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Potential Biological Properties of Sulphated Polysaccharides Extracted from the Macroalgae *Ulva lactuca* L.

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Abstract: Sulphated polysaccharides (SPS) were obtained from the macro-algae *Ulva lactuca* L (*Ul*-SPS) grown in both artificial and natural seawater media, by hot water and 85% ethanol extraction followed by ethanol absolute precipitated and its chemical characteristic and biological activities were evaluate. The different *Ul*-SPS extracts were characterized by HPLC and IR spectroscopy and show to be contains the similar type of polysaccharides family, with the compositional of six mono-saccharides: rhamnose, xylose, glucouronic acid, mannose and fucose, as well as sulphates sugars. Moreover, IR spectra of these *Ul*-SPS extracts had typical specific absorbance of standard ulvan type polysaccharides. The different *Ul*-SPS extracts exhibited significant remarkable scavenging effects in DPPH and ABTS radicals scavenging assays and showed prominent anticoagulant property. All *Ul*-SPS also had potent effect on the inhibition cell proliferation of two culture human cancer cell lines (breast adenocarcinoma cells, MCF-7 and hepatocellular carcinoma cells, HepG2), with an IC_{50} values ranged from 0.54 to 9.22 μg/ml. In addition, the various *Ul*-SPS extracts showed high levels of antiviral activity against herpes simple virus-1 and significant differences were observed among all extracts,

Key words: *Ulva lactuca* · Sulphated Polysaccharides characterization · Ulvan · Antiproliferation · HPLC · Anticoagulant and antiviral

INTRODUCTION

The green algae, Ulvales (Chlorophyta) are very common seaweed distributed worldwide and considered as an important food source in many parts of the world as a marine vegetable [1]. *Ulva* spp. (sea lettuce) is a rich natural source of carbohydrates, protein, essential vitamins like B, B1, C, E and B9 etc., amino acids, trace elements like zinc and iron etc., dietary fibers and pigments, while containing low levels of lipids [2-4]. Moreover, it has been used as a drug in traditional Chinese medicine for hyperlipidemia, sunstroke and urinary diseases, etc [5]. In addition, *Ulva* spp. is a very interesting natural sources of new potential valuable compounds with biological properties in agriculture, food and pharmaceutical application [3,6,7].

Green microalgae belong to *Ulvales* contains unique multitude bioactive compounds such as phenolics and sulphated polysaccharides that might have a broad range of important biological activities comprising anticarcinogenic, antiviral, antioxidants, antihyperlipidimic, antihypertensive and anticoagulant [3,4,8,9]. Polysaccharide extracted from *Ulva* spp. is a group of hetero-polysaccharide, mainly composed of rhamnose, xylose, glucose, glucuronic acid and sulphate [10]. For instance, ulvan (sulphated rhamnoglycuronans) represents about 8-29% of the *Ulva* algal dry weight. It is composed of different repeating chemical sequences mostly based on disaccharides made of rhamnose, glucuronic acid, iduronic acid, xylose and sulphate (the mainly repeating disaccharide units are ([β-d-GlcpA-(1→4)-α-L-Rhap3S] and [-α-L- IdopA-(1-4)- α-L -Rhap 3S]) [7,11].

Reactive oxygen species (ROS) formed, e.g., by metabolic process or induced by external factors such as environmental pollutants, radiation, dietary habits, etc., capable of causing damage to DNA, which can lead to cell injury and death and create oxidative stress which have been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age [5,12,13]. Therefore, radical scavenging compounds such as vegetable and fruits can indirectly reduce cancer formation in human body. Hence, the antioxidants are essentially needed to reduce this

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negative effect of oxidative stress in body system. BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are synthetic antioxidants, commonly used to maintain foodstuff, but those have been suspected of toxicity and are responsible for liver damage and carcinogenesis [14,15]. Thus, current attention is focusing on develop and utilize effective of natural antioxidants [3]. Algal polysaccharides have been demonstrated to play an important role as free-radical scavengers *in vitro* and antioxidants for the protecting living organisms against the ROS [5,13]. Moreover, marine algae have attracted attention in the search for bioactive compounds to develop new drugs and health foods [6,12,8].

The objective of this work is to investigate the chemical characteristic of sulphated polysaccharides obtained by hot water and 85% ethanol extraction followed by ethanol absolute precipitated, from *U. lactuca* grown in natural and artificial seawater media, as well as to evaluate its antioxidant, antiviral, anticoagulant and antiproliferative activities.

