Structural Characteristics, Antitumor and Antioxidant Properties of Polysaccharides isolated from the brown algae *Stypopodium schimperi* growing on the Lebanese coast.

Original Research Article

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Name of the Author:

Haddad[1] M., Zein[1] S., Hazimeh[1] G., Karaki[1] R., Krivoruchko[3] E., Makhour[4] Y., Kassem[2] Z. Kanaan*[1] H.

[1]Laboratory of Chemical synthesis and extraction of polysaccharides from seaweed, Faculty of Pharmacy, Lebanese University

[2]PRASE, Platform of Research and Analysis in Environmental Sciences, Doctoral School of Sciences and

[3]Department of Pharmacognosy, National University of Pharmacy, (Ukraine)

[4]Faculty of Sciences I, Lebanese University, Hadath campus, Beirut,(Lebanon.)

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ABSTRACT

Objective: The purpose of this study was to extract, to determine the functional groups and to examine the antioxidant and anti-proliferative effect of the water-soluble polysaccharides fucoidan and sodium alginate extracted from the brown algae *Stypopodium schimperi* growing on the Lebanese coast.

Materials and Methods: Fucoidan and sodium alginate were analyzed using ^{1}H NMR, and FTIR methods to reveal their structures. Antioxidant effects were also tested, using DPPH method. The polysaccharides were tested in-vitro for anti-proliferative activity against the colorectal cancer cell lines (HT 29 and HCT 116) for 24h, 48h and 72h respectively, by the MTT cell viability assay.

Results: The extraction yield of fucoidan and sodium alginate was 7.4% and 21% respectively. The structure of α-L fucose unit of fucoidan was confirmed through the spectrum of ^{1}H NMR. M/G ratio of sodium alginate was calculated and found to be 0.96 which allows the formation of strong and heat stable gel. Both polysaccharides have an antioxidant effect with fucoidan the most pronounced. For the anti-proliferative effect, sodium alginate was able to inhibit the growth of HT 29 and HCT 116 more than fucoidan at the different time intervals.

Conclusion: This study discusses the structure, the functional groups, the antioxidant and anti-proliferative effect of fucoidan and sodium alginate. Both polysaccharides showed an antioxidant effect which is dose dependent and they were able to inhibit the growth of HT 29 and HCT 116 with difference in the degree of inhibition between sodium alginate and fucoidan. These effects make them candidate in the field of medicine and food industry.

Keywords:

Stypopodium schimperi, fucoidan, sodium alginate, anti-proliferative, antioxidant.

I. INTRODUCTION

The ocean covers more than 70% of Earth's surface and is characterized by a wide diversity of marine organisms that offer a rich source of natural products *(Wijesekara et al, 2011)*. Among marine resources, algae are well known natural sources of polysaccharides *(Cunha et al, 2016)*. They are commercially important and renewable marine resource that is being studied on the use of many industrial applications such as in food, cosmetics, and medicine *(Burtin, 2003)*. In addition, due to the multiple side effects of chemically synthesized drugs, their toxic effects and their costs, medicinal plants have been widely developed in recent decades *(Tiwari et al, 2011)*. 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)*.

In Lebanon, we studied the structure and pharmacological activities of water-soluble polysaccharides, fucoidan and sodium alginate extracted from marines algae (brown and red). (kanaan et al, 2011, 2012, 2013, 2014, 2015) .We have followed the studies on the brown algae, *Stypopodium Schimperi* which is from the family dictyotaceae, and grows in shallow, in deeper water and inhabits tropical and sub-tropical seas such as in Europe, Africa, Atlantic Islands, and South West Asia. A remarkable feature of the alga is its phosphorescent color, a brilliant turquoise when immersed in seawater *(Kanaan et Belouse, 2015)*. The aim of this work was to evaluate the antioxidant, and anti-proliferative effects of polysaccharides extracted from the brown algae "*Stypopodium Schimperi*".

II. MATERIALS AND METHODS

2.1. Sample collection

Stypopodium schimperi was collected from "Barbara" region near "Batroun" in May 2015. To proceed in the extraction, the algae was washed and cleaned with water and dried at room temperature.

2.2. Extraction of polysaccharides from *stypopodium schimperi*

9.5 g of *stypopodium schimperi* were extracted in 250 ml absolute ethanol (96%) two times at 40°C for 3 hours each time, in order to remove pigments, fatty acids, and oligoelements, and centrifuged at 3000 rpm for 20 minutes, supernatant obtained was discarded. Then, the residues were also extracted with 150 ml of diluted hydrochloric acid (HCl) (PH=2) two times at 60°C for 3 hours each time for further depigmentation, then centrifuged at 3000 rpm for 20 minutes. Supernatant (a) obtained after centrifugation contains the complex fucoidan, laminaran and mannuloran (FLM), while the residues (b) contain alginate.

