Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1099-1111

ISSN: 0975-4873

Research Article

In vitro Antiviral, Cytotoxic, Antioxidant and Hypolipidemic Activites of Polysaccharide Isolated From Marine Algae

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Available Online: 30th September, 2015

ABSTRACT

Cold and hot aqueous extraction method (CEM and HEM) of polysaccharides of *Dictyopetris membraceae*, *Padina pavonia*, *Colpomenia sinusa*, *Enteromorpha intestinalis*, *Corallina officinalis*, *Petrocladia capillraceae* and *Jania rubes* were performed and their physicochemical characterization were studied. The average molecular weight of polysaccharides was (2.15×10^3 to 6.08×10^5 $gmol^{-1}$) and composed of sugars (39.40 - 69.92% w/w), protein (4.0-25.0%), sulfur (0.87-11.59%), and ash (7.91-30.24%). The polysaccharides devoid antiviral effect on HCV, whereas, HEM of *P. capillraceae* inhibited adenovirus type 40 into 46.6%. Both polysaccharide extracts of *J. rubens* and HEM of *C. sinuosa* exhibited most potent cytotocicity on HepG2 with an IC_{50} 10.73, 38.47 and 38.75 µg/ml, respectively. Polysaccharides from *J. rubens*, *C. sinuosa*, *C. officinalis* and *P. pavonia* showed various scavenging abilities against $DPPH^{\bullet}$ (9.10-58.97%) and nitric oxide (28.57-92.86%). While, polysaccharides of *C. sinuosa*, *D. membranacea* and *E. intestinalis* displayed in vitro hypolipidemic effect. Polysaccharides might become important source for drug development for treating hepatic disease.

Keywords: Marine algae; polysaccharide; antiviral; cytotoxic; antioxidant; hypolipidemic activity.

INTRODUCTION

Several algal species belonging to Phaeophyta, Rhodophyta and Chlorophyta, have been recognized as crucial sources of bioactive sulfated polysaccharides (SP)[1]. The structure of algal sulfated polysaccharides varies according to their species of algae[2] and can be classified into three categories. Sulfated polysaccharides of Phaeophyta are found as fucans and fucoidans, which comprise of water soluble polydisperse molecules based on sulfated L-fucose[2-5].

However, sulfated polysaccharides of Rhodophyta are composed mainly from galactans, which have a linear backbone of alternating 3-linked β-D-galactose and 4-linked α-D-galactose residues[6] and various hydroxyl groups may be substituted by a sulfate ester, methyl group, or pyruvic acid[7]. The major structural variation in these polysaccharides is due to the sulfation pattern[8].

The principle of polysaccharides in Chlorophyta are hetero-polysaccharides named Ulvan, are composed of disaccharide repetition moieties made up of sulfated rhamnose linked to either glucuronic acid, iduronic acid, or xylose[9].

Sulfated polysaccharides have a wide range of important biological properties such as antioxidant, antitumor, immunomodulatory, anti-inflammatory, lipid lowering, in addition, nano medicine applications have been discovered[1,10].

These polymers are known to suppress the replication of enveloped viruses including herps simplex virus, human immunodeficiency virus, human cytomegalovirus, dengue virus and respiratory syncytial virus[11-14]. However, the cytotoxic activity of polysaccharide has been previously investigated[15,16]. Moreover, Sulfated polysaccharides had scavenging abilities on superoxide radical, hydroxyl radical and hypochlorous acid[17]. So, the antioxidant activity of these polysaccharides plays an important role in hepatoprotection which reduced hepatic inflammation and fibrosis[18].

Accordingly from the above mentioned valuable bioactivity of polysaccharide, the aim of the present studies was conducted to isolation, characterization and *in vitro* evaluation of water soluble polysaccharides from Egyptian marine algae as antiviral, antioxidant, cytotoxic and hypolipidemic for managing hepatic disease.

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Experimental

Materials

Algal materials

Phaeophyceae; *Dictyopteris membranacea* (Stackh.) Batt. (A02), *Padina pavonia* (A03), *Colopmenia sinuosa* (A04), Chlorophyceae; *Enteromorpha intestinalis* (A05), and Rhodophyceae; *Corallina officinalis* L. (A06), *Pterocladia capillaceae* (SG Gmelin) Bornet (A07) and *Jania rubens* (A08) were collected from along the coast of Abo-Qir, Alexandria and Hurghada, Egypt. The algal were washed thoroughly with tape water, and epiphytes, barnacle, gastropod were removed. The cleaned algae were air dried in the shade at room temperature, milled coarsely powdered and stored in polyethylene plastic bags in a dry place. Herbarium specimens of the alga were identified by Dr. S. A. Shaalan, Professor of Phycology, Faculty of Science, Alexandria University and have been deposited under nº. (A02 - A08, respectively) at Pharmacognosy Department, NRC, Egypt.

Culture cells for in vitro antiviral

Continuous cell lines; Human larynx carcinoma cell line (Hep-2) and Human hepatocyte cell line (Huh 7.5) (obtained from the Lab. of Prof. Dr. Charles Rice, The Rockefeller University, USA) were cultured using specific growth media (10% Foetal calf serum) and will be kept in CO_2 incubator. The cells were prepared in 96-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37°C in a humidified 5% (v/v) CO_2 atmosphere cell monolayer was confluent, the medium was removed from each well.

Culture cells for in vitro cytotoxic activity

Human hepatocarcinoma cell line (HepG2) was obtained from the American Type Culture Collection, University Boulevard, Manassas, USA.

Material for in vitro hypolipidemic activity:

DL-3-Hydroxy-3-methyl-glutaryl coenzyme A sodium salt (HMG-CoA) (Sigma-USA), NADPH- MP (Biomedicals, France), EDTA (El Nasr Pharmaceutical Chemicals Co., Egypt), dithiothreitol (Sigma, USA), bovine serum albumin(Sigma, USA), potassium dihydrogen phosphate (El Nasr Pharmaceutical Chemicals Co., Egypt) and dipotassium hydrogen phosphate (Fluka, Germany).

Methods

Estimation of total polysaccharide of dried powdered of algae

Quantitative estimation of total polysaccharide content in each dried algal sample was determined with the phenol-sulfuric method[19].

Extraction and purification of water soluble polysaccharide:

Extraction and purification of the polysaccharides were carried out according to the method of Luo *et al.*[20]. The dried powdered algal samples extracted with petroleum ether for two hours, and further extracted with 80% ethanol at 90°C for 2 h in order to remove phenolic compounds, monosaccharides, amino acids and other related molecules. After filtering, the residue was dried on oven at 40°C. Further the dried residue was soaked in 30 volume (w/v) of distilled water and kept overnight at 4 to 5°C, stirred well and allowed to return to room temperature. The slurry was first filtered through muslin cloth and then with Whatman no.1 filter paper (particle retention 11µm). The process was repeated till complete exhaustion (-ve molish test). Extract was concentrated to 1/4 of its volume under reduced pressure using a rotary evaporator at 45°C. The polysaccharide was precipitated by adding 4-fold volume of 95% (v/v) ethanol (Doummar & Sons Co., Syria). The algal residue was soaked in distilled water and incubated at 80°C for 3h and hot water extract was obtained following the same procedure used for the cold water extract. The precipitate was collected by centrifugation at 3,000 rpm for 10 min, washed successively with acetone and ethanol, and the procedure of precipitation was repeated, and then dissolved in water and dialyzed against deionized water for 72 h, then dried by freeze dryer (Virtis, Gardiner, USA), to yield the crude polysaccharide cold (CPE) and hot (HPE) extracts then kept in refrigerator for chemical and biological evaluation. The polysaccharide was tested for the phenolic compounds content using the ferric chloride color method.

Physico-Chemical Characterization

Chemical analysis:

Moisture content was determined after heating 0.5 g at 105°C for 24 h. Ash content was quantified gravimetrically after heating for 12 h at 550°C and further 4 h at 900°C. Nitrogen, carbon, hydrogen and sulfate content were determined by Elemental Microanalysis (Elementary Vario EL). The total sugar content was determined by the phenol-sulfuric method[19] using glucose as standard. Protein content was calculated from %N using the correction factor of 6.25, as mentioned by Maciel et al.[21]. The degree of substitution (DS), which is the average number of sulfate groups on each sugar residue, was calculated from the sulfur content using the following formula[22]:

$$DS = 2.25 \times \frac{S\%}{C\%} \qquad (1)$$

The Fourier transform IR spectra (FT-IR) were recorded with a FT/IR-6100 (JASCO, Japan) between 400 - 4000 cm^{-1}. The samples were analyzed as KBr pellets.

Molar mass distribution:

The peak molar masses (Mpk) were estimated by gel permeation chromatography (GPC) with Agilent 1100 series (Germany) according technique described in Matloub et al[23] using ASTRA 1.4 software (Wyatt, USA).

