tumor cells (Dias *et al*., 2005), inhibit the attachment of virus infected cells (Zhu *et al*., 2004), alter the physiology of the kidney (Sousa *et al*., 2007) and reduce the expression of genes at the DNA level (Liu *et al*., 2008). For the first time in Lebanon we have studied two species of brown algae *Sargassum* and *Padina Pavonica* which grow in abundance on the Lebanese coast. The results of our study showed that *Sargassu*m is rich in polysaccharides whose main component is alginic acid, as well as water-soluble polysaccharides, such as fucoidan, laminaran and mannuroran. These polysaccharides, particularly fucoidans have a significant antitumor effect demonstrated by in vitro cultures of cancer cells RPMI-7951 human melanoma type (Sokolova *et al*., 2011). Moreover, the variation in the composition of polysaccharides from the brown algae *Padina pavonica* was studied as a function of harvesting seasons (April to July). It has been shown that the main polysaccharide (8.0 to 13.3% dry weight of algae) was the

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alginic acid; its content has not undergone significant changes from April to July. The content of water-soluble polysaccharides (laminarans and fucoidans) was low (<0.3% of dry seaweed). The content of fucoidans in the samples of algae increased from May to July. Therefore, it has been shown that *Padina pavonica* was a promising source of alginic acids with a high gelling capacity, and a source of fucoidans which exhibit antitumor activity against RPMI-7951 human melanoma cells (Men'Shova *et al*., 2012). We have also demonstrated that this brown algae is rich in fatty acids, 11 types including palmitic and oleic acids predominated (39.5% and 24% respectively), and quantitatively rich in carotenoids and chlorophyll (420.4 mg / g and 435.7 mg / g of dry seaweed respectively). Continued work on *Padina* has recently demonstrated its richness in a complex polysaccharide and lipophilic extract; the presence of 19 macro-and micro-elements with high proportions of Ca, Mg, Si, Na and K (Kanaan *et al*., 2008) and 15 amino acids of which 9 are essential for nutrition (Kanaan *et al*., 2009). The goal of our current work is to perform the extraction and isolation of polysaccharides from the brown algae *Dictyopteris polypodiodes* growing on the Lebanese coast and explore the potential biological activities of its extracts.

MATERIALS AND METHODS

The brown seaweed *Dictyopteris polypodioides* freshly collected from the coast of the town of Batroun in May and July 2011 (Table 1), were cleaned and washed with water, set to dry at room temperature and then extracted according to the method of Imbs *et al*., 2009.

Table 1: The nature of water and other local factors present while taking samples on the Lebanese coast of Batroun

coordinates	batroun
Longitude	35°39.413'
Latitude	34°15.090'
at ° C	23
Oxygen (ml/l)	7.20
water t°	20
PO_4 (µg at/l)	1.60
NO_3 (µg at/l)	0.40
NH_4 (µg/l)	2.55
Salinity %	40

The following chemicals were purchased from Sigma Aldrich-Lebanon, and used in the extraction-purification processes: EtOH 90%; HCl; Na_2CO_3; NaOH; $NaHCO_3$. Activated partial thromboplastin and human control plasma were measured using the test of Human Gesellschaft, Germany.

Extraction of alginates from *Dictyopteris polypodioide*s

20 mg of dry algae were extracted twice by ethanol (96%) for 3 hours at 40°C (algae: ethanol 1:0.8 w/w) to remove low molecular weight compounds. The supernatant was separated by centrifugation (3000 rpm, 20 minutes) then the algae was dried and extracted for 3 hours, 2 times by 150 ml HCl (pH 2.0-2.3) at 60ºC to separate the supernatant 1 (used for the extraction of FLM) from the pellet 1 (used for the extraction of alginate). The pellet 1 was extracted successively with aqueous solutions (100ml) of Na_2CO_3 3% and 1.5% for 8 hours with 60ºC and rinsed by water. The extracts were dialyzed for 24 hours using a membrane with a retention limit of 100 KDa; then precipitated with absolute ethanol. The precipitate was dissolved in water and the pH was adjusted to 2 by HCl (12%). The obtained precipitate of alginic acid was then dissolved in a minor amount of water with addition of NaOH to pH= 8.6. The final solution was lyophilized to obtain the alginate powder.

Extraction of Mannuronan from *Dictyopeteris polypodioides*

The supernatant 1 was neutralized by an aqueous solution of $NaHCO_3$ (3%) to pH (5.7-6.1), dialyzed for 24 hours, then lyophilized to obtain the powder of complex FLM (fucoidan (F), laminaran (L), mannuroran (M)). 0.2 g of FLM powder was then dissolved in 50 ml of HCl (0.02M) and left in the refrigerator for 6 hours, then the mixture underwent a centrifugation (3000 rpm, 20 minutes). The complex was then dissolved in HCl and centrifugated (3000 rpm, 20 minutes) to get the mannuronan (M) in the pellet and the FL fraction in the supernatant.