2.2.1. Extraction and isolation of fucoidan

Supernatant (a) was neutralized with 3% sodium bicarbonate ($NaHCO_3$). Then, Rota vapored in order to increase concentration of the sample by evaporating water, then dialyzed for 24 hours in order to remove impurities by using a dialysis tube SPECTRAPOR (standard cellulose dialysis tubing, dry cylinder diameter: 20,4 mm, dry thickness: 0.0009, MW cutoff: 12000-14000),and lyophilized to obtain FLM powder. After that, 50 ml of diluted HCl (PH=2) was added in order to precipitate M, then centrifuged at 3000 rpm for 20 minutes and the residues that contains mannuloran were discarded, and supernatant that contains "fucoidan" our molecule of interest was taken. Finally, lyophilize to obtain fucoidan in powder (figure 10). The powdered product was weighed to calculate fucoidan yield. Yield of fucoidan (%) = actual weight × 100/ theoretical weight.

2.2.2. Extraction and Isolation of Alginate

Residues (b) were extracted with 3% Na_2CO_3 then with 1.5% Na_2CO_3 at 60°C for 8 hours each, rinsed with water, centrifuged at 3000 rpm for 20 minutes and the residues were discarded. Then, supernatant was Rota vapored, dialyzed for 24 hours, precipitated with a certain volume of ethanol (1:1) in order to precipitate alginic acid and centrifuged at 3000 rpm for 20 minutes and residues were taken. They were dissolved with water, then few drops of HCl (PH=2) was added and centrifuged at 3000 rpm for 20 minutes. Residues obtained are alginic acid. To obtain sodium alginate: dissolve alginic acid with water, then add few drops of sodium hydroxide NaOH (PH=8), dialyze for 24 hours and finally lyophilize to obtain powder of sodium alginate. The powdered product was weighed to calculate sodium alginate yield. Yield of sodium alginate (%) = actual weight × 100/ theoretical weight.

2.3. Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)

In order to determine the position of proton in sodium alginate and fucoidan, 3 mg of the samples were dissolved in 0.5 ml deuterium oxide (D_2O), and the NMR spectra of the samples were recorded using Ultra shield Broker 300 spectrometer at room temperature, with a frequency of 300 MHZ, an acquisition time of 5.2 seconds and a pulse duration of 11 milliseconds. Tetramethylsilane was used as an internal standard.

2.4. Fourier transforms infrared spectrometer (FTIR)

Fucoidan and sodium alginate were mixed with potassium bromide (KBr), in a way that the % of sample/ KBr = 2%. The FTIR spectrum of the samples was recorded on a JASCO FT/IR 6300 spectrometer. The resolution was 4 cm^{-1} and data were collected in the range of 4000-400 cm^{-1}.

2.5. Antioxidant activity: 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity

The radical scavenging activity of fucoidan and sodium alginate depend on their capacities to scavenge DPPH free radicals. DPPH is a purple colored crystalline powder that changes to yellow color in case the sample has an antioxidant effect through gaining a proton (DPPH-H). *It should be freshly prepared and protected from light.*

A total of 2.62 mg DPPH was dissolved in 50 ml methanol and 5 concentrations of fucoidan and sodium alginate (0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, 0.4 mg/ml, and 0.5 mg/ml) were prepared, then 1 ml of each concentration was added to 1ml of DPPH solution, agitated and incubated for 30 minutes in the dark. Then the absorbance was measured at 517 nm using a UV-visible spectrometer (Shi-madzu UV-1605, Japan). Control used was 1 ml DPPH with 1 ml of the solvent by which the samples were dissolved.

The DPPH scavenging capacity was calculated as follows:

DPPH scavenging capacity (%) = [Absorbance of control – Absorbance of sample/ Absorbance of control] × 100.

2.6. Cell culture

The cell lines used in this study were human colorectal cancer cell lines HCT 116 and HT 29 and murine macrophage cell line RAW 264.7. The cells were grown in plastic bottles (75 cm^2) containing Dulbecco's modified Eagle's medium complete media supplemented with 10% fetal bovine serum, 1% penicillin streptomycin (10000 IU/ml), and were maintained at 95% humidity in a CO_2 incubator at 37°C. During the experiments, cells were allowed to grow till 80-90% confluency, where all available space of the culture vessel is covered due to cellular expansion before passage. Cells were passaged 8 times in order to prolong life of the cells and allow them to proliferate.

2.7. Anti-proliferative activity: MTT cell viability assay

In order to determine the anti-proliferative effect of fucoidan and sodium alginate on HT 29 and HCT 116, MTT assay was performed. This test is a good index of mitochondrial activity and thus of cell viability. It is based on the ability of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes in metabolically active cells to reduce the yellow tetrazolium dye MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) to its insoluble formazan, which has a purple color. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. HCT 116, and HT 29 cell lines were seeded in 96-well plates at a density of 15 000 cells/well and incubated in DMEM complete media for 24 hours. After incubation, media were aspirated and test compounds (fucoidan and sodium alginate) were prepared with different concentrations (5mcg/ml, 25mcg/ml, 50mcg/ml, 100mcg/ml, and 200mcg/ml) and 100 µl were distributed into the wells in triplicate and incubated for 24 hours, 48 hours, and 72 hours. Control groups were treated with DMEM. After incubation is completed, 10 µl of MTT was added to each well. Three hours later, 100 µl of stop solution (HCl + Sodium Dodecyl Sulfate) was added and incubated for 1 hour to solubilize formazan. Then, the optical density (OD) of the samples was determined at 570 nm using enzyme-linked immunosorbent assay (ELISA) reader. Data were analyzed and percentages of cell viability were determined for the tested compounds.

$$\% \text{ of cell viability} = \frac{\text{Mean OD of treated Cells-Mean OD of blank} \times 100}{\text{Mean OD of Control-Mean OD of blank}}$$

Where the blank was the acidified isopropanol.