X-ray diffraction investigations:

X-ray diffraction (XRD) patterns were measured using A Philips PW 1390 X-ray diffractometer (USA), adopting Ni-filtered Cu radiation with tube voltage of 40 kV and a current of 25 mA. The X-ray diffraction patterns were recorded in a 2θ and over a Bragg angle range $4° < 2\theta < 70°$.

Biological Activity

Antiviral Activity

Determination of the non toxic dose on Hep-2 and Huh 7.5 human cell lines

Each polysaccharide extracts (0.1 g) was dissolved in 500 - 1000 µl water. 12µl of 100x of antibiotic-antimycotic mixture (10,000U penicillin G sodium, 10,000 µg streptomycin sulfate and 250 µg amphotericin B) was

Table 1: Quantitative estimation of total carbohydrate, soluble sugars and total polysaccharides contents of dried powder tested algae and yields% of their water soluble polysaccharides

Algal sample	*Dictyopteris membranacea*	*Padina pavonia*	*Colpomenia sinuosa*	*Enteromorpha intestinalis*	*Corallina officinalis*	*Pterocladia capillaceae*	*Jania rubens*
Total Carbohydrate[a]	9.41 ± 0.06	10.84± 0.05	13.11 ± 0.07	20.34 ± 0.10	6.29 ± 0.02	40.29 ± 0.04	12.21± 0.09
Free Sugars[b]	0.06 ± 0.01	2.48± 0.03	1.37 ± 0.03	0.94 ± 0.09	2.31 ± 0.05	1.90 ± 0.02	2.04 ± 0.07
Total polysaccharide[c]	9.41	8.36	11.74	19.40	3.98	38.39	10.17
Yield of cold water extract[d]	3.67	5.69	7.80	4.93	2.38	6.15	2.63
Yield of hot water extract[d]	1.17	1.20	2.64	2.22	0.98	2.95	1.07
Crude Water Soluble Polysaccharide[e]	51.43	82.42	88.93	36.86	84.42	23.70	46.21

[a] Each value represents the mean of total carbohydrate calculated as glucose of three replicates ±SEM (Standard Error of Mean) expressed as w/w% of dried powdered of algae.
[b] Each value represents the mean of free sugars calculated as glucose of three replicates ±SEM (Standard Error of Mean) expressed as w/w% of dried powdered of algae.
[c] Each value represents the difference between total carbohydrate and free sugars expressed as w/w% of dried powdered of algae.
[d] Each value expressed as w/w% of dried powdered of algae.
[e] Each value expressed as w/w% the total polysaccharide

added. Ten fold dilutions of decontaminated samples were inoculated in Hep-2 and Huh 7.5 human cell lines to estimate the non toxic dose of the algal polysaccharide extracts. Cytotoxicity assay was carried out using cell morphology evaluation by inverted light microscopy[24] and cell viability test trypan blue dye exclusion method[25].

Antiviral assay on infectious Adenovirus Type 40 using cell culture-PCR (CC-PCR) technique

The antiviral activity was accomplished according to Esawy et al. & Abdo et al.[26-27].

Determination of antiviral effect on Hepatitis C virus genotype 4a replicon (ED-43/SG-Feo (VYG) replicon)

ED-43/SG-Feo (VYG) replicon of HCV genotype 4a (obtained from the Lab. of Prof. Dr. Charles Rice, The Rockefeller University, USA) was treated with the non-toxic doses of tested samples. HCV RNA was quantified as initial titers and after treatment with the non toxic doses of the tested samples according to Saeed et al.[28].

Cytotoxic activity on hepatocarcinoma human cell line

Cytotoxic effect was accomplished on HepG2 human cell line. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan[30].

Data were subjected to paired-samples SPSS Statistical Software Package (version 8.0). P<0.005 was regarded as significant. Also, a probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

Antioxidant activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity

The DPPH free-radical scavenging activity was assessed according to McCue et al. [30]. Serial concentrations of the isolated polysaccharides (10, 50, 100, 500 & 1000 µg/ml) were evaluated and ascorbic acid was used as a reference drug.

The scavenging ability of DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{A0-A1}{A0} \times 100 \qquad (2)$$

where A_0 is the absorbance of DPPH• solution (without the tested polysaccharides) and A_1 is the absorbance of the tested polysaccharides with DPPH• solution.

Nitric oxide radical-scavenging assay

The nitric oxide radical-scavenging activity was assessed according to Menaga et al[31]. Serial concentrations of the isolated polysaccharides (10, 50, 100, 500 & 1000 µg/ml) were evaluated and Na_2NO_2 was used as a reference drug.

The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{A0-A1}{A0} \times 100 \qquad (3)$$

where A_0 was the absorbance of the control (blank, without polysaccharide extract) and A_1 was the absorbance of the tested polysaccharides.

The experiment was repeated in triplicate. Statistical analysis was tested between samples and control using paired t-test, where $P \leq 0.05$ is considered significance (SPSS version 8 computer programs).

Hypolipidemic activity

The hypolipidemic activity of polysaccharide extracts were evaluated by the colorimetric method[32] using fluvastatin as reference drug. The β-hydroxy- β- methyl glutaryl co A reductase **(**HMG-CoA reductase) inhibitory activity was estimated by measuring the rate of decrease in the absorbance at 340 nm due to the oxidation of NADPH[33].

The HMG-CoA reductase inhibitory activity was calculated according to the following equation:

Table 2: Chemical characterization of the isolated polysaccharides from tested algae

Algal Sample	Extract	% of the isolated polysaccharides								
		Total Carbo-hydrate	Moisture	Ash	C	H	S	N	Protein	Degree of Sulfation
Dictyopteris membranacea	Cold	59.57 ±0.2	7.90±0.2	9.60±0.4	25.90±0.7	5.60±0.5	4.30±0.2	1.70±0.6	10.63	0.373
	Hot	63.25 ±0.2	7.29±0.2	20.64±0.6	27.50±0.5	6.40±0.2	2.80±0.2	1.50±0.3	9.38	0.229
Padina pavonia	Cold	49.51 ±0.6	18.81±0.5	22.25±0.8	14.77±0.4	2.23±0.6	11.5±0.9	0.64±0.7	4.00	1.751
	Hot	67.29 ±0.3	14.66±0.4	15.33±0.2	23.66±0.2	5.09±0.3	2.3±0.2	2.19±0.4	13.69	0.220
Colpomenia sinuosa	Cold	51.40 ±0.3	17.07±0.2	16.75±0.4	13.65±0.3	2.55±0.3	7.55±0.6	1.40±0.4	8.75	1.240
	Hot	60.30 ±0.1	11.46±0.2	16.51±0.3	26.22±0.2	4.63±0.5	0.87±0.3	2.44±0.2	15.25	0.070
Enteromorpha intestinalis	Cold	46.77 ±0.5	4.88±0.1	18.64±0.1	15.50±0.4	2.40±0.6	10.80 ±0.7	-	-	1.567
	Hot	55.2 ±0.4	3.75±0.1	29.38+0.3	24.00±0.5	3.60±0.8	3.30±0.3	2.40±0.6	15.00	0.309
Corallina officinalis	Cold	47.84 ±0.3	6.09±0.1	30.24±0.2	20.80±0.4	4.20±0.3	2.80±0.3	4.00±0.4	25.00	0.302
	Hot	56.58 ±0.2	13.00±0.3	7.91±0.1	24.60±0.4	3.40±0.4	2.10±0.6	2.40±0.4	15.00	0.192
Pterocladia capillaceae	Cold	44.85 ±0.5	13.97±0.1	19.73±0.2	19.50±0.6	7.40±0.4	7.50±0.2	2.10±0.5	13.13	1.865
	Hot	69.92 ±0.5	6.14±0.6	16.14±0.1	30.40±0.7	4.20±0.6	2.30±0.7	2.30±0.5	14.38	0.170
Jania rubens	Cold	39.40 ±0.8	10.85±0.3	28.31±0.3	12.94±0.2	2.68±0.8	8.88±0.3	1.93±0.2	12.06	1.540
	Hot	50.81 ±0.5	6.92±0.38	22.43±0.4	22.09±0.4	4.35±0.3	1.89±0.5	2.44±0.3	15.25	0.19

Note: Each value represents the mean of three replicates ±SEM (Standard Error of Mean) expressed as w/w% of isolated polysaccharides.

Enzyme activity µmol/mg protein =

$$\frac{\Delta A}{E} \times \frac{1}{mg\ of\ isolated\ polysscharide} \qquad (4)$$

ΔA = the difference between consecutive absorbance.

E = Extinction coefficient of NADPH at 340 nm (6.22×10^{-3} μM^{-1} cm^{-1}).