MATERIALS AND METHODS

Algal Source: The green algal, *Ulva lactuca* L (sea lettuce), of 5-8 cm in height were collected on September 2007 from an exposed rocky site near the edge of beach Shat El-Gharam (Mediterranean Sea), Marsa-Matroh government, Egypt.

Cultivation of *Ulva lactuca*: The collected *U. lactuca* was transported in sea water to the laboratory. The collected sample was washed with seawater and deionized water to remove extraneous matter such as epiphytes and contamination from other algae species, in order to avoid microbial contamination in the following cultures. After two weeks (adapted period to aquarium condition), the selected thallus segments were cultivated in an aerated an aquarium (30 L) containing natural sea water (NSW, 25 L), illuminated by ten cool-white fluorescent lamps (40 W, Philips) regularly spaced around the culture and maintained at 25°C±3°C. The medium was weekly changed. Two weeks after, in the aquarium, healthy thallus segments of 5 cm diameter were cut out and divided into two parts (10 g each); first one was grown in natural sea water and the second one was grown in nutrient enriched artificial sea water as described by Provasoli [16]. All algal cultures were maintained for 30 days under laboratory conditions as described before and stopped 24 h after exponential algal growth phase [17].

Extraction of Polysaccharides from *U. Lactuca* (*Ul*-Pss): Thallus segments of *Ulva* algae (250 g) grown either in NSW or ASW medium were washed with fresh water and air shade dried. Then, the *Ul*-PSs was extracted from dried samples with either hot distilled water or 85% ethanol as procedure described by Yu *et al.* [18] and Li *et al.* [19], respectively.

Hot Water Extraction: Dried algae samples (25 g, n=3) were roughly cut and homogenized with distillated water (100 ml). The slurry sample was transferred quantitatively into the extraction Erlenmeyer flask and then refluxed for 3 h at 60°C, under constant stirring. After cooling, the supernatant was separated from the algae residues by centrifugation. The resultant solution was dialyzed in cellulose membrane tubing (molecular weight was cut off 3800 Da, Spectrum Co.) against distilled water for successively three times (4 liters each). Then, the crude polysaccharides in the extract were precipitated with three volume of 99.5% ethanol (absolute) and recovered by filtration. The recovered SPS precipitate was washed three times with absolute ethanol and then dried at 40°C to obtain *Ul*-PSs preparation [18].

Ethanolic Extraction: A 30 g dried sample (n=3) was dipped into 10 volumes of 85 % ethanol (v/v) and kept at room temperature for 2 h. Then the algal homogenized and the slurry was refluxed at 60°C for 3 h. After cooling and centrifugation, the supernatants were concentrated to about 50 ml under reduced pressure. The resultant solution was dialyzed against distilled water and then the crude *Ul*-PSs were precipitated with two volume of absolute ethanol and recovered by filtration. The crude *Ul*-PSs was dried at 40°C. The yields of *Ul*-PSs were calculated based on the dry weight (d.w.) of algae samples.

Determined of Total Sugar Content (TSC): Total sugar content was determined by the phenol–sulphuric acid assay using glucose as the standard [20].

Determined of Total Sulphate Content: Sulphate content of polysaccharide was determined after hydrolysis with 1 mol/L HCl (for 2 h at 100°C) according to the methods of Therho and Hartiala [21].

Analysis of Monosaccharide's by HPLC: The polysaccharides extracts of *Ulva* SPS were subjected to acid hydrolysis. The hydrolysate was quantified and qualified the monosaccharide's content by HPLC, using Shimadzu Shim-Pack SCR-101N column (7.9 mmX 30 cm

i.d., 5 μm particle size). Samples were dissolved in deionized water and eluted with the same deionized water as the mobile phase (flow rate 0.5 ml/ min) and detected with a refractive index detector El-Sayed *et al.* [22]. Peak area/retention times were compared with the following MS as references: rhamnose, fucose, xylose, galactose, arabinose, glucose and glucouronic acid (Sigma chemical Co, USA).