2.8. Statistical analysis

The data obtained in the study were expressed as Mean ± SEM and were analyzed using one-way ANOVA test and student's t- test compared to the control. A value is considered significant if $P \leq 0.05$.

III. RESULTS

3.1. Percentage yield of polysaccharides

The quantity of fucoidan obtained from 9.5 g *stypopodium schimperi* is: 0.7 g with a yield of 7.4% (0.7/9.5 × 100). While, the quantity of sodium alginate is: 2 g with a yield of 21% (2/9.5 × 100).

3.2. Determination of the proton position: ^{1}H NMR analysis of fucoidan

The ^{1}H NMR spectrum of fucoidan signals spread from 5.0 to 5.4 ppm, the presence of these signals at 5.0 to 5.4 ppm is consistent with the presence of α-L-fucopyranosyl. Signals of the fucose methyl group appear in the 1.0-1.3 region. Moreover, a signal at 4.65 ppm can be attributed to H_4 of 4 sulfated fucose (figure 1).

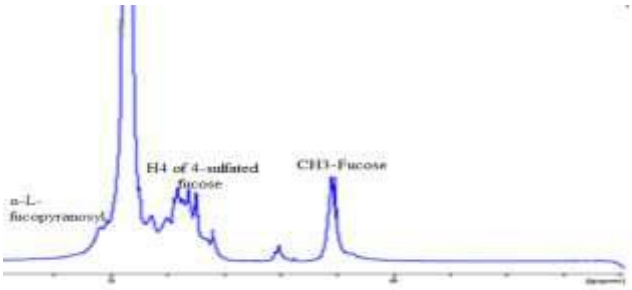


Figure 1: ^{1}HNMR of fucoidan isolated from *stypopodium schimperi.*

3.3. Determination of the proton position: ^{1}H NMR analysis of sodium alginate

The composition and also the block structures of alginate molecules are often determined by ^{1}H NMR spectroscopy. The method proposed by Grasdalen (1983) and Grasdalen et al. (1979) makes it possible to calculate the *M/G* ratio and to determine the block structure, where the following notations are used: H-1 G = the peak corresponding to the hydrogen in C-1 in guluronic acid residues; H-1 M = the peak corresponding to the hydrogen in C-1 in mannuronic acid residues; H-5 GM = the peak corresponding to the hydrogen in C-5 in alternating blocks of mannuronic and guluronic acid residues; H-5 G = the peak corresponding to the hydrogen in C-5 in guluronic acid residues and IA, IB, IC are the peak areas corresponding to H-1 G, H-1 M + H-5 GM and H-5 G, respectively.

FG = IA / (IB + IC) FM=1-FG M / G = FM/FG = (1-FG) / FG

The ^{1}HNMR spectrum of sodium alginate (figure 2) 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

Our results showed that the fraction of guluronic acid is higher than mannuronic acid which means that our extract has a high ability to form strong and heat stable gels, because guluronate blocks allows a high degree of coordination of the divalent ions and form hydrogels.

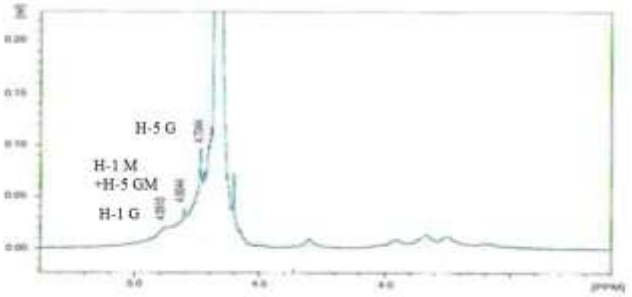


Figure 2: ^{1}HNMR of sodium alginate isolated from *stypopodium schimperi.*

3.4. Determination of the functional groups: FTIR spectroscopic analysis of fucoidan

The FTIR spectrum of fucoidan showed a wide band centered at 3448.1 cm-1 which is assigned to the hydrogen bonded O-H stretching vibration. A weak signal at wavelength 2928.38 cm-1 indicated the presence of C-H. The band at 1634.38 cm-1 indicates the absorbance of uronic acid (carboxylate O_ C _ O asymmetric stretching vibrations). The weak band at 1252.54 cm-1 indicated the presence of S=O stretching vibration of sulphate group. The band at 1043.3 cm-1 is assigned to the sulfate ester group. The band at 618.074 cm-1 may be due to C - C -H stretching vibration (figure 3).