RESULTS AND DISCUSSION

The total carbohydrate, total polysaccharide and soluble sugar contents of tested algae as well as their cold and hot water soluble polysaccharides yields % are given in Table (1). The higher total carbohydrate content was found in dried powdered of *Pterocladia capillaceae* and *Enteromorpha intestinalis* (40.29 and 20.34% w/w of dried powder, respectively).

The air dried powdered of algae were subjected to many purification procedures before isolation of crude polysaccharides to remove fat-soluble molecules, monosaccharides and phenolic compounds. The polysaccharide precipitate was washed successively with acetone and ethanol, and then the residue was dissolved in water and dialyzed against deionized water for 72 h, followed by freeze-drying to yield the polysaccharide. Further, the detection of phenolic compounds by the ferric chloride color method revealed that the isolated polysaccharide did not contain any phenolic compounds.

In the present study, the polysaccharide represents the main content of algal carbohydrate (Table 1). Our observation revealed that the yield of polysaccharides obtained from the cold water extract is higher and ranged from 3.67 to 7.80% w/w of dried powder, than that of the hot water extract (0.98 – 2.95% w/w). Contradictory of Abou Zeid et al and Siddhanta et al[16,34] were reported that the yield of hot water polysaccharide is more than that of cold water polysaccharide. This may be due to complete extract of cold water polysaccharide till exhaustion which may be partially extracted in other studies. On the other hand, the water soluble polysaccharides of *Colpomenia sinuosa* and *Corallina officinalis* (88.93 & 84.42%, respectively) represent the predominant content of total polysaccharide.

From Table (2), the total sugar contents of cold polysaccharide extract were ranged from 39.40 to 59.57% and the highest content was found in cold *Dictyopteris membranacea* extract, while cold *Jania rubens* extract possesses the lowest content.

The total carbohydrate contents of hot polysaccharides extracts were 50.81- 69.92% where, *Pterocladia*

Table 3: FT-IR analysis of the polysaccharide extracts isolated from tested algae

Algae	Extracts	Assignment wave number (cm^{-1})									
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Dictyopteris membranacea	Cold	3421.10	2925.51	1639.2	-	1422.24	1252.54	1092.48	836.95	754.03	652.78
	Hot	3440.39	2936.16	1641.13	1510.95	-	1251.58	1043.3	836.95	-	574.68
Padina pavonia	Cold	3393.14	2979.48	1639.20	1536.02	1449.24	-	1142.62 1098.26	870.70	754.03	652.78
	Hot	3425.92	2932.23	1635.34	1518.67	1426.10	1254.47	1042.34	813.81	-	619.03
Colpomenia sinuosa	Cold	3370.00	2973.70	1645.95	1536.02	1423.21	1322.93	1132.01 1097.30	-	754.99	650.85
	Hot	3410.49	2937.06	1647.88	1515.78	1426.10	1255.43 1323.89	1040.41	859.13	-	584.32
Enteromorpha intestinalis	Cold	3403.74	2928.38	1646.91 1635.34	1569.77 1552.42 1519.63	1448.28	1253.50	1145.51 1098.26	870.70	755.95	-
	Hot	3426.89	2933.2	1640.16	1519.63	1438.64	1253.50	1056.8	858.16	780.06	-
Corallina officinalis	Cold	3425.92	2962.13	1653.66	1519.63	1425.14	1251.58	1082.83	874.56	-	-
	Hot	3433.64	2962.13	1648.84	-	1459.85	1256.40	1076.08	873.59	-	-
Pterocladia capillaceae	Cold	3387.35	2939.95	1646.9	1519.63	1423.21	1256.40 1323.89	1198.58 1137.8 1091.51	825.38	753.06	625.78 599.75
	Hot	3430.74	2925.48	1628.59	-	1428.99	1256.40	1081.87	876.48	-	-
Jania rubens	Cold	3366.14	2965.02	1647.88	-	1428.03	-	1094.40	873.59	755.95	-
	Hot	3403.74	2935.13	1649.80	1519.63	1434.78	-	1040.41	837.59	751.13	-

(1)The assignment for stretch vibration of O-H (hydroxyl groups). (2)The assignment for stretch vibration of C-H. (3)The assignment for asymmetric stretch vibration of $–COO^-$ (uronic acids). (4)The assignment for Amide II (N-H bending of amino acid group). (5)The assignment for symmetric stretch vibration of $–COO^-$ (uronic acids). (6)The assignment for stretching vibration of S=O (esterified sulfate). (7)The assignment for C-O-C bending mode in glycosidic linkages. (8)The assignment for bending vibration of C-O-S of the sulfate in axial position. (9)The assignment for bending vibration of C-O-S of the sulfate in equatorial position. (10)The assignment for asymmetric deformation of O-S-O groups.

capillaceae stood the highest whilst the lowest content was found in *Jania rubens* extract. Ash content of cold water polysaccharides of *Corallina officinalis* (30.24%) & *Jania rubens* (28.31%) as well as hot water polysaccharide of *Enteromorpha intestinalis* (29.38%) was high in comparison to the others.

The nitrogen content of isolated polysaccharides were 0.64 - 4.0% corresponding to 4.0 - 25% of protein. Being higher in the polysaccharide extracts of *Corallina officinalis* (25% protein in CEM), *Jania rubens* (15.25% protein in HEM) and *Colpomenia sinuosa* (15.25% protein in HEM) than other polysaccharide extracts. The higher protein

Table 4: The gel permeation chromatography of polysaccharide extracts isolated from tested algae

Algal	polysaccharide extract	Peaks	Integration (min)	Mn	Mw	Mz	Mp	D	A (ml*V)
				g mol^{-1}					
Dictyopteris membranacea	Cold	peak 1	5.96-8.40	4.11×10^4	6.38×10^4	1.00×10^5	3.14×10^4	1.55	3.00×10^4
		peak 2	8.50-10.53	2.38×10^3	3.37×10^3	4.39×10^3	3.09×10^3	1.41	1.13×10^4
	Hot	peak 1	6.28-8.03	5.80×10^4	7.00×10^4	8.30×10^4	6.51×10^4	1.21	5.64×10^4
Padina pavonia	Cold	peak 1	6.86-8.79	1.48×10^4	1.90×10^4	2.49×10^4	1.62×10^4	1.28	2.93×10^4
		peak 2	8.81-10.31	2.27×10^3	2.79×10^3	3.34×10^3	2.72×10^3	1.23	2.58×10^4
	Hot	peak 1	5.50-8.19	7.67×10^4	1.53×10^5	2.61×10^5	6.05×10^4	1.99	6.83×10^3
		peak 2	8.47-10.47	2.53×10^3	3.55×10^3	4.51×10^3	3.47×10^3	1.41	4.48×10^3
Colpomenia sinuosa	Cold	peak 1	6.92- 8.63	2.11×10^4	2.65×10^4	3.31×10^4	2.12×10^4	1.25	1.93×10^4
		peak 2	8.68-10.39	2.17×10^3	2.79×10^3	3.49×10^3	2.84×10^3	1.29	1.79×10^4
	Hot	peak 1	6.86-9.17	4.59×10^3	1.25×10^4	2.15×10^4	1.78×10^4	2.71	3.75×10^3
Enteromorpha intestinalis	Cold	peak 1	8.93-10.33	1.75×10^3	2.15×10^3	2.61×10^3	2.04×10^3	1.23	2.86×10^4
	Hot	peak 1	6.55-7.49	4.69×10^4	5.19×10^4	5.64×10^4	5.77×10^4	1.10	1.38×10^3
Corallina officinalis	Cold	peak 1	8.54-10.34	2.33×10^3	3.02×10^3	3.78×10^3	2.81×10^3	1.29	1.36×10^4
	Hot	peak 1	4.31-7.79	1.43×10^5	6.08×10^5	2.19×10^5	2.08×10^5	4.25	2.29×10^3
Ptercladia capilleace	Cold	peak 1	6.83-8.89	1.45×10^4	2.10×10^4	3.12×10^4	1.39×10^4	1.45	5.29×10^4
		peak 2	8.89-10.35	1.99×10^3	2.56×10^3	3.13×10^3	2.64×10^3	1.28	3.24×10^4
	Hot	peak 1	6.92-8.39	8.78×10^4	5.16×10^5	1.71×10^6	3.91×10^5	5.88	4.64×10^4
Jania rubens	Cold	peak 1	6.90 - 8.54	2.50×10^4	2.88×10^4	3.32×10^4	2.67×10^4	1.15	8.92×10^3
		peak 2	8.56-10.29	2.28×10^3	2.90×10^3	3.61×10^3	3.26×10^3	1.27	9.94×10^3
	Hot	peak 1	4.34-8.46	7.09×10^4	3.18×10^5	1.65×10^6	1.19×10^5	4.48	1.33×10^4
		peak 2	5.16- 8.49	6.58×10^4	1.62×10^5	3.80×10^5	1.19×10^5	2.47	1.23×10^4
		peak 3	8.49-10.42	2.21×10^3	3.05×10^3	3.94×10^3	2.72×10^3	1.37	5.57×10^3

Mn: The number-average molecular weights, Mw: The weight-average molecular weights, D: Polydisperity of a polymer-mixture [ratio Mw/Mn], Mp: The molecular weight of the standard at the peak maximum, Mz: average molecular weight and A: Area under peak.