FTIR Spectroscopy analysis

The infrared spectra were recorded on a JASCO FT/IR 6300 spectrometer. The resolution was 4 cm^{-1}. Data was collected in the range of 4000-400 cm^{-1}. All the alginate samples were prepared for measurement in the form of KBr pellets.

H^1NMR spectra

H^1NMR spectra of sodium alginate and mannuronan were recorded using Ultrashield 300 Bruker spectrometer at room temperature, with a frequency of 300 MHZ, an acquisition time of 5.29 s and duration of impulse of 11 µs. 3mg of each sample was dissolved in 0.5ml of 99 % D2O. Tetramethylsilane (TMS) was used as internal standard.

Extraction of trace elements from *Dictyopteris polypodioides*

The following test was conducted in the institute of Monocrystals, Kharkov, Ukraine. 2g of *Dictyopteris polypodioide*s powder were taken and placed in a capillary tube of fluorized polymers for dispersion under pressure and microwaves. Afterwards, 5ml of HNO3 (70%) were added and the capillary was firmly closed and put in a stream room for 20 min, under a pressure not exceeding 120 psi. After cooling and filtration, the substance was put in a 50 ml tube. Water was added afterwards to complete the volume (50ml) which allowed us to obtain the liquid number (1). Then we took 1ml of liquid (1), put it in a 100ml tube and completed the volume with water obtaining therefore liquid number (2) (Kanaan *et al*., 2005). In order to determine the percentages of macro elements, a Thermo Jarrel Ash, CWA spectrometer was used. With liquid (1) we determined the percentage of: Fe, Zn, Cu, Ni, Mn, Al and Se. With liquid (2) we determined the percentage of Ca, Mg, K and Na.

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

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

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

Extraction of Fatty acid from *Dictyopteris polypodioides*

In order to obtain fatty acids, approximately 1.2g (the accurate weighted portion) of the raw material previously powdered to the particle size of 0.5mm was extracted by a methanol-chloroform mixture in portions of 10 ml, three times for 3h. The combined extract was filtered through a paper filter with 1g of anhydrous sodium sulfate to a previously weighted flask. The extract obtained was evaporated at 60˚C in the nitrogen stream until dryness (a residue of 40 mg). To the residue, 1 ml of diethyl ester, 5 ml of methanol and 0.2 ml of acetyl chloride were added, and the mixture was heated on a glycerin bath at reflux at 70ºC in the atmosphere of nitrogen, for 45 min. Afterwards, the solution was evaporated to the volume of about 0.3 ml and 2 ml of cyclohexane were added and shaken for 1 min. Then, the upper cyclohexane layer was taken away and filtered through the filter with 0.2g of sodium sulfate. The obtained solution was studied chromatographically on Shimadsu GC-14B, FID chromatography according to the following conditions: capillary column (60m x 0.32 mm HP-23; 0.25 µm); the column temperature was 175ºC; the process occurred for 2 min then, the temperature was increased to 225ºC with the rate of 3ºC/min: the injector temperature was 240ºC; the detector temperature was 250ºC; the rate of the gas-carrier (nitrogen) was 1.0 ml/min; the flow distribution was 1:60. The content of each fatty acid was calculated by the ‘‘internal rating’’ (Kanaan *et al*., 2005).

Antioxidant activity

The physiological Tyrode solution was first prepared from: 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). The electrolysis of physiological solution was carried out in a bath containing 20 ml of the prepared solution provided with two platinum electrodes maintained at distance of 2 cm from each other, and connected by electric wires to a stimulator adjusted to 10 mA by a sensitive multimeter. A cascade of free radicals (FR) will be as a result generated by electrolysis in the tyrode solution and a magnetic stirrer to speed up mixing and homogenizing the medium (Chahine *et al*., 1998).

A constant current of 10 mA generated by the stimulator was applied during 4 min in 20 ml of tyrode solution to generate free radicals and their derivatives. Every minute during electrolysis, 1 ml was taken from the physiological solution and added to 2 ml of N,N- diethyl-P-phenylenedialanine (DPD) (25 mg/ml) in a specific tube, vortexed and the absorbance was measured using a spectrophotometer at 515 nm (control). Similarly, the electrolysis of the physiological solution was performed in the presence of different concentrations (0.1, 0.2 and 0.4 g/l) of the isolated polysaccharides (FLM and M). Absorbance measured indirectly FR formation.

In vitro coagulation assay

Activated partial thromboplastin time (APTT) assays were carried out according to the method of Anderson *et al*. (1976). 100 µl of normal human plasma were incubated with 10 µl of a solution of polysaccharides (FLM and M) (0.05, 0.5, 2.5, 5 µg) at 37ºC for 2 min. Then 100 µl of APTT reagent were added and incubated for 2 min at 37ºC. After that, 100 µl of 0.25 M $CaCl_2$ were added to the mixtures and the clotting time was recorded using a KC1A coagulometer (Thrombotimer “Behnk elektronik’’).