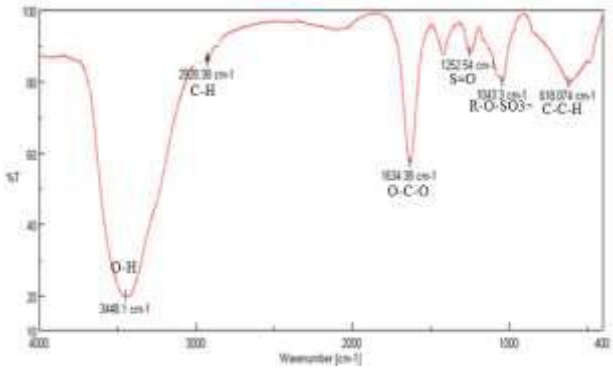


Figure 3: FTIR spectrum of fucoidan isolated from *stypopodium schimperi.* %T: % Transmittance.

3.5. Determination of the functional groups: FTIR spectroscopic analysis of sodium alginate

The FTIR spectrum of sodium alginate is shown in figure 4. The FT-IR analysis of sodium alginate of *stypopodium schimperi* represented that in 3600–1600 cm^{-1} region, four bands appeared with a broad band centered at 3423.03 cm^{-1}. It is assigned to hydrogen bond (O–H) stretching vibrations, the weak signal at 2927.41 cm^{-1} is due to C–H stretching vibrations, the wavelength at 2100.1 cm^{-1} indicated the presence of C=C=O and the asymmetric stretching of carboxylate O–C–O vibration at 1635.34 cm^{-1}. The band at 1416.46 cm^{-1} may be due to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The weak band at 1121.4 cm^{-1} may be assigned to C–O stretching, and C–C stretching vibrations of pyranose rings; the band at 1028.84 cm^{-1} may also be due to C–O stretching vibrations. The spectrum showed a band at 942.056 cm^{-1}, which is assigned to the C–O stretching vibration of uronic acid residues, and one at 864.917 cm^{-1} assigned to the C1–H deformation vibration of b-mannuronic acid residues. The band at 621.931 cm^{-1} may be due to C - C-H stretching vibration.

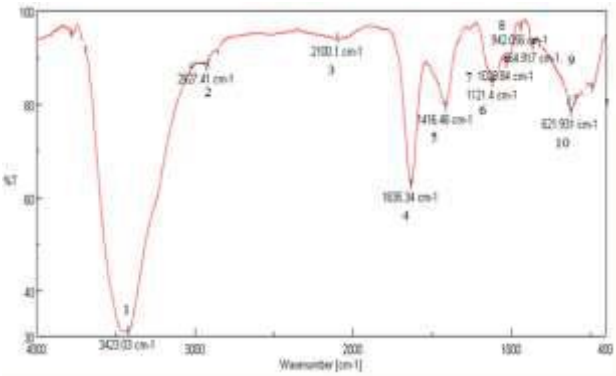


Figure 4: FTIR of sodium alginate extracted from *stypopodium schimperi*. %T: % Transmittance. 1: O-H, 2: C-H, 3: C=C=O, 4: O-C-O, 5: C-OH, 6: C-C stretching of pyranose ring, 7: C-O, 8: C-O stretching of uronic acid, 9: mannuronic acid, and 10: C-C-H.

3.6. Antioxidant activity: DPPH radical scavenging activity

The results obtained from DPPH assay showed that the scavenging activity of fucoidan is dependent on its concentrations. As the concentration increases from 0.05 mg/ml to 0.5 mg/ml, the antioxidant effect also increases from 15.17% to 44.13% respectively (figure 5). Each value represents the average of three independent experiments.

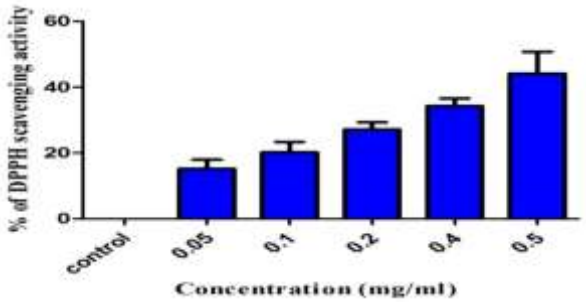


Figure 5: DPPH radical scavenging activity of fucoidan isolated from *stypopodium schimperi.* The results are expressed as mean+ SEM and analyzed using the t-test compared to the control. *P<0.05; **P<0.01; ***P<0.001. Each value represents the average of three independent experiments.

For sodium alginate, the results showed that the scavenging activity is dependent on its concentrations. At 0.05 mg/ml and 0.1 mg/ml there is no antioxidant effect compared to control. Starting from 0.2 mg/ml, the effect appears (2.8%), and it continues to increase with the concentrations to reach 6.7%, and 9.8% at 0.4 mg/ml and 0.5 mg/ml respectively. This increase is significantly different compared to control ($P < 0.01$ at 0.4 mg/ml and $P < 0.001$ at 0.5 mg/ml). Each value represents the average of two independent experiments (figure 6).