Mn: The number-average molecular weights, Mw: The weight-average molecular weights, D: Polydisperity of a polymer-mixture [ratio Mw/Mn], Mp: The molecular weight of the standard at the peak maximum, Mz: average molecular weight and A: Area under peak.

content bound to polysaccharide were also found in *Ulva fasiata* (36.25%)[23,34].

The S% and C% found were 0.87-11.59%, and 12.94-30.40%, respectively. The degree of substitution for sulfate (DS sulfate) was found to be 0.07-1.751 calculated based on Liu et al[22], according to Eq. (1).

The FT-IR spectrum data (Table 3) of cold and hot polysaccharide extracts gives a complete "molecular fingerprint" of the studied samples. The spectra scanned between wavenumber from 4000 to 400 cm^{-1} showed major absorption bands around 3366.14 - 3440.39 cm^{-1} represents O-H stretching of hydroxyls which overlaps in part with the CH stretching peak of CH_2 groups appearing at around 2925.48-2979.48 cm^{-1}.

The IR spectra between 1800 and 500 cm^{-1} revealed, most pronounced functional groups for tested polysaccharides, bands around 1628.59-1653.66 cm^{-1} indicated uronic acid (COO^- stretching), 1510.95-1569.77 cm^{-1} assigned to the amide-II band (N-H bending of amino acid group) and this result confirmed the fact that polysaccharide extracts contain protein[35,36] and absorption band at 1422.24 - 1459.85 cm^{-1} due to the symmetric stretch vibration of COO^- and the stretch vibration of C-O within COOH. Moreover, the IR spectra of isolated polysaccharides showed an absorption band at 1251.58 - 1256.40 cm^{-1} and 1322.93 - 1323.89 cm^{-1} were assigned as S=O stretching vibration indicating the presence of esterified sulfate. Whereas, bands around 825.38 - 876.48 cm^{-1} could be assigned to sulfate groups in the axial of either C-6, C-4 and C-2 positions[37,38] whereas 813.81 cm^{-1} assigned to equatorial primary sulfate group. Also, the signals 751.13 - 780.06 cm^{-1} might correspond to the bending vibration of C-O-S of sulfate in equatorial position. Further, 574.68 - 652.78 cm^{-1} attributed to the asymmetric deformation of O-S-O groups[39]. While, the vibration of the C-O-C bridge of glucosides were recorded at wavenumbers 1040.41-1098.26 cm^{-1} and 1137.8 - 1146.47 cm^{-1}.

The gel permeation chromatogram (GPC) of isolated polysaccharides revealed one to three major peaks, and their characterizations were summarized in Table (4).

The average molecular weights of cold polysaccharide extracts were 2.15×10^3- 6.38 $\times 10^4$ g mol^{-1} while the average molecular weights of hot polysaccharide extracts were 3.05×10^3- 6.08×10^5 g mol^{-1} by GPC. High molecular mass polysaccharides have been described for other sulfated polysaccharides from seaweeds with values larger than 100 kDa[40].

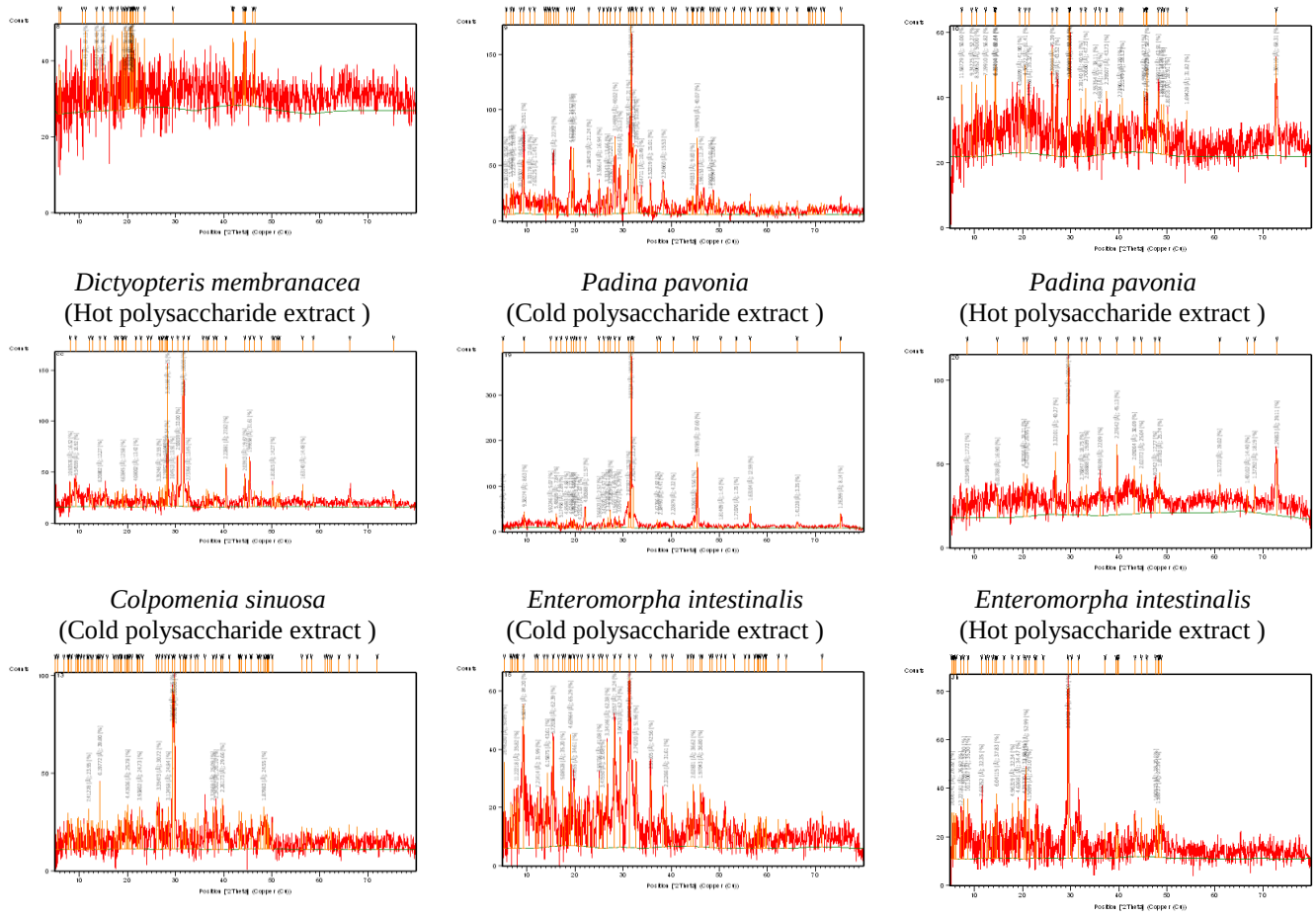
This amalgamation indicates that the isolated polysaccharides, behave as a heterogeneous system similar to other natural polysaccharides, containing sulfate ester and associated with protein.