RESULTS AND DISCUSSION

Extraction of polysaccharides from Dictyopteris polypodioides

According to the method of Imbs *et al*. (2009), the amount of sodium alginate obtained from 20g of brown alga *Dictyopteris polypodioides* collected from the Lebanese coast in May and July was respectively: 0.92 g and 1.25 g, with respective yields of 4.6% and 6.25%. 0.75g of FLM were isolated in May against 1.15 g in July, with respective yields of 3.75% and 5.8%. From 0.2 g of FLM, the extraction yield of mannuronan was 0.38% and 0.67% respectively in May and July.

Comparing our results with those of another species of brown algae, collected from the Lebanese coast, *Padina Pavonica*, we noticed that our species *Dictyopteris polypodioides* contained a higher amount of FLM (3.75% and 5.8% in May and July respectively) than that found in *Padina pavonica* (2.25% and 3.5% in May and July respectively).

The main polysaccharide of *D. polypodioides* was the alginate (11% of dry weight of algae) whose content varies with species, seasons, altitude and ecological systems (Obluchinskaya *et al*., 2002). Referring to the literature, the alginic acid content of the genus *Sargassum* varies from 3 to 17% (Sinha *et al*., 2010).

Determination of fatty acids from Dictyopteris polypodioides

Marine macroalgae are known to be a source of polyunsaturated fatty acids which are associated with the prevention of inflammation, cardiovascular diseases and mental disorders (Van Ginneken *et al*., 2011).

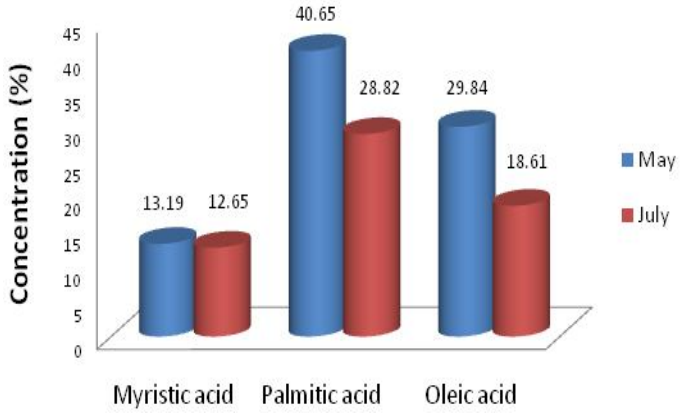

Fig. 1: comparison of the most abundant fatty acids contained in the brown algae *Dictyopteris polypodioides* in May and July.

Thus the fatty acids detected in algal sample were determined. 10 items were obtained in both seasons (data not shown). The palmitic acid was the major fatty acid in both seasons (40.65 % in May and 28.82 % in July). Moreover, the percentage of myristic

and oleic acids in May (13.19 % and 29.84% respectively) was higher than that in July (12.65 % and 18.61 % respectively) (Summarized in Figure 1**)**. Whereas, the study made on *Padina pavonica* showed that 25 fatty acids were obtained in April and 14 in July. The palmitic acid presented a high percentage (41%) in both seasons whereas the percentage of oleic (22.98 %) and myristic acids (8.133 %) showed a higher percentage in July (Yassine *et al*., 2012).

Determination of trace elements from Dictyopetris polypodioides

Minerals are essential for the proper functioning of the body. Therefore, the trace elements were detected in the algal sample and their concentrations were determined and shown in figure 2. Fifteen trace elements were found in *Dictyopteris polypodioides* analyzed by atomic microscopy in each season**.** *Dictyopteris* appeared rich in Fe, Si, P, Mg, Ca, Na, K, Sr. Minerals such as Calcium (2080 mg/100g), Strontium (200 mg/100g), Phosphorus (110 mg/100g), were found at a larger amount in May, while Magnesium (595 mg/100g), potassium (4250 mg/100g), sodium (1020 mg/100g), silicon (1190 mg/100g) and iron (170 mg/100g) showed higher amounts in July (Figure 2). According to the literature, *Padina pavonica* showed larger amounts of Silicon (3330 mg/100g), Calcium (3530 mg/100g) and Iron (125 mg/100g) being at their highest levels in April, whereas Mg (1600 mg/100g) and K (2140 mg/100g) in July (Yassine *et al*., 2012). These results indicate that the mineral composition of seaweeds varies with seasons, harvesting time and species.