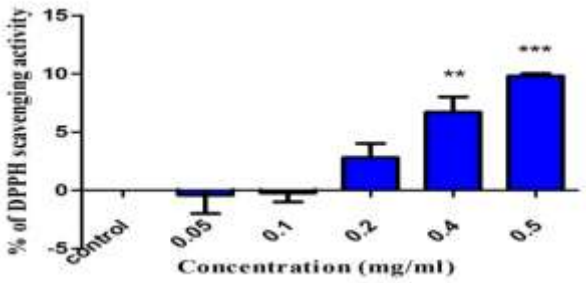


Figure 6: DPPH radical scavenging activity of sodium alginate isolated from *stypopodium schimperi*. The results are expressed as mean+ SEM and analyzed using the t-test compared to the control. *P<0.05; **P<0.01; ***P<0.001. Each value represents the average of two independent experiments.

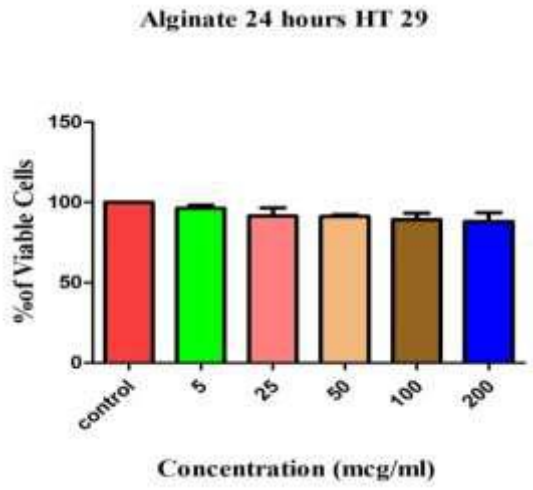
3.7. Anti-proliferative activity: MTT Cell viability assay

In order to examine the anti-proliferative effect of fucoidan and sodium alginate, MTT cell viability assay was conducted on HCT 116 and HT 29 cells for 24, 48, and 72 hours. The results obtained are presented in figure 7, 8, 9, and 10.

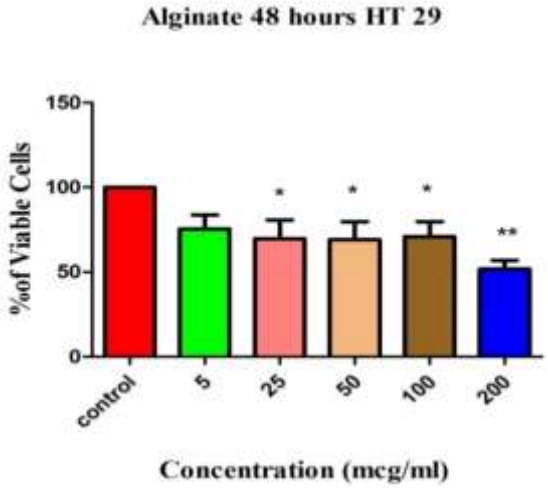
3.7.1. Anti-proliferative effect of sodium alginate on HT 29 (n=3)

Results of sodium alginate showed that the effect on HT 29 was dose and time-dependent. At 24 hours there is no significant effect (figure 7a), while at 48 and 72 hours a significant decrease of cell viability was seen compared to control. At 48 hours the % of cell viability was reduced by 24.47% at 5mcg/ml and significantly by 48.18% at 200mcg/ml ($P< 0.01$) (figure 7b). At 72 hours, the % of cell viability was significantly reduced by 23.95% and 40.67% at 5mcg/ml ($P< 0.05$) and 200mcg/ml ($P< 0.01$) respectively compared to control. In addition, at concentrations 25mcg/ml, 50mcg/ml, and 100mcg/ml the reduction was almost 30% at 48 and 72 hours compared to control (figure 7c).

a)



b)



c)

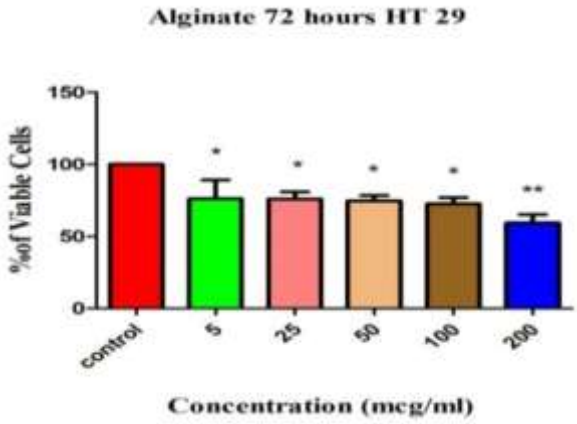
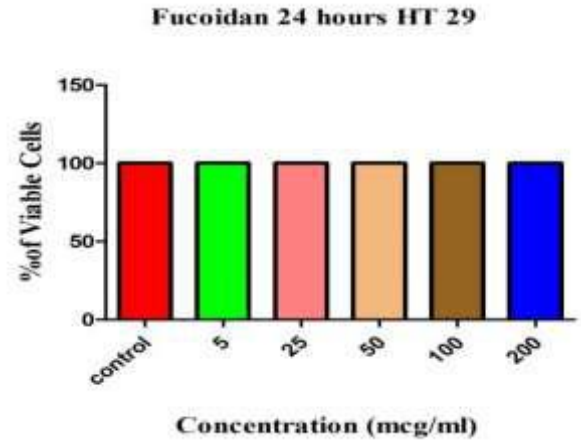