Antioxidant Assays

DPPH Radical Scavenging Assay: The free-radical scavenging of the various *Ul*-SPS extracts toward DPPH (2,2-diphenyl-1-picrylhydrazyl) was estimated following a previously reported method [23]. Brief, two ml of each U*l*-PS extracts (5, 25, 50, 100 and 150 and 200 μg/ml) was added to 3 ml of 0.1 mM DPPH solution. The absorbance of the mixture at ambient temperature was measured at 519 nm, for 60 min at 10 min intervals. The scavenging of DPPH was calculated according to the following equation: scavenging percentage (%) = [(A_{517} control - A_{517} sample) /A_{517} control] X 100; where: A control = absorbance of DPPH radical + methanol; A sample = absorbance of DPPH radical + USPS/standard. The IC_{50} of scavenging capacity of tested samples was determined terms of the amounts μg/ml of extracts necessary for inhibiting 50% of the scavenging of DPPH solution. BHT, BHA and α-tocopherol were used as positive controls.

Measurement of Total Antioxidant Activity: Total antioxidant activity was determined according to the Trolox(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) assay [24]. The extracts and reagents were prepared in 0.1 M phosphate buffer saline (PBS, pH 7.4). $ABTS^+$ solution (2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) was prepared by mixing 2.5 mM potassium persulfate with 7.0 mM $ABTS^+$ in a 1:1 (v/v) ratio and leaving the mixture for 8 h. The absorbance of the radical solution at 743 nm was 0.7±0.03. The solution was stored at room temperature and protected from light. Trolox standard curve (ranged 0.5-20 μg/ml) was prepared. The reduction in the absorbance of the $ABTS^+$ solution (1.9 ml) at different concentrations of Trolox (2-30 μg/ml) over a period of 60 min was measured and plotted. The TEAC values of UCPS (5 mg/ml) were determined in the same way and expressed as μM Trolox equivalents.

Evaluation of Antiproliferative Activity: Antiproliferative activity of the crude *Ulva* sulphated polysaccharides (*Ul*-SPS) extracts were tested against two human cancer cell lines MCF-7 (Human breast adenocarcinoma) and HepG2 (Human hepatocellular liver carcinoma) cell line using colorimetric method. The absorbance of cells lines treated with UCPS (0- 10 μg/ml), after staining with sulphorhodamine B (SRB) was recorded in an ELIZA reader [25]. The viability was expressed as a percentage of control cells. The $IC_{50,}$ the antiproliferative activity of tested samples was determined terms of the amounts μg/ml of extracts necessary for inhibiting 50% of the cell growth. All determination was carried out in triplicate.This assays were done in National Cancer Institute, Cairo University. Cairo, Egypt.

Antiviral Assays: Antiviral activity of USPS was tested using Herpes simplex virus type-1 (HSV-1) as a model of DNA virus. The virus was isolated and propagated in the Sera and Vaccinated laboratory, Ministry of Health. African green monkey cells (Vero) were used. Cells were grown in minimum essential medium with Hank's buffer (HMEM) supplemented by 1% antibiotic-antimycotic mixture (GIBCO-BRL), 8% fetal bovine serum and the pH of mixtur was adjusted to 7.2±0.2 by 7.5% sodium bicarbonate solution. Cells were grown as monolayer sheets dissociated by trypsin-versine solution (0.15% trypsin and 0.04% ethylene diamine tetraacetic acid). The dissociated cells were sub-cultured in a 96-well plate to measure the antiviral activity of the algal extracts. The 50% effective concentration (IC_{50}) is the concentration of the tested samples required to reduce viral plaques by 50% when compared with the virus control [26].

Anticoagulant Assay: These assays were based on determined of clotting time (CT) as described by Matsuhio *et al.* (1996). In briefly, 0.2 ml of rat's plasma and USPS extracts (1-100 μg/ml) were mixed in 3 ml test tube and incubated at 37 °C for 30 min. Then, 0.1 ml of 3-4 units of thrombin (Sigma Co., St. Louis, MO, USA) solution was added. Heparin was used as a positive control.

Infra-red (IR) Spectroscopy Analysis: For IR spectroscopy, samples were mixed with KBr, grounded and pressed into a disc to form a film. The IR spectra of *Ul*-SPS were recorded on a Jacsco, FT/IR- 6100 type A. The spectra were recorded in transmittance mode in the whole IR region (400-4000 cm^{-1}).

Statistical Analyses: Data obtained from measurements and biochemical analyses for each variable were subjected to analysis of variance using the COSTAT computer package (Cohort Software, CA, USA). The mean values were compared with LSD test.