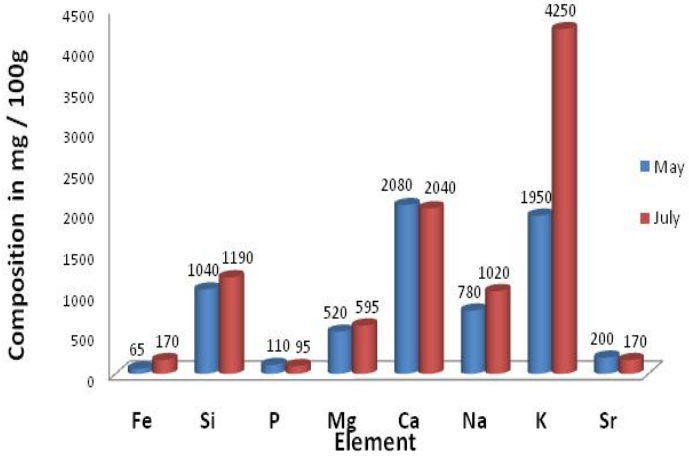

Fig. 2: Comparison of trace elements from *Dictyopteris polypodioides* in May and July.

[1]HNMR spectroscopy of alginates

[1]H NMR spectroscopy is considered to be the most reliable method to determine the composition and the detailed structure of alginates (Heyraud *et al*., 1996), which are typically described by their mannuronic / guluronic (M/G) ratio, the distribution of their M and G units along the chain and their average molecular weight, since these parameters are closely related to the properties of alginates like solubility, interaction with metals, gel-forming properties and viscosity (Stokke *et al*., 1991). The ability of alginic acids to form a gel depends on the content of guluronic acid (20-25%) thereby on the M/G ratio. For a smaller ratio, the alginate forms a brittle gel but a higher ratio leads to a more elastic gel (Sokolova *et al*., 2011**).** The composition and sequential structure including M/G ratio and the properties of alginates vary with extraction procedures (Gomez *et al*., 2009), age (Honya *et al*., 1993), season (Jothisaraswathi *et al*., 2006), and geographic variability of alginate for the same species of brown algae (Torres *et al*., 2007). Moreover, the alginate mainly formed of polymannuronate blocks, shows antitumor effects (Sousa *et al*., 2007) whereas that composed mainly of polyguluronate blocks presents biosorptive properties (Davis *et al*., 2004).

The [1]H NMR spectra give us the two values of monad (FM and FG) and the four frequencies of diad (FGG, FMM, FMG, FGM) (Draget *et al*., 2002). Usually the ratio M/G is calculated using the method proposed by Grasdalen *et al*., 1979 and Grasdalen 1983, where the following notations are used: HG-1 = the peak corresponding to the hydrogen in C-1 in guluronic acid residues; HM-1 = the peak corresponding to the hydrogen in C-1 in mannuronic acid residues; HGM-5 = the peak corresponding to the hydrogen in C-5 in alternating blocks of mannuronic and guluronic acid residues; HG-5 = the peak corresponding to the hydrogen in C-5 in guluronic acid residues and IA, IB, IC are the peak areas corresponding to HG-1, HM-1 + HGM-5 and HG-5, respectively.

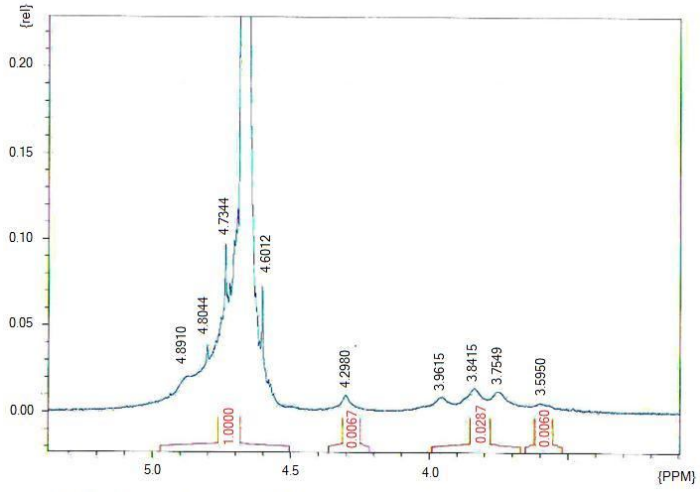

Fig 3: [1]H NMR spectra of alginate isolated from *Dictyopteris polypodioides*

Figure 3 shows the NMR spectrum which is usually used to calculate the composition of sodium alginate by comparing the signal areas of HG-1 (IA), HM-1 + HGM-5 (IB), and HG-5 (IC) using the equations proposed by Gradsalen *et al*., 1979.

$$FG = IA / (IB + IC) \qquad M / G = (1-FG) / FG$$

The NMR spectrum (Figure 3) corresponding to the sodium alginate isolated showed a peak around 4.7344 ppm corresponding to IC, a peak around 4.8044 ppm corresponding to IB, and a small peak around 4.8910 ppm corresponding to IA.