Table 5: Antiviral activity of the isolated water soluble polysaccharides against Adenovirus type 40 and Hepatitis C virus at Non-toxic Concentration

Algae	Extract	Adenovirus 40					Hepatitis C virus				
		Non toxic concentrations mg/ml	Initial infectious titre	Final infectious titre	% of reduction	Average	Non toxic concentrations mg/ml	Initial infectious titre	Final infectious titre	% of reduction	Average
Dictyopteris membranacea	Cold	2	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	1	$1X10^5$ $1X10^5$ $1X10^5$	$9X10^4$ $9X10^4$ $9X10^4$	10% 10% 10%	10%	1	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Padina pavonia	Cold	2	$1X10^5$ $1X10^5$ $1X10^5$	$9X10^4$ $9X10^4$ $9X10^4$	10% 10% 10%	10%	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	1	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $9X10^4$	0% 0% 10%	3.3%	1	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Colpomenia sinusa	Cold	n.t.	n.t.	n.t.	n.t.	n.t.	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	n.t.	n.t.	n.t.	n.t.	n.t.	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Ulva fasciata	Cold	0.2	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	0.2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	0.5	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	0.5	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Enteromorpha instinalis	Cold	0.2	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	0.2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	0.2	$1X10^5$ $1X10^5$ $1X10^5$	$9X10^4$ $9X10^4$ $1X10^5$	10% 10% 0%	6.6%	0.2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Carolina officinalis	Cold	0.02	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	0.02	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	2	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $9X10^4$ $1X10^5$	0% 10% 0%	3.3%	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Pterocladia capillaceae	Cold	1	$1X10^5$ $1X10^5$ $1X10^5$	$1X10^5$ $1X10^5$ $1X10^5$	0% 0% 0%	0%	1	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	2	$1X10^5$ $1X10^5$ $1X10^5$	$5X10^4$ $6X10^4$ $5X10^4$	50% 40% 50%	46.6%	2	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
Jania rubens	Cold	n.t.	n.t.	n.t.	n.t.	n.t.	1	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%
	Hot	n.t.	n.t.	n.t.	n.t.	n.t.	1	$1X10^3$ $1X10^3$ $1X10^3$	$1X10^3$ $1X10^3$ $1X10^3$	0% 0% 0%	0%

n.t.= not tested

The X-ray diffraction (XRD) analysis was applied to determine the crystallinity degree of the isolated polysaccharide. The XRD patterns of HEM of *D. membranacea*, *P. pavonia*, and CEM of *P. capillaceae*, presented in Fig. (1), are typical for crystalline polymer and show major crystalline reflections at (11.28°, 13.62°, 14.87°, 18.82°, 19.57°, 19.94°, 20.56°, 21.11°), (14.22°, 14.37°, 26.21°, 29.68°, 29.84°, 45.85°) and (9.30°, 15.49°,



Dictyopteris membranacea (Hot polysaccharide extract)
Padina pavonia (Cold polysaccharide extract)
Padina pavonia (Hot polysaccharide extract)
Colpomenia sinuosa (Cold polysaccharide extract)
Enteromorpha intestinalis (Cold polysaccharide extract)
Enteromorpha intestinalis (Hot polysaccharide extract)
Corallina officinalis (Cold polysaccharide extract)
Ptercladia capilleace (Cold polysaccharide extract)
Jania rubens (Hot polysaccharide extract)

Figure 1: X-ray diffraction of some isolated polysaccharides

19.12°, 26.67°, 28.19°, 29.35°, 31.28°, 32.65°), respectively. Further, according to XRD pattern of both polysaccharide extracts of *E. intestinalis*, (CEM) of *P. pavonia, C. sinuosa, C. officinalis* and (HEM) *J. rubens*, revealed that the structure are poor crystalline.

Algal polysaccharides have been a surge in interest to tap these unexploited inventions to develop novel therapeutics for treating hepatic disease. So, they were subjected to primary evaluation as anti-HCV, anti-Adenovirus type 40, anti-hepatocarcinoma, antioxidant, hypolipidemic *in vitro*. For antiviral evaluation, the nontoxic dose of the isolated polysaccharides was determined on Hep-2 and Huh 7.5 cell lines and accomplished in Table (5). The results of cytotoxicity assay against both cell lines showed the same toxicity for each isolated polysaccharide and their non toxic concentration were ranged from 0.02 to 2 mg/ml. The antiviral activity of algal water soluble polysaccharide against adenovirus type 40 and hepatitis C virus is compiled in Table (5). The hot polysaccharide extract of *Pterocladia capillaceae* showed a considerable antiviral activity; inhibit adenoviral replication about 46.6% in comparison with other species. At the same time the other compounds showed no or weak reduction of initial adenovirus titre ranged from 0 to 10%. However, the tested polysaccharides didn't exhibit antiviral activity on hepatitis C virus. On the other hand, there is no comparison with antiviral reference drug as there is no antiviral drug against enteric viruses worldwide. So, it gives a great importance to the studies of natural products as antiviral candidate compounds.

The variation in antiviral activity of the sulfated polysaccharides depending on the viral serotype and the host cell[41]. Many viruses display affinity for cell surface heparin sulfate proteoglycans with biological relevance to virus entry. This raises the possibility of application of sulfated polysaccharides in antiviral therapy[42]. The virus-cell complex is formed by ionic interaction between the anionic (mainly sulphate) groups in the polysaccharide and the basic amino acids of the glycoprotein, and non-ionic ones depending on hydrophobic amino acids interspersed between the basic ones in the glycoprotein- binding zone. In addition, the virucidal activity of sulfated polysaccharides due to formation of a stable virion-sulfated polysaccharide complex where binding is not reversible[43], hence the sites on the viral envelope required for virus attachment to host cells are occupied[13]. Furthermore, degree of sulfation of polymer has a major impact on the antiviral activity i.e. sulfate content higher than 20 (mol %) have a clear tendency to show an antiviral activity, but in our study, the isolated polysaccharides has low sulfation degree. Moreover, high-molecular-weight polysaccharides appear to possess the most pronounced inhibitory activity toward viral receptor binding and entry[44].

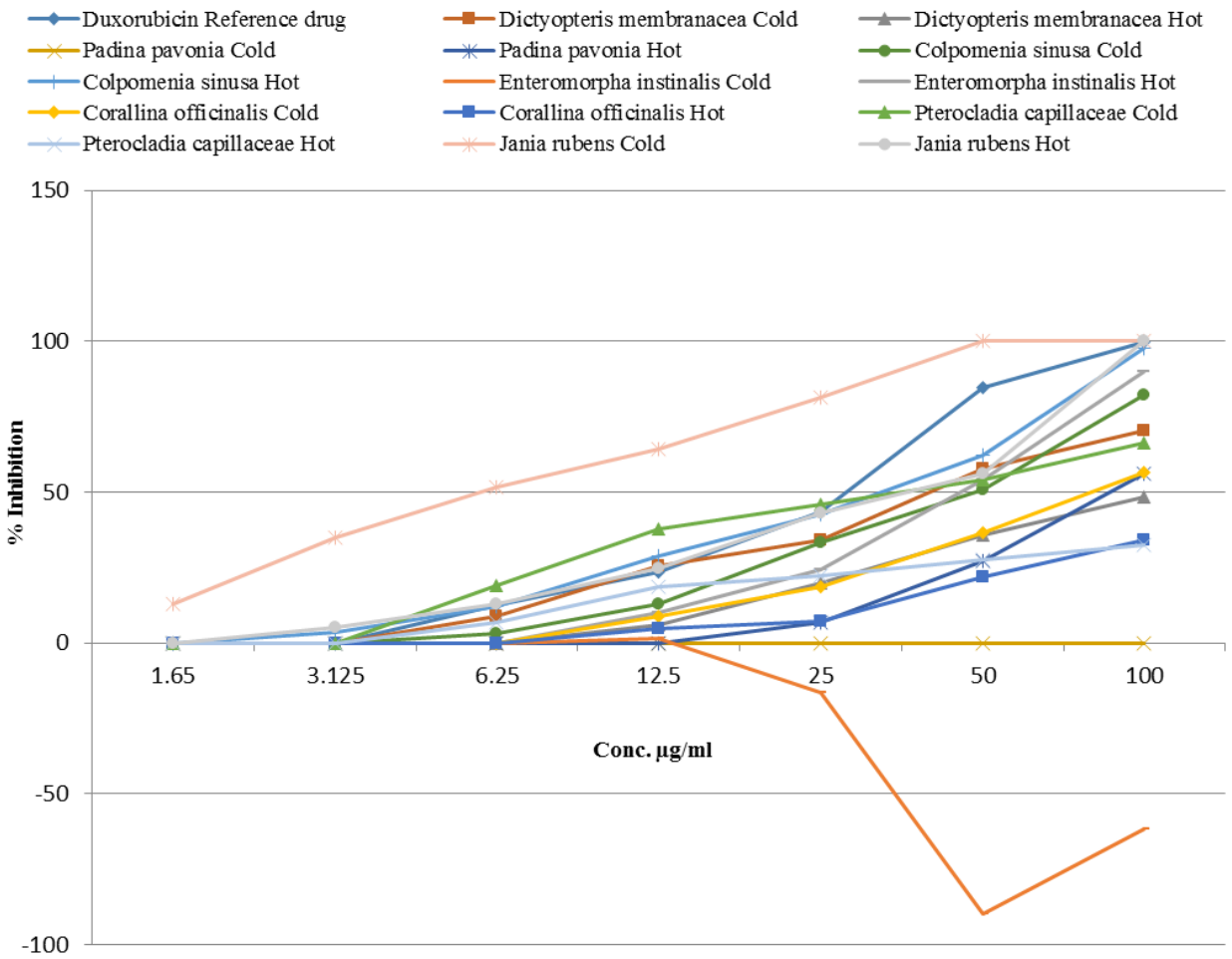


Figure 2. Cytotoxic activity of polysaccharide extracts of the tested algae on HepG2 Cell Line in Vitro

For cytotoxic study, the cold and hot polysaccharide extracts isolated from tested algae were evaluated *in vitro* for cytotoxic activity on HepG2 cultured. The percentages of growth inhibition are shown in Fig. 2 and IC_{50} and IC_{90} are summarized in Table 6.