RESULTS AND DISCUSSION

A wide variation in polysaccharides (SPS) was observed among different *Ulva* species, differences were significantly in response to various a biotic stresses, environmental factors and extraction methods [28,3]. The composition of growth nutrients medium and extraction procedures are supposed to allow the isolation of polysaccharides with different structure. In this study, the *Ul*-SPS extracted from *U. lactuca* algae grown in natural seawater (NSW) or artificial sea water (ASW), by hot water (HW) and 85% ethanol extraction may be first assessed. The yields of crude SPS extracts (CSPS) are given in Table 1. The CSPS extracted into HW and ethanol from *Ulva* grown in NSW and ASW (in parentheses) were amounted to 14.52 % (16.82 %) and 10.32% (11.33%, of the algal dry weight d.w.), respectively. Thus, the yields of extraction by water are relatively higher than that of water/ethanol mixture (15:58, v/v). However Andriamanantoanina, *et al.* [28] obtained the similar results for isolation of polysaccharides from *Gracilaria corticata*, that yield changed substantially with extraction conditions. The total sugars (TS), sulphated (SC), uronic acid (UAC) contents found in different *Ul*-SPS extracts, derived from *Ulva* algae grown in NSW and ASW (in parentheses) extracted with HW were 51.35% (66.54 %), 12.45% (14.33%) and 19.87% (22.59%, relative % to yield of crude SPS extracts), respectively. Corresponding values for 85% ethanolic extraction were 37.14% (43.24%), 8.52 %(9.21%) and 12.52 % (13.69 % relative % to yield of crude SPS extracts), respectively. These results revealed that the levels of TPS, SC and UAC constituents of *Ulva algae* were significantly changed in relation to composition of nutrients media. Moreover, hot water had high ability to extractable large amounts of the total TPS, SC and UAC than that of ethanol extracts. Token together, the amounts of photochemical compounds produce by *Ulva lactuca* were largely dependent upon media composition and the relative ratios of their constituent were affected by extraction method. However, this phenomenon is partly in accordance to those found by Li *et al.*, [19] and Andriamanantoanina, *et al.* [28]. They reported that the extraction temperature and time, solvent/solid ratio had a significant effect on the yield, purity and composition of polysaccharides. Much resent studies have shown that *U. lactuca* grown in artificial seawater exhibited a higher ability to accumulate large amounts of secondary metabolites than that of algae grown in natural seawater and their effect could be due to its role in growth and regulation of metabolism pathway [3].

Chemical Composition: The different *Ulva* sulphated polysaccharides hydrolysate was analyzed by HPLC (Table 2) glucouronic acids (12.52-22.59% w/w) and rhamnose (7.55-3.58 %, w/w) were found to be predominant (>10% 0f total percentage) in all samples. Glucose (1.64-2.65%) and galactose (0.98 - 3.33%) was presented as a minor (<10% - >1.0%) constituents. In addition, SPS extracted into hot water was characterized by contained fucose (0.78-1.85%) and mannose in small amounts (0.14-0.42%). The molar ratios between uronic acid to rhamnose in different SPS extracted from *Ulva* grown in NSW and NSW media was significantly affected by the extraction procedures. The present results suggested that the *Ul*-SPS extracted with hot-water and ethanol from *Ulva* algae had a approximately the similar chemical composition to that found in other *Ulva* species, that was characterized by contains large amounts of rhamnose and uronic acid containing sulfated polysaccharide and their structure appeared to be a type homo-polysaccharides [5]. However, the composition of the different *Ul*-SPS extracts generally could be changes depending on the algal species, the extraction procedures, the seasons of harvest and the local climatic conditions [34]. Furthermore, the different *Ul*-SPS extracted were compared with that of ulvan standard with infrared analysis.