So FG = IA / (IB + IC) = 4.8910/9.5388 = 0.51

FM = 1- FG = 1-0.51= 0.49

M/G = FM/ FG = 0.49/0.51 = **0.96**

Thus, the studied alga was a promising source for obtaining alginic acid that can be used as bioadsorbant of heavy metals such as Cd^{2+} and with a high ability to form a gel whereas the study of Men'shova *et al* (2012) showed that the M/G ratio for *Padina Pavonica* collected in July was about 1.33. According to the literature the M/G ratio for many algae of the genus Sargassum varies from 0.19 to 1.5 (Sokolova *et al*. 2011).

Infrared spectrum of sodium alginate

The FT-IR spectrum of sodium alginate isolated from *Dictyopteris polypodioides* is presented in Figure 4 A. In the region between 3600-1600 cm^{-1} three bands appear: a broad band centered at 3432.67 cm^{-1} assigned to hydrogen bounded O-H stretching vibrations, the weak signal at 2929.34 cm^{-1} is due to C-H stretching vibrations, and the asymmetric stretching vibration of O-C-O is centered at 1612.2cm-1. The band at 1415.49 cm-1 may be due to the C-OH bending vibration with contribution of carboxylate group O-C-O (Mathlouthi *et al*., 2001; Silverstein *et al*., 1991). Or the weak bands at 1322.93 cm^{-1}, 1125.26 cm^{-1}, 1091.51 cm^{-1}, may be assigned to C-C-H and O-C-H deformation and C-O stretching vibrations of pyrannose rings respectively; the band at 1029cm^{-1} may be also due to C-O stretching vibration. Moreover, the anomeric region between 950 and 750 cm^{-1} is the most discussed of carbohydrates (Mathlouthi *et al*., 1986). The spectrum showed a band at 945.9 cm-1, which was assigned to C-O stretching vibration of uronic acid residues, and one at 903.487cm^{-1} assigned to the C-H bond of α-L-guluronic acid. According to Mackie, the alginate has two characteristics bands in the IR spectra, one at 808 cm^{-1} assigned to ß-mannuronic acid, and the other one at 787 cm^{-1}, which is a weak band assigned to the Cl-H deformation vibration of α-L-guluronic acid. (Mackie *et al*., 1971).

Infrared spectrum of Mannuronan

The FT-IR spectrum of Mannuronan isolated from *Dictyopteris polypodioides* is presented in (Figure 4 B). A broad band centered at 3372.89cm^{-1} is assigned to the hydrogen bonded O-H stretching vibration; a weak band at 2924.52 cm^{-1} is assigned to a C-H stretching vibration. The band centered at 1728.87cm^{-1} is assigned to the carbonyl group C═O, the one at 1612.2 cm^{-1} is assigned to the C_O bond of the carboxylate group. The band at 1270.86 cm^{-1} corresponds to the bond C-H, the fingerprint in the region 1000-400 cm^{-1} is difficult to analyze since no previous work on mannuronan has been done nor its structure has been identified vet.