Figure 7: Viability of HT 29 cells treated with different concentrations of sodium alginate for a) 24 hours, b) 48 hours, and c) 72 hours. Control group is untreated cells. The results are expressed as mean+ SEM and analyzed using the t-test compared to the control. *P<0.05; **P<0.01; ***P<0.001. Each value represents the average of three independent experiments.

3.7.2 Anti-proliferative effect of fucoidan on HT 29 (n=2)

The results showed that there is only an effect at 48 hours, and almost the same between the different concentrations: 79.5% at 5 mcg/ml, 79.9% at 24 mcg/ml, 84.15% at 50 mcg/ml, 77.355 at 100 mcg/ml, and 87.25% at 200 mcg/ml (figure 8b).

a)



b)

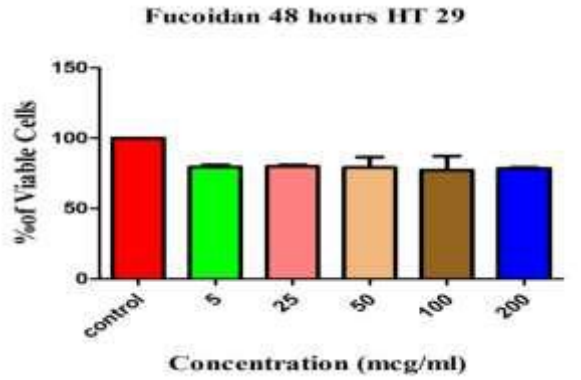
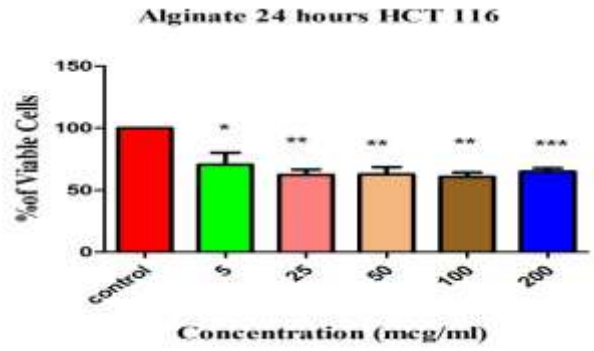


Figure 8: Viability of HT 29 cells treated with different concentrations of fucoidan for a) 24 hours, and b) 48 hours. Control group is untreated cells. The results are expressed as mean+ SEM and analyzed using the t test compared to the control. *P<0.05; **P<0.01; ***P<0.001. Each value represents the average of two independent experiments.

3.7.3 Anti-proliferative effect of sodium alginate on HCT 116 (n=3)

Results showed that at 24 hours, the effect was significantly different compared to control with all the concentrations. The % of cell viability was reduced by 29.57%, 37.87%, 37.27%, 39.37%, and 35.07% at 5 mcg/ml, 25 mcg/ml, 50 mcg/ml, 100 mcg/ml, and 200 mcg/ml respectively (figure 9a). At 48 hours the % of cell viability was significantly (P< 0.05) reduced by 29.28%, 30.27%, 28.5%, 28.84%, and 31.88% at 5 mcg/ml, 25 mcg/ml, 50 mcg/ml, 100 mcg/ml, and 200 mcg/ml respectively (figure 9b). At 72 hours the effect is dose-dependent and significantly different at all concentrations except for 5mcg/ml, the % of cell viability was reduced by 22.67%, 23.63%, 21.72%, and 39.26% at 25 mcg/ml, 50 mcg/ml, 100 mcg/ml, and 200 mcg/ml respectively (figure 9c).

a)



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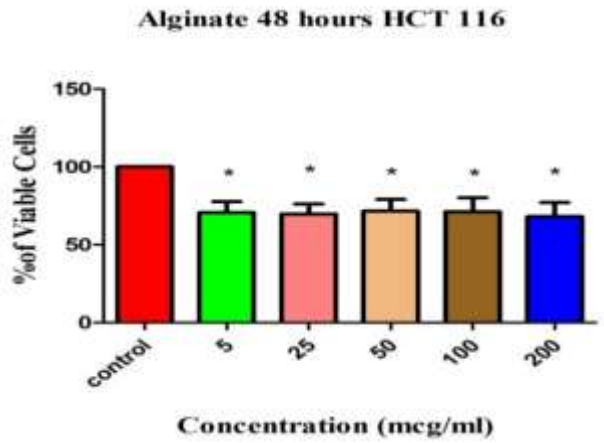