Infrared Spectroscopy of *Ulva* SPS: IR spectrum was applied for all SPS extracted from *Ulva lactuca* (Fig. 1). All the *Ul*-SPS extracts showed similar IR spectra. They had a several peaks corresponding to sulfate ester: the intense band at 851 and 1256 cm^{-1} derived from the bending vibration of C–O–S of sulfate in axial position and stretching vibration of S–O of sulfate, respectively. In addition, strong bands at 1649 cm^{-1} was due to asymmetric stretch vibration of COO^- of uronic acids; 1430 cm^{-1}, symmetric stretch vibration of COO^- and stretch vibration of C-O within COOH. The large absorption band centered on 3100-4100 cm^{-1}, which caused by a large amount of O–H stretching. The absorbance band at 1050 cm^{-1} was characterized the stretching vibration of C-O. About other absorption, there were a weak peaks were appeared at 2925 and 2852 cm^{-1}, due to the present of asymmetrical and symmetrical C-H stretching vibrations of aliphatic CH_2. Also, C-H bending of aliphatic CH_2 was presented at 1375-1385 cm^{-1} caused by. Moreover, the absorbance attributions were done by comparison with those reported previously for natural ulvan polysaccharides in the literature [11,29,1]. These results revealed that *Ul*-SPS showed typical absorbance of ulvan; the most common characteristics polysaccharides isolated from many *Ulva* species,

Table 1: Total sugars, total sulphate contents and yield of sulphated polysaccharides extracts of *Ulva lactuca* grown in natural sea water (NSW) and artificial sea water (ASW)

Treatments	Polysaccharides extracts	Polysaccharide[a] Yield %	Total sugars %	Total Sulphate %
U. lactuca grown in NSW	Hot water extract	14.52	51.35	12.45
	Ethanol extract	10.32	37.14	8.52
U. lactuca grow in ASW	Hot water extract	16.82	66.54	14.33
	Ethanolic extract	11.33	43.24	9.21
LSD at level (P< 0.01)		1.92	2.11	1.81

All values show mean of three replicates, ± standard deviation

Values are significantly different at (P≤ 0.01), ± SD

[a]: Relative % to algal dry weight

[b]: Relative % to yield of crude SPS extracts

Table 2: HPLC profile of polysaccharides in *Ulva lactuca* extracts grown in natural sea water (NSW) and artificial sea water (ASW)

	Concentration %			
	Ulva grown in NSW		*Ulva* grown in ASW	
Compounds	Hot water extract	Ethanolic extract	Hot water extract	Ethanolic extract
Glucouronic acid	19.87	12.52	22.59	13.69
Rhmnose	6.96	3.58	7.55	4.66
Mannose	0.14		0.42	
Glucose	1.8	1.64	2.65	1.96
Galactose	1.52	0.98	2.4	3.33
Fuecose	1.85		0.78	
Glc UA / Rha	2.85	3.49	2.99	2.9

Molar ratios Glc UA / Rha: Glucouronic acid / Rhmnose

Table 3: Antiproliferative activity of *Ulva lactuca* sulphated polysaccharides extracts against HePG2 and MCF-7

		IC_{50} µg/ ml	
Treatments	Polysaccharides extracts	HEPG2	MCF7
Ulva lactuca grown in NSW	Hot water extract	7.85	0.54
	Ethanolic extract	9.22	0.85
Ulva lactuca grown in ASW	Hot water extract	5.70	0.74
	Ethanolic extract	7.32	0.91

Table 4: Scavenging activity of effect of *Ulva lactuca* polysaccharides extracts on DPPH●

Treatments	polysaccharides extracts	Scavenging activity %[a]	IC_{50}[b] mg /ml
Ulva lactuca grown in NSW	*Hot water extract*	87.63	0.57
	Ethanolic extract	61.74	0.81
Ulva lactuca grown in ASW	*Hot water extract*	93.09	0.41
	Ethanolic extract	64.36	0.78
BHA		92.10	0.29
BHT		94.00	0.23
α-Tocopherol		90.20	0.35

[a]: Percentage of antioxidant inhibition was calculated from following equation:

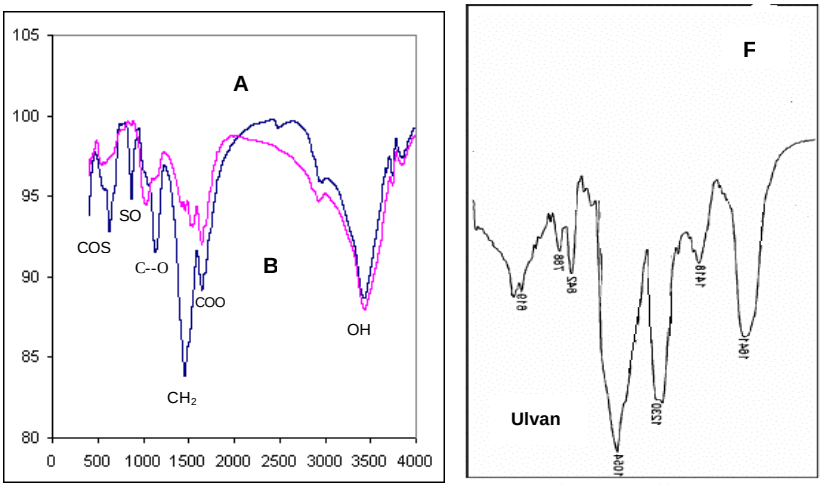
$\%^a = (A_{blank} - A_{sample} / A_{blank}) \times 100$ Where A_{blank} = absorbance of methanolic DPPH· A_{sample} = absorbance of DPPH· radical + samples

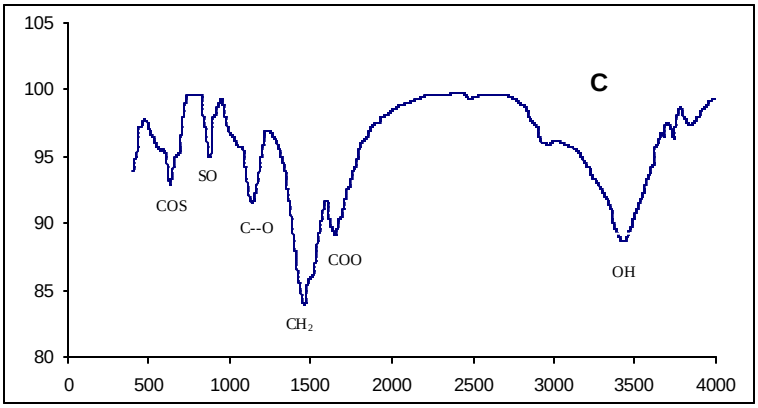
IC_{50}[b]: Concentration (mg/ml) for a 50% inhibition was calculated from the plot of inhibition (%) against *Ulva* extracts concentration tests were carried out in triplicate

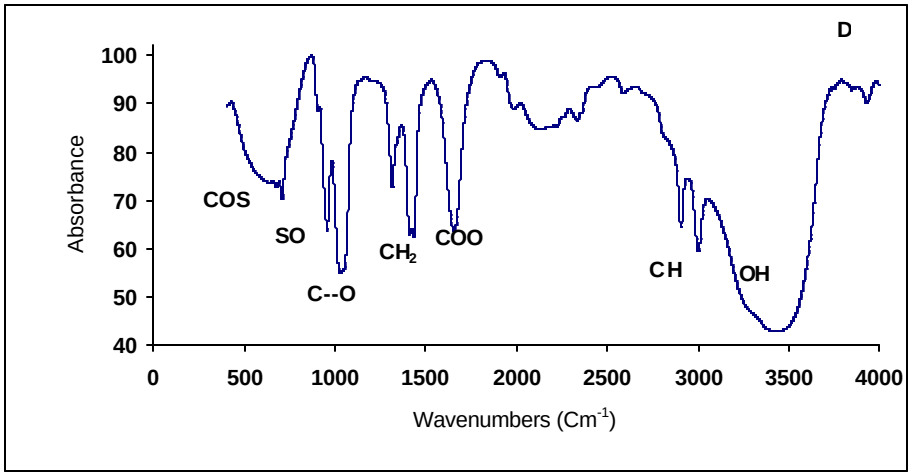
Table 5: $ABTS^+$ Scavenging capacity of *Ulva lactuca* polysaccharides extracts

Treatments	Polysaccharides extracts	$ABTS^+$ scavenging activity, activity, µmol trolox/g grain *Ulva* cells
Ulva lactuca grown in NSW	*Hot water extract*	15.25
	Ethanolic extract	19.55
Ulva lactuca grown in ASW	*Hot water extract*	13.54
	Ethanolic extract	17.66
LSD at level (P< 0.01)		1.23

$ABTS^+$ measured the scavenging capacity against this cation radical expressed as µmol trolox equivalent (TE) per g *Ulva* cells d.w