The cold and hot polysaccharide extracts of *Jania rubens* and *Colpomenia sinusa* (IC_{50}: 10.73, 38.47, 57.39 and 38.75 µg/ml, respectively) as well as the hot polysaccharide extract of *Enteromorpha intestinalis* and cold polysaccharide extract of *Dictyopteris membranacea* (IC_{50}: 53.64 & 58.90 µg/ml, respectively) have promising cytotoxic activity on HepG2 *in vitro* compared with doxorubicin as a reference drug. Also, the cold polysaccharide extract of *Corallina officinalis*, *Pterocladia capillaceae* and hot polysaccharide extract of *Padina pavonia* showed lower cytotoxic activity on HepG2 with ID_{50} of 82.72, 56.54 and 87.75 µg/ml, respectively.

However, the cold polysaccharide extract of *Padina Pavonia* didn't exhibit cytotoxic activity on HepG2 *in vitro.* Indeed, the hot polysaccharide extracts of both brown and red algae have good cytotoxic activity on HepG2 with lowest inhibition than that of cold extract.

The water polysaccharide extracts mainly consisted of sulfated polysaccharide - protein complex. The polysaccharide - protein complex was isolated previously from mashrooms which have immunomodulation and anticancer effects[45].

Although the isolation process, structural characterization and antitumor activity of polysaccharides have been extensively investigated, the relationship between the

Table 6: LC_{50} and LC_{90} of the tested algal polysaccharide extracts on HepG2 in Vitro

Sample	Extract	Conc. µg/ml	
		LC50	LC90
Duxorubicin	Reference drug	29.58	53.16
Dictyopteris membranacea	Cold	58.9	123.79
	Hot	98.72	168.82
Padina pavonia	Cold	-	-
	Hot	87.75	134.68
Colpomenia sinusa	Cold	57.39	100.15
	Hot	38.75	71.42
Enteromorpha intestinalis	Cold	-	-
	Hot	53.64	89.51
Corallina officinalis	Cold	82.72	144.95
	Hot	-	-
Pterocladia capillaceae	Cold	56.54	142.73
	Hot	-	-
Jania rubens	Cold	10.73	25.83
	Hot	38.47	69.86

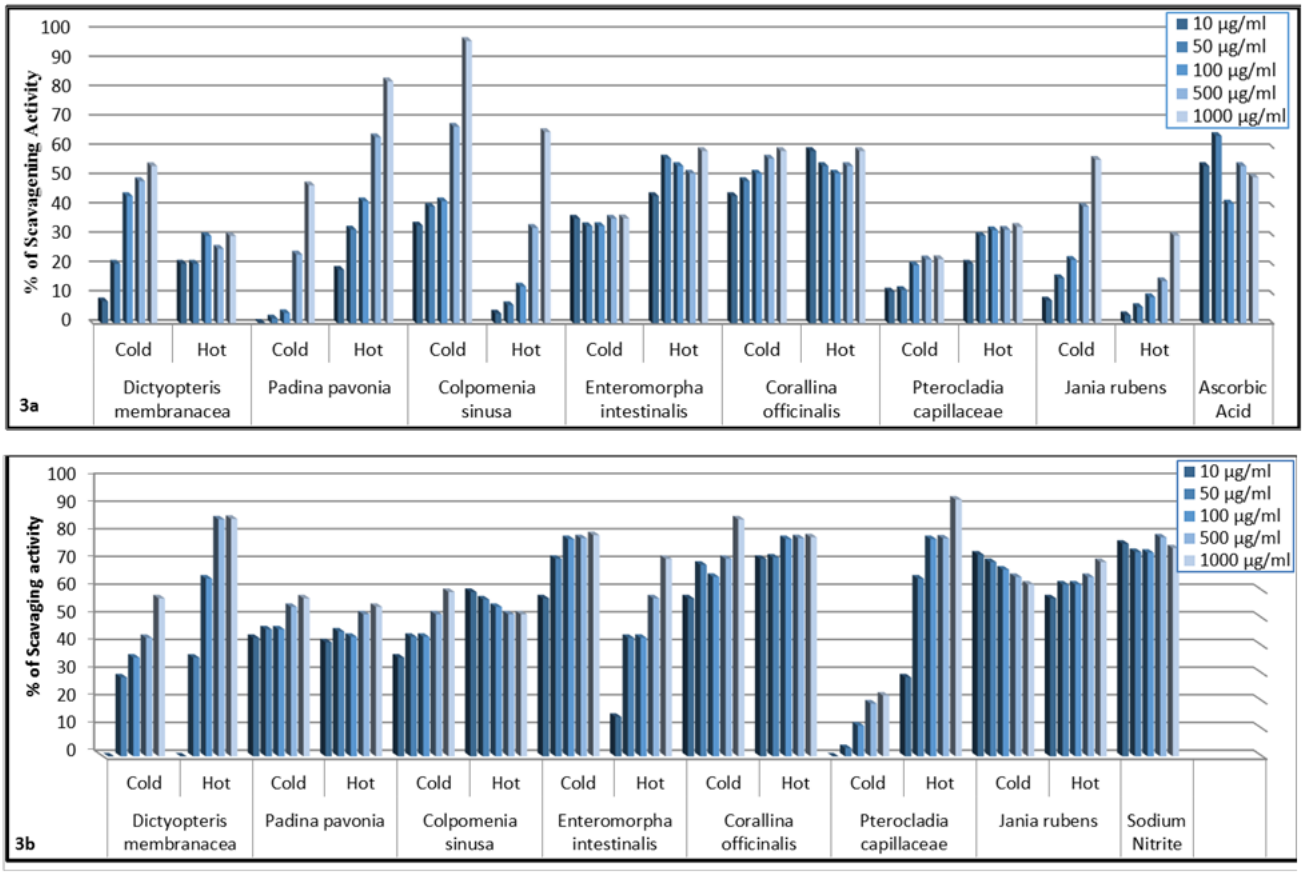


Fig.3. Antioxidant activity of the isolated polysaccharides: (a) DPPH scavenging activity. (b) Nitric oxide scavenging activity.

Table 7: *In vitro* hypolipidemic activity of isolated polysaccharide obtained from marine algae

Species	PolysaccharideExtracts	Enzyme activity (µmol/mg ± SEM)	% of Inhibition
Fluvastatin (reference drug)	-	1.51±0.16	90.58
Dictyopteris membranacea	Cold	4.82±0.20*	70.00
	Hot	3.22±0.03	79.99
Padina pavonia	Cold	12.86±0.06*	19.97
	Hot	12.86±0.06*	19.98
Colpomenia sinuosa	Cold	3.21±0.15	80.02
	Hot	1.60±0.09	90.04
Enteromorpha intestinalis	Cold	3.22±0.08	79.96
	Hot	3.22±0.02	79.96
Corallina officinalis	Cold	14.46±0.05*	10.02
	Hot	3.21±0.01	80.02
Pterocladia capillaceae	Cold	6.43±0.04*	59.98
	Hot	27.33±0.11*	+70.06
Jania rubens	Cold	3.21±0.14	80.02
	Hot	12.80±0.08*	20.35

Each value represents the mean of percentage of enzyme activity of three replicates ±SEM (Standard Error of Mean)
*Significantly different from Fluvastain as reference drug at $p < 0.005$ according to paired-sample *t*-test

antitumor activity and the chemical composition as well as the high order structure of their active components is still not well established. Stevan et al[46] mentioned that the degree of sulfation in sulphated polysaccharides is not a prerequisite for the manifestation of cytotoxic effect. But the cytotoxic effects can be correlated with the carbohydrate composition, position of the sulfate groups, backbone type and/ or molecular conformation. Further, some sulfated polysaccharides can induce apoptosis and differentiation of tumour cells and can enhance the innate and adaptive immune response for tumour cells[47]. In addition, sulphated polysaccharides inhibited the

migration of the carcinoma cell line, increased the anti-invasive property and these results indicate that sulfated polysaccharides are potential molecular leads for antimetastatic drug development[48].

A sulfated polysaccharide extracted from *Enteromorpha intestinalis* had antitumor activity on human hepatoma HepG2 cell line using MTT assay[49] and this results was agree with our results. Wang et al[49] explained that the antitumor activity due to increase of the apoptosis of tumour cells, decreased protein expression of Bcl-2, increased in Bax, cleaved caspase-3, cleaved caspase-9 and cleaved poly(ADP-ribose) polymerase. Also, the polysaccharide caused a loss of mitochondrial memberane potential i.e the polysaccharide induces apoptosis in HepG2 cells involving a caspases-mediated mitochondrial signaling pathway. In addition, Devaki et al[50] found that sulfated polysaccharide play crucial role in stabilizing the functional status of mitochondrial and microsomal membrane by prevention of the oxidative stress.