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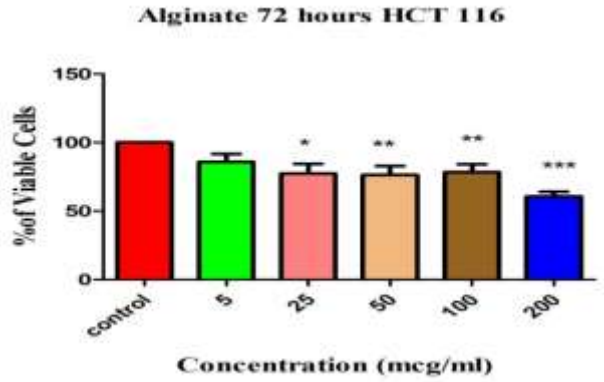
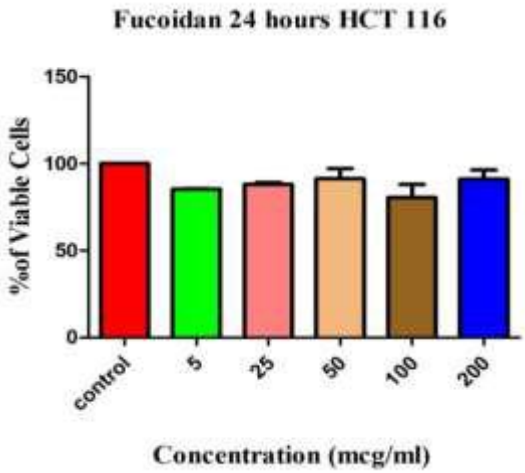


Figure 9: Viability of HCT 116 cells treated with different concentrations of sodium alginate for a) 24 hours, b) 48 hours and c) 72 hours. Control group is untreated cells. The results are expressed as mean+ SEM and analyzed using the t-test compared to the control. *P<0.05; **P<0.01; ***P<0.001. Each value represents-the average of three independent experiments.

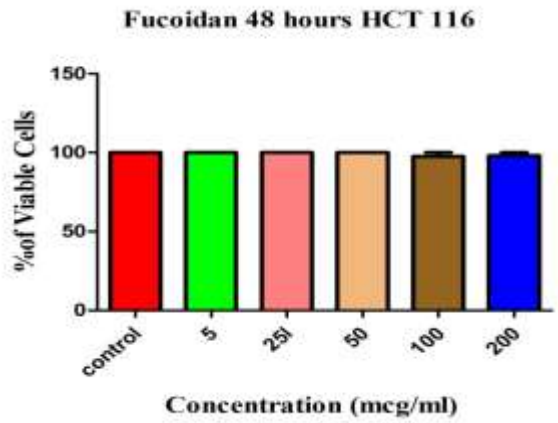
3.7.4 Anti-proliferative effect of fucoidan on HCT 116 (n=2)

With HCT 116, at 24 and 48 hours there is no effect and the % of cell viability was the same as the control (100%) (figure 10a, and b), especially at 48 hours. At 72 hours, results showed that there is a decrease in the % of viability, but this reduction is not significant and there is only a reduction by 17% at 200mcg/ml (figure 10c).

a)



b)



c)

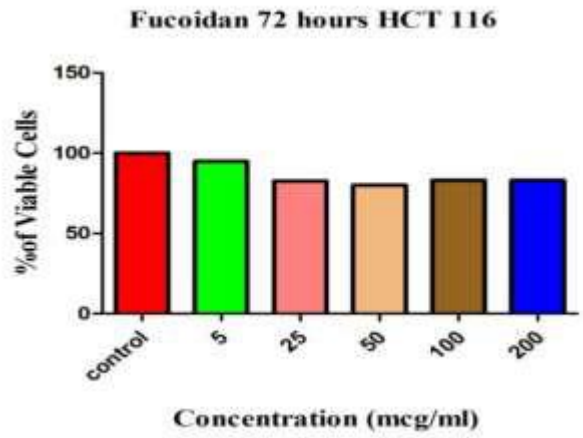


Figure 10: Viability of HCT 116 cells treated with different concentrations of fucoidan for a) 24 hours, b) 48 hours and c) 72 hours. Control group is untreated cells. Each value represents the average of two independent experiments.

IV. DISCUSSION

In the present study the polysaccharides such as fucoidan, and sodium alginate were extracted from brown seaweed *stypopodium schimperi* and characterized through FT-IR and 1H NMR analysis and their antioxidant, anticancer and anti-inflammatory effects were studied.