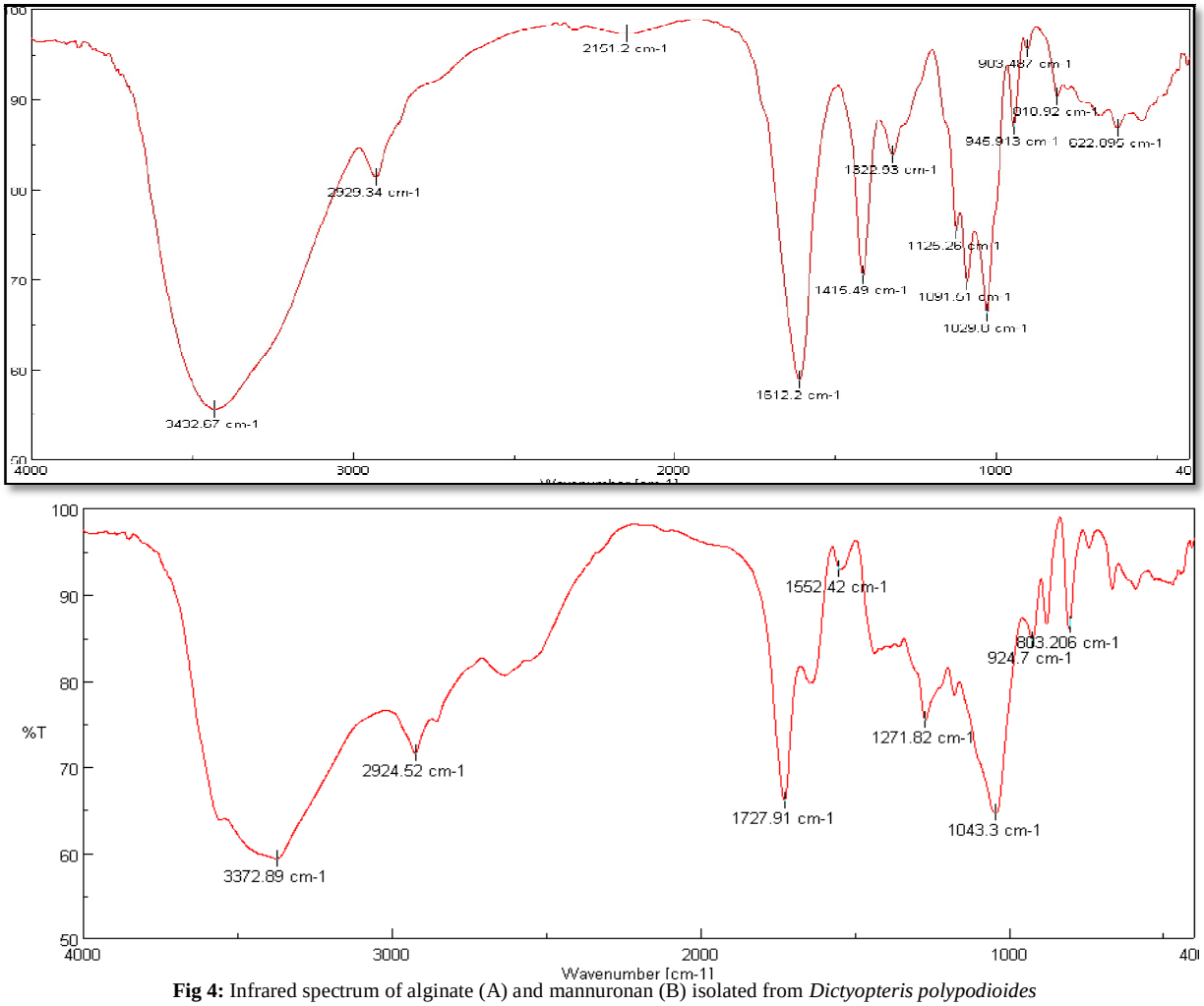

Fig 4: Infrared spectrum of alginate (A) and mannuronan (B) isolated from *Dictyopteris polypodioides*

Antioxidant activity

The electrolysis of the Tyrode solution generates FR (free chlorine, hypochlorous acid, and different reactive oxygen species) which react instantaneously with the DPD reagent giving through absorbance a red color proportional to the generated FR.

For the control curve without adding any active molecules, absorbance increases with time reaching a maximal value of 0.41 at the fourth mine of electrolysis. For the same initial concentration 4 mg/ml, while the concentration of FLM in both seasons increases from 0.1 g/l to 0.4 g/l, absorbtion decreases dramatically and reaches a maximal value at the fourth min of electrolysis about 0.1 and 0.07 respectively for FLM in May and FLM in July at 0.4 g/l (Figure 5 A and B). These lower levels compared to control showed that FLM exhibits free radicals scavenger effect directly proportional to its concentration in the solution. Thus, the increased concentration of polysaccharides allows more FR trapping, and high antioxidant activity. Moreover, we can conclude that the antioxidant activity of FLM extract from *D. polypodioides* at the end of the season is higher than the effect of FLM obtained from *D. polypodioides* at the beginning of the season. As well, we can notice that Mannuronan in both seasons exerts a scavenger effect on FR (Figure 5 C and D). Furthermore, the formation of FR is highly attenuated in the presence of mannuronan M from *D. polypodioides* in July (Figure 5 D). Indeed, in the presence of M obtained in May, the maximal absorbance at the fourth min of electrolysis was 0.34 (0.1 g/l), 0.3 (0.2 g/l) and 0.2 (0.4 g/l) while in the presence of M obtained in July a gradual lowering of the maximal absorbance was observed with 0.28 (0.1g/l), 0.22 (0.2 g/l) and 0.11 (0.4 g/l) (Figure 5 C and D). Hence, it is advisable to collect the seaweed *Dictyopteris polypodioides* at the end of the season in July. On the other hand, the comparison of the antioxidant effect between mannuronan alone and FLM compound in both seasons showed that mannuronan (FR inhibition 87 % for M5 and 93 % for M7) exhibits a greater antioxidant activity than FLM (FR inhibition 75 % for FLM 5 and 83 % for FLM 7) (Figure 6).