The antioxidant activity of the isolated polysaccharides (10-1000µg/ml) can be expressed as their abilities to scavenging either DPPH and/ or Nitric oxide free radical. The DPPH free radical is a stable free radical, when it encounters a proton-donating substance (sulfated polysaccharides), the radical would be scavenged and the absorbance at 518 nm is reduced. The DPPH• scavenging % was calculated according to (Eq.2) and was illustrated in Fig. (3a).

On the other hand, nitric oxide is a free radical product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[51]. In the present study, the nitrite produced by the incubation of solutions of sodium nitropriusside in standard phosphate buffer at 25°C, was reduced by the tested polysaccharide extracts which suppress the released NO. The nitric oxide scavenging % was calculated according to (Eq. 3) and was illustrated in Fig. (3b). Either DPPH or Nitric oxide free radicals scavenging abilities of all tested polysaccharides were in a concentration-dependent fashion. *Corallina officinalis* (CEM and HEM) as well as *Enteromorpha intestinalis* (HEM), *Padina pavonia* (HEM) and *Colpomenia sinuosa* (CEM) had higher DPPH• scavenging capacities than that of ascorbic acid at concentration of inhibitors 100- 1000 µg/ml. It was found that, *Jania rubens* and *Corallina officinalis* cold and hot polysaccharide extracts as well as hot polysaccharide extracts of *Pterocladia capillaceae* and *Dictyopteris membranacea* exhibited potent antioxidant activities in scavenging NO and were found to have higher activity than sodium nitrite standard at the same concentrations. Also, the present data declare that, nitric oxide scavenging capacity of cold polysaccharide extract from *Enteromorpha intestinalis* was significantly potent than sodium nitrite at the same concentration of inhibitors. So, it could be provide in medication as powerful agents against various disorders in which oxidative stress is predominant. While, the other polysaccharides exhibited fluctuate activities as antioxidant. However, sulfated polysaccharides were demonstrated stronger antioxidant capacities than de-sulfated polysaccharides[52,53]. Thus, the high degree of sulfation and low molecular weight showed the best antioxidant capacities[54].

For hypolipideamic study, the hypolipideamic activity of cold and hot polysaccharide extracts was calculated according to (Eq. 4) and was compiled in Table (7). Both polysaccharide extracts of *Colpomenia sinuosa & Enteromorpha intestinalis* as well as cold polysaccharide extract of *Jania rubens* and hot polysaccharide extract of *Dictyopteris membranacea & Corallina officinalis* exhibited hypolipidemic activity insignificantly different in compared with Fluvastatin as reference drug. On the contrary, the hot polysaccharide extract of *Pterocladia capillaceae* showed hyperlipidemic activity.

Generally, the biological activity of sulfated polysaccharides from marine algae is related to the molecular size, type of sugar, sulfate content, sulfate position and type of linkage, also, molecular geometry are known to play a role in activity[55].

CONCLUSION

The three main divisions of marine macroalgae (Chlorophyta, Phaeophyta and Rhodophyta), are crucial sources of structurally diverse bioactive sulfated polysaccharides and remain largely unexploited in nutraceutical and pharmaceutical areas. These sulfated polysaccharides exhibit many beneficial biological activities. From our data, these biopolymers frequently show cytotoxicity on HepG2 cell line, radical scavenging and hyopolipidemic properties and didn't show antiviral activity against hepatitis C virus and adenovirus 40 except hot polysaccharide extract of *Pterocladia capillaceae* reduced 46.6% adenovirus 40 replication. Although isolated polysaccharides contain sulfate group, the bioactivity do not rely particulary on degree of sulfation. Therefore, algal sulfated polysaccharides have great therapeutically potential in drug development for the prevention of hepatocarcinoma and could be used as hepatoprotective and hypolipidemic agent in near future.

ACKNOWLEDGMENTS

The authors acknowledged the National Research Centre for the financial support grant (No: 9080104).

REFERENCES

1. Patel, S. Therapeutic importance of sulfated polysaccharides from seaweeds: updating the recent findings. *3 Biotech*, 2012; 2(3): 171-185.
2. Bilan, M. I., & Usov, A. I. Structural analysis of fucoidans. *Nat Prod Commun*, 2008; 3: 1639–1648.
3. Rocha, H. A., Bezerra, L. C., de Albuquerque, I. R., Costa, L. S., Guerra, C. M., de Abreu, L. D., Nader, H. B., & Leite, E. L. A xylogalactofucan from the brown seaweed *Spatoglossum schroederi* stimulates the synthesis of an antithrombotic heparan sulfate from endothelial cells. *Planta Med*, 2005; 71: 379–381.
4. Li, B., Lu, F., Wei, X., & Zhao, R. Fucoidan: Structure and Bioactivity. *Molecules*, 2008; 13: 1671-1695.
5. Costa, L. S., Fidelis, G. P., Telles, C. B. S., Santos, N. D., Camara, R. B. G. C., Cordeiro, S. L., Costa, M. S.

S. P., Lima, J. A., Silveira, R. F. M., Oliveira, R. M., Albuquerque, I. R. L., Andrade, G. P. V., & Rocha, H. A. O. Antioxidant and antiproliferactive activities of Heterofucans from the Seaweed *Sargassum filipendula*. *Mar Drugs*, 2011; 9(6): 952-966.

6. Tuvikene, R., Truus, K., Vaher, M., Kailas, T., Martin, G., & Kersen, P. Extraction and quantification of hybrid carrageenans from the biomass of the red algae *Furcellaria lumbricalis* and *Coccotylus truncatus*. *Proc Estonian Acad Sci Chem*, 2006; 55: 40-53.
7. Usov, A. I. Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloid*, 1998; 12: 301–308.
8. Pereira, M. G., Benevides, N. M., Melo, M. R., Valente, A. P., Melo, F. R., & Mourão, P. A. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? *Carbohyd Res*, 2005; 340: 2015-2023.
9. Lahaye, M., & Robic, A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules*, 2007; 8(6): 1765-1774.
10. Jiao, G., Yu, G., Zhang, J., & Ewart, H.S. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. *Mar Drugs*, 2011; 9: 196-223.
11. Witvrouw, M., & De Clercq, E. Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen Pharmacol*, 1997; 29(4): 497-511.
12. Ponce, N. M. A., Pujol, C. A., & Damonte, E. B. Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies. *Carbohyd Res*, 2003; 338: 153-165.
13. Damonte, E. B., Matulewicz, M. C., & Cerezo, A. S. Sulfated seaweed polysaccharides as antiviral agents. *Curr Med Chem*, 2004; 11(18); 2399-2419.
14. Ohta, Y., Lee, J. B., Hayashi, K., & Hayashi, T. Isolation of sulfated galactan from *Codium fragile* and its antiviral effect. *Biol Pharm Bull*, 2009; 32(5): 892-898.
15. Awad, N. E., Motawe, H. M., Selim, M. A., & Matloub, A. A. Antitumourigenic polysaccharides isolated from the brown algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe. *Medicinal Aromatic Plant Sci Biotech*, 2009; 3 (special issue 1): 6-11.
16. Abou Zeid, A. H., Aboutabl, E. A., Sleem, A. A., & El-Rafie, H.M. Water soluble polysaccharides extracted from *Pterocladia capillacea* and *Dictyopteris membranaceae* and their biological activities. *Carbohydr Polym*, 2014; 113: 62-66.
17. Zhao, X., Xue, C. H., Li, Z. J., Cai, Y. P., Liu, H. Y., & Qi, H. T. Antioxidant and hepatoprotective activities of low molecular weight sulfated polysaccharide from *Laminaria japonica. J Appl Phycol*, 2004; 16: 111-115.
18. Wong, C. K., Ooi, V. E. C., & Ang, P. O. Hepatoprotective effect of seaweeds methanol extract against carbon tetrachloride-induced poisoning in rats. *Hydrobiologia*, 2004; 512: 1- 3.
19. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. Colorimetric method for determination of sugars and related substances. *Anal Chem*, 1956; 28: 350-356.
20. Luo A, Luo A, Huang J, Fan Y. Purification, characterization and antioxidant activities *in vitro* and *in vivo* of the polysaccharides from *Boletus edulis* Bull. *Molecules*, 2012; 17: 8079-8090.
21. Maciel, J. S., Chaves, L. S., Souza, B. W. S., Teixeira, D. I. A., Freitas, A. L. P., Feitosa, J. P. A., & De Paula, R. C. M. Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from seaweed *Gracilaria birdiae*. *Carbohydr Polym*, 2008; 71: 559-565.
22. Liu, J. G., Zhao, H. J., Liu, Y. J., & Wang, X. L. Effect of selenium-enriched malt on VEGF and several relevant angiogenic cytokines in diethylnitrosamine induced hepatocarcinoma rats. *J Trace Elem Med Bio*, 2010; 24(1): 52-57.
23. Matloub, A. A., El-Sherbini, M., Borai, I. H., Magda, K. E., Rizk, M. Z., Aly, H. F., & Fouad, G. I. Assessment of anti-Hyperlipidemic effect and physco-chemical characterization of water soluble polysaccharides from *Ulva fasciata* Delile. *J Appl Sci Res*, 2013; 9(4): 2983-2993.
24. Simões, C., Amoros, M., & Girre, L. Mechanism of antiviral activity of triterpenoid saponins. *Phytother Res*, 1999; 21: 317–325.
25. Walum, E., Strenberg, K., & Jenssen, D. *Understanding cell toxicology: Principles and practice*. NewYork: Ellis Howood., 1990; pp. 97–111.
26. Esawy, M. A., Ahmed, E. F., Helmy, W. A., Mansour, N. M., El-Senousy, W. M., & El-Safty M. M. Production of a halophilic levansucrase from novel honey *Bacillus subtilis* isolates capable of producing antiviral levans. *Carbohydr Polym*, 2011; 86: 823-830.
27. Abdo, S. M., Hetta, M. H., El-Senousy, W. M., Salah El Din, R. A., & Ali, G. H. Antiviral activity of freshwater algae. *J Appl Pharm Sci*, 2012; 2: 21-25.
28. Saeed, M., Scheel, T. K. H., Gottwein, J. M., Marukian, S., Dustin, L. B., Bukh, J., & Rice, C. M. Efficient Replication of Genotype 3a and 4a Hepatitis C Virus Replicons in Human Hepatoma Cells. *Antimicrob Agents Ch*, 2012; 56(10): 5356-5373.
29. Mosmann, T. Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 1983; 65: 55-63.
30. McCue, P., Horii, A., & Shetty, K. Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by *Rhizopus oligosporus*: role of carbohydrate-cleaving enzymes. *J Food Biochem*, 2003; 27(6): 501-514.
31. Menaga, D., Rajakumar, S., & Ayyasamy, P. M. Free radical scavenging activity of methanolic extract of *Pleurotus florida* mushroom. *Int J Pharm Pharm Sci*, 2013; 5: 601-606.
32. Zhang, R., Liu, Y., & Liu, B. The colorimetric method for measuring activities of lipoprotein lipase and