By using the DPPH radical scavenging assay, our results showed that fucoidan has an antioxidant effect which is dose-dependent, with the maximum effect at 0.5mg/ml (44.13%).The antioxidant properties of fucoidan are affected by their structural characteristics, in fact a study done on fucoidan extracted from the brown seaweed *Laminaria japonica*, showed that the content of sulfate group, the molar ratio of sulfate/fucose and sulfate/total sugar, and the molecular weight played an important role on antioxidant *(Wang et al, 2010)*. This study correlates with our results where the extracted fucoidan contains a sulfate group which increases its effect. Also, another study showed that the antioxidant effect of fucoidan is affected by the method of extraction and the chemical composition (fucose, polyphenol, uronic acid, sulfate…). It was found that the fractions of fucoidan from *Fucus evanescens* obtained by different extraction methods had different amount of polyphenols, and the antioxidant activity of these fucoidan was strongly correlated with polyphenol content which increase its effect compared to pure fucoidan *(Imbs et al, 2015)*. For sodium alginate, the antioxidant effect was not high and it was only 9.8% at 0.5mg/ml. In contrast, a study done by Chmayssem et al showed that purified alginate extracted from *Sargassum vulgare and depolymerized (by hydrolysis of the glycosidic bonds between M and G blocks) into*

polyguluronate has the higher hydroxyl radical scavenging activity (~92 % at 2 mg/mL). This discrepancy between our results and this study can be explained as the follow: the extracted sodium alginate contains both blocks mannuronate (MM), guluronate (GG), in addition to MG block, however in the study of Chmayssem the sodium alginate contains only the GG blocks that has increased the antioxidant effect.

For the anti-proliferative effect, the human colorectal cancer cell lines (HT 29 and HCT 116) were treated with our samples for 24, 48, and 72 hours. HT 29 is more adhesive than HCT 116, and more resistant to chemotherapy. For fucoidan, it was found that the effect was not pronounced for the two cell lines and the decrease in the % viability of HT 29 was approximately the same between the different concentrations, where the highest decrease in the % of viability was at 100 mcg/ml(22.65%).

For HCT 116, there is no significant decrease in the % of viability and there is only a decrease by 17% at 200 mcg/ml. In addition, the effect was seen with HT 29 at 48 hours while with HCT 116 at 72 hours. Our results showed that, the degree of inhibition of the growth was smaller in HCT116 cells than was noted with the HT-29 cells, this is consistent with a study done by Kim et al, where fucoidan has suppressed the growth of HT 29. Several authors investigated the effect of fucoidan from Fucus vesiculosus on colorectal cancer growth. The fucoidan was shown to suppress the growth of human colon carcinoma HCT 15 cells on 62% at concentration 100 mcg/ml (Hyun et al, 2009). Another study done by Han et al showed that Fucoidan treatment significantly inhibited growth, and suppressed cyclin and cyclin-dependent kinase expression in HT29 colon cancer cells. Also, intraperitoneal injection of fucoidan reduced tumor volume; associated with induction of apoptosis and decreased angiogenesis (Han et al, 2015). Because of the heterogeneity in structural characteristics within seaweed, differing extraction conditions used by researchers can give rise to the isolation of distinct fucan forms (Li et al, 2008). This fact can explain difference in doses of fucoidan using for the treatment of the same cell lines. For sodium alginate, it was found that it has a more significant effect than fucoidan. With HT 29, the effect was seen at 48 hours and 72 hours, with the highest decrease in the % of viability at 200 mcg/ml (48.18% and 40.67% respectively). With HCT 116, the effect of sodium alginate was more significant than HT 29 and the effect was seen at the three intervals (24, 48, and 72 hours). A study done by Alessendra et al showed that alginates extracted from Sargassum Vulgare with different viscosities (high and low) inhibited growth of sarcoma 180 while alginate with low viscosity was more active than that with high viscosity. In addition, previous work had discussed the influence of the content of D-mannuronic and L-guluronic acid blocks in alginates antitumor activity. According to these authors, the higher content of MM blocks in alginate correlates with the higher antitumor activity (Fujiihara et al, 1993). In contrast, the extracted alginate is rich in GG blocks this can be one of the causes that the anti-proliferative effect of sodium alginate is not higher enough.

V. CONCLUSION

Brown algae are commercially important and renewable marine resources that have played a critical therapeutic role for a long time. In this context, *stypopodium schimperi*, one of those algae have been studied. In the present study, the yield of fucoidan was 7.4% and that of sodium alginate 21%. M/G ratio of sodium alginate was found to be 0.96 by which it can form strong gels. The functional groups of both polysaccharides were also determined. For the antioxidant effect both polysaccharides have an effect, which give the advantage for being used as natural antioxidants in treating many human diseases. Also, they have anti - proliferative effect against human colorectal cancer cells (HT 29, HCT 116); sodium alginate was found to be more potent than fucoidan at different time intervals (24, 48, and 72 hours) and HT 29 was more resistant to treatment than HCT 116. This study once again allows the development of the exploitation of polysaccharides extracted from *Stypopodium Schimperi* growing on the Lebanese coast in the pharmaceutical and cosmetic fields. At the same time, it confirms that they can be used, after further analyses in the fields of medicine, pharmacy and food industry.

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Corresponding author : Kanaan H
Laboratory of Chemical synthesis and extraction of polysaccharides from seaweed, Faculty of Pharmacy, Lebanese University , Lebanon