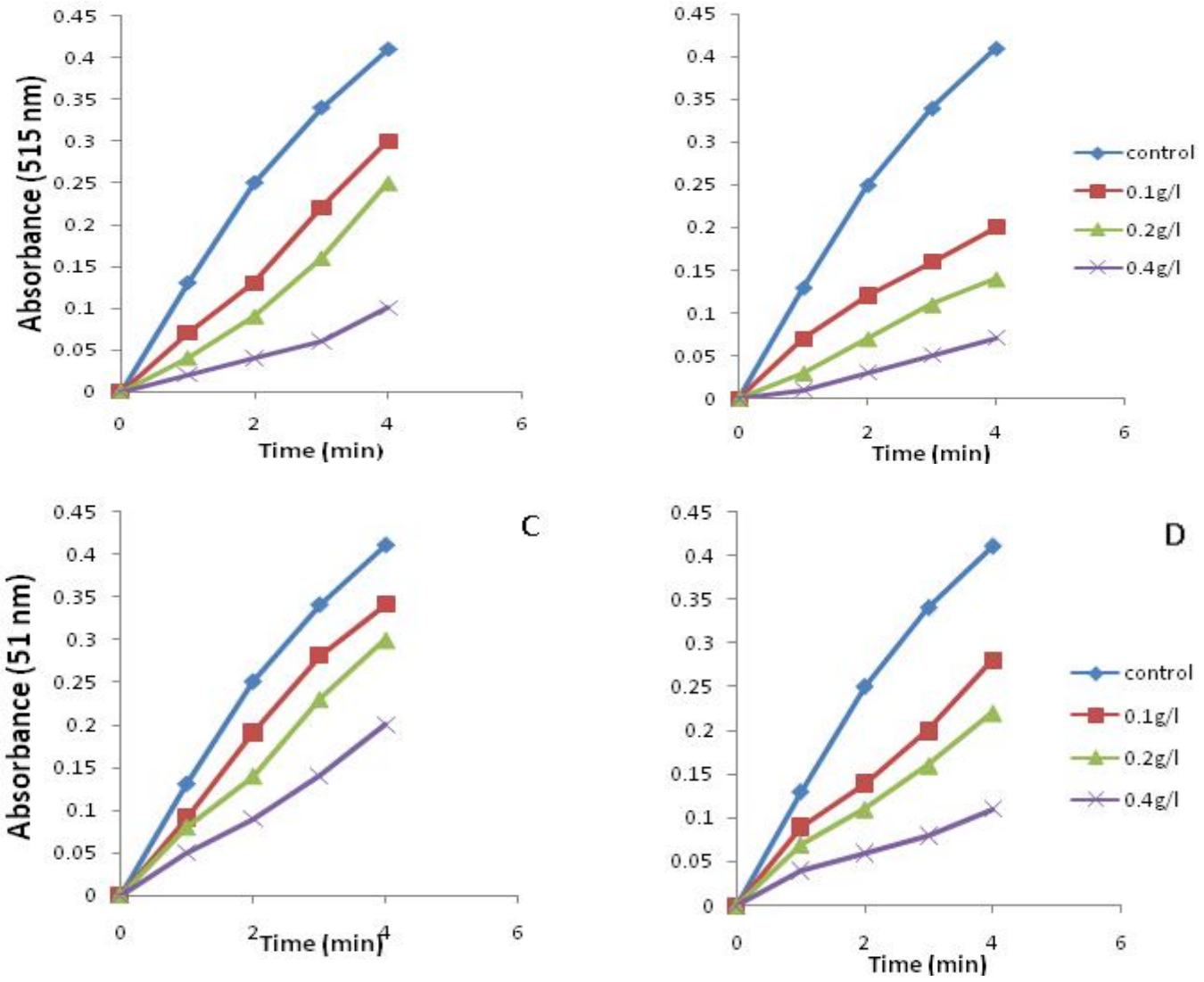

Fig 5: Variation of the antioxidant effect of FLM collected in May (A) and July (B) and Mannuronan collected in May (C) and in July (D)