hepatic lipase in plasma. *Hua Xi Yi Ke Da Xue Xue Bao*, 1996; 27: 106–110.
33. Kim, H. J., Lee, D. H., Hwang, Y. Y., Lee, K. S., & Lee, J. S. Characterization of β – hydroxy-β-methylglutaryl coenzyme A reductase inhibitor from *Pueraria thunbergiana*. *J Agr Food Chem*, 2005; 53: 5882-5888.
34. Siddhanta, A. K., Goswami, A. M., Ramavat, B. K., Mody, K. H., & Mairh, O. P. Water soluble polysaccharides of marine algal species of *Ulva* (Ulvales, Chlorophyta) of Indian waters. *Indian J Mar Sci*, 2001; 30: 166-172.
35. Guezennec, J. G., Pignet, P., Raguenes, G., Deslandes, E., Lijour, Y., & Gentric, E. Preliminary chemical characterization of unusual eubacterial exopolysaccharides of deep-sea origin. *Carbohydr Polym*, 1994; 24: 287-294.
36. Kumirska, J., Czerwicka, M., Kaczyński, Z., Bychowska, A., Brzozowski, K., Thöming, J., & Stepnowski, P. (). Application of spectroscopic methods for structural analysis of chitin and chitosan. *Mar Drugs*, 2010; 8(5):1567-1636.
37. Patankar, M. S., Oehninger, S., Barnett, T., Williams, R. L., & Clark, G. F. A revised structure for fucoidan may explain some of its biological activities. *Biol Chem*, 1993; 268: 21770-21776.
38. Ale, M. T., Maruyama, H., Tamauchi, H., Mikkelsen, J. D., & Meyer, A. S. Fucose-containing sulfated polysaccharides from brown seaweeds inhibit proliferation of melanoma cells and induce apoptosis by activation of Caspase- 3 in vitro. *Mar Drugs*, 2011; 9(12): 2605-2621.
39. Matsuhiro, B. Vibrational spectroscopy of seaweed galactans. *Hydrobiologia*, 1996; 326/327, 481-489.
40. Barros, F. C. N., da Silva, D. C., Sombra, V. G., Maciel, J. S., Feitosa, J. P. A., Freitas, A. L. P., & de Paula, R. C. M. Structural characterization of polysaccharide obtained from red seaweed *Gracilaria caudata* (J Agardh). *Carbohydr Polym*, 2013; 92: 598–603.
41. Talarico, L. B., Pujol, C. A., Zibetti, R. G., Faria, P. C., Noseda, M. E., Duarte, M. E., & Damonte, E. B. The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. *Antivir Res*, 2005; 66(2-3): 103-110.
42. Bandyopadhyay, S. S., Navid, M. H., Ghosh, T., Schnitzler, P., & Ray, B. Structural features and in *vitro* antiviral activities of sulfated polysaccharides from *Sphacelaria indica*. *Phytochemistry*, 2011; 72(2-3): 276-283.
43. Harden, E. A., Falshaw, R., Carnacchan, S. M., Kern, E. R., & Prichard, M. N. Virucidal activity of polysaccharide extracts from four algal species against *Herpes Simplex* Virus. *Antivir Res*, 2009; 83 (3): 282-289.
44. Ghosh, T., Chattopadhyay, K., Marschall, M., Karmakar, P., Mandal, P., & Ray, B. Focus on antivirally active sulfated polysaccharides: from structure- activity analysis to clinical evaluation. Glycobiology, 2009; 19(1): 2-15.
45. Ooi, V. E. C., & Liu, F. Immunomodulation and anticancer activity of polsaccharide- protein complexes. *Curr Med Chem*, 2000; 7, 715-729.
46. Stevan, F. R., Oliveira, M. B. M., Bucchi, D. F., Noseda, M. D., Iacomini, M., & Duarte, M. E. R. Cytotoxic effects against Hela cells of polysaccharides from seaweeds. *J Submicr Cytol Path*, 2001; 33(4), 477-484.
47. Wu, X.Z., & Chen, D. Effects of sulfated polysaccharides on tumour biology. *W Indian Med J*, 2006; 55(4), 270-273.
48. Delma, C., Ramalingam, K., Pandian, V., Baskar, A., Savarimuthu, I., Thangavelu, B., & Somasundaram, S. Angiogenesis and Invasion: Abstract A4: Antagonistic effects of sulphated polysaccharides from *Turbinaria conoides* (J. Agardh) on tumor cell migration and angiogenesis". *Cancer Prev Res* 1 (Meeting Abstract Supplement), 2008: A4, November 1.
49. Wang, X., Chen, Y., Wang, J., Liu, Z., & Zhao, S. Antitumour activity of a sulfated polysaccharide from *Enteromorpha intestinalis* targeted against hepatoma through mitochondrial pathway. *Tumor Biol*, 2014; 35: 1641-1647.
50. Devaki, T., Sathivel, A., & Raghavendran, H. R. B. Stabilization of mitochondrial and microsomal function by polysaccharide of *Ulva lactuca* on d-Galactosamine induced hepatitis in rats. *Chem Biol Interact*, 2009; 177: 83–88.
51. Ialenti, A., Moncada, S., & Di Rosa, M. Modulation of adjuvant arthritis by endogenous nitric oxide. *Brit J Pharmacol*, 1993; 110: 701–706.
52. Hu, T., Liu, D., Chen, Y., Wu, J., & Wang, S. Antioxidant activity of sulfated polysaccharide fractions extracted from *Undaria pinnitafida* in vitro. *Int J Biol Macromol*, 2010; 46(2): 193-198.
53. Yang, Y., Liu, D., Chen, Y., & Wang, S. In *vitro* antioxidant activities of sulfated polysaccharide fractions extracted from *Corallina officinalis*. *Int J Biol Macromol*, 2011; 49(5): 1031-1037.
54. Wang, X., Wang, J., Zhang, J., Zhao, B., Yao, J., & Wang, Y. Structure- antioxidant relationships of sulfated galactomannan from guar gum. *Int J of Biol Macromol*, 2010; 46(1): 59-66.
55. Shanmugan, M., & Mody, K. H. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr Sci India, 2000; 79: 1672-1683.