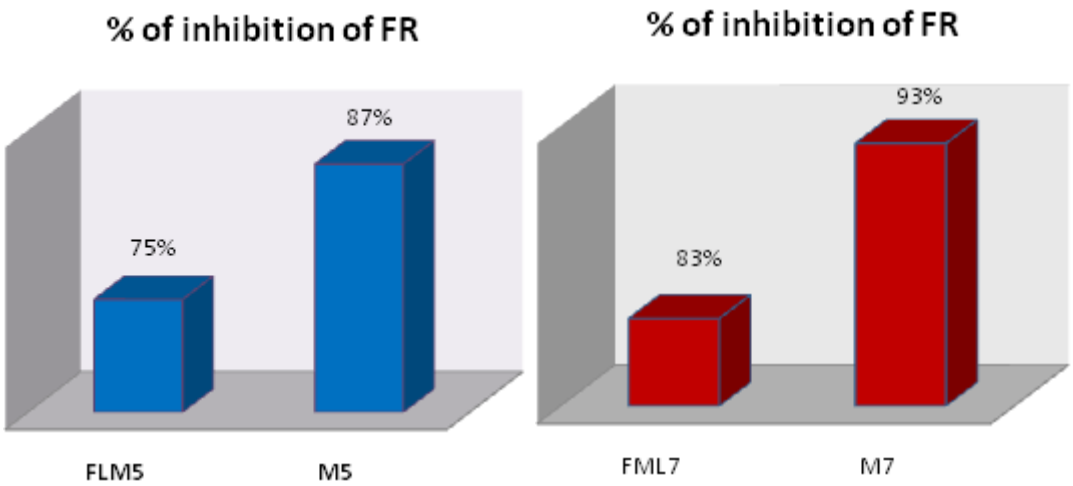

Fig 6: Comparison of the percentage of inhibition of FR by FLM and M collected in May and July. **FLM5:** FLM in May. **FLM7**: FLM in July. **M5**: Mannuronan in May. **M7**: Mannuronan in July.

Table. 2: Comparison of the anticoagulant activity of polysaccharides isolated from *Dictyopteris polypodioides.*

Quantity (µg)	Polysaccharides	APTT (s)	Ratio
0.05	FL	40.5	1.35
	FLM	40.8	1.36
Quantity (µg)	**Polysaccharides**	**APTT (s)**	**Ratio**
0.5	FL	41.3	1.38
	FLM	42.2	1.41
	M	39.8	1.33
Quantity (µg)	**Polysaccharides**	**APTT (s)**	**Ratio**
2.5	FL	42.5	1.42
	FLM	43.1	1.44
	M	42.1	1.40
Quantity (µg)	**Polysaccharides**	**APTT (s)**	**Ratio**
5	FL	40.8	1.45
	FLM	43.4	1.55
	M	42.2	1.44

APTT was measured in second by a coagulometer. Ratio was calculated by the formula: Ratio = APTT measured / APTT control = APTT measured / 30.
FL (Fucoidan + Laminaran); FLM (Fucoidan + Laminaran + Mannuronan); M (Mannuronan)

Anticoagulant activity

Heparin has been widely used in anticoagulant therapy for more than 50 years. The major mechanism by which unfractionated heparin exerts its anticoagulant effect is by accelerating serine proteinase inhibitor plasma factor such as thrombin (factor IIa) and factor Xa. But being from animal origin, heparin can induce diseases in mammals, such as avian influenza and bovine spongiform encephalopathy (Mendes *et al*. 2009). These reasons reinforce the need to find a new anticoagulant and antithrombotic agent replacing heparin. Previous studies have shown that sulfated polysaccharides or chemically modified naturally exhibit anticoagulant activities attributed to the substitution of sulfate group of glucosamine residue. The anticoagulant activity of the sulphated polysaccharides depends on their degree of substitution, their molecular weights and the position of the sulfate group (Jiraporn *et al*., 2009). Therefore we have been investigating the anticoagulant activity of polysaccharides isolated from *D. polypodioides* using the activated partial thromboplastin time APTT clotting assays (summarized in Table 2). A compound exhibits an anticoagulant activity when the ratio APPT / APTT control is greater than 1.2. The control has a normal APTT of 28-38 sec, and this value depends on the laboratory techniques and reagents used. 30 seconds were used as APTT control in our experiments. We noticed that as far as the quantity of the polysaccharides isolated increases the APTT and the ratio increase and consequently the anticoagulant effect increases as well (Table 2).

In this work it has been demonstrated for the first time that mannuronan shows a significant anticoagulant activity. We noticed also that FLM mixture shows the most important anticoagulant effect compared to other compounds.

This might be due to to the presence of sulphates groups in the fucoidan. In fact, the sulphate content of monosaccharides of algae influences the anticoagulant activity (Li *et al*., 2008). These results suggest that the FL and even M from *Dictyopteris polypodioides* may constitute an alternative to the anticoagulant heparin.

CONCLUSION

The brown algae *Dictyopteris polypodioides* growing on the Lebanese coast consist of mineral or inorganic components and organic components, the latter consisting mainly of alginates, fucoidans, mannuronans and other carbohydrates. These polysaccharides show a wide variety of physical properties such as gelling and emulsifying agents as well as biological properties such as antioxidant and anticoagulant activities. Further investigations are imperative in order to develop new molecules based on these polysaccharides to be used as specific drugs or for healthcare in general. It might be also interesting to determine the antioxidant activity of the complex FL and compare it to that of FLM and M to specify whether M synergists FL or diminishes its effect. Given that mannuronans have never been studied before; it will be very interesting to perform the 2D NMR (COSY, NOESY, HMQC, HSQC) and mass spectroscopy to determine their structures. Therefore they can be a valuable source for further studies on biological activities such as anti-inflammatory, antiviral and antibacterial. It is also important to analyze the content of heavy metals in brown algae that could be useful for environmental studies as an indicator of pollution of the Lebanese coast.

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