A: *Ulva lactuca* grown in *ASW Ethanolic extract*
B: *Ulva lactuca* grown in *NSW Ethanolic extract*
C: *Ulva lactuca* grown in *NSW Hot water extract*
D: *Ulva lactuca* grown in *ASW Hot water extract*
F: *Ulvan* standard

Fig. 1: The FT-IR spectra of *Ulva lactuca* polysaccharides extracts

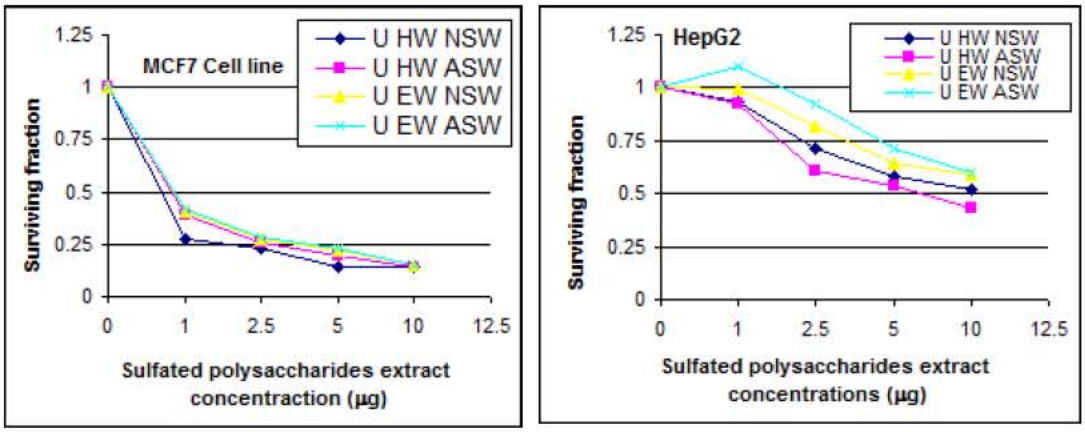


Fig. 2: Antiproliferative activity of *Ulva lactuca* polysaccharides extracts against HepG2 and MCF7

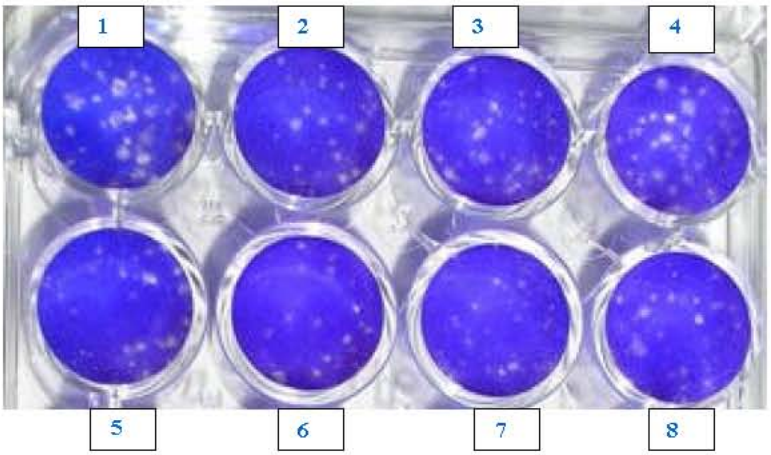


Fig. 3: Antiviral activity of Ulva lactuca polysaccharides extracts against HSV-1

1 Ulva lactuca grown in NSW Hot water extract at concentration 100 µg/ml
2 Ulva lactuca grown in NSW Ethanolic extract at concentration 100 µg/ml
3 Ulva lactuca grown in ASW Hot water extract at concentration 100 µg/ml
4 Ulva lactuca grown in ASW Ethanolic extract at concentration 100 µg/ml
5 Ulva lactuca grown in NSW Hot water extract at concentration 200 µg/ml
6 Ulva lactuca grown in NSW Ethanolic extract at concentration 200 µg/ml
7 Ulva lactuca grown in ASW Hot water extract at concentration 200 µg/ml
8 Ulva lactuca grown in ASW Ethanolic extract at concentration 200 µg/ml

Color wells = No viral growth and dotted wells = obvious growth

which composes of large amounts of ulvan [1,11,29]. Finally, there are some shifting in the values of absorption and relative intensity of function group as compared with other polysaccharides. This could be explained with the results reported by Silverstein and Webstar [30], that the stretching and bending modes of vibration with single function group is normally coupled with the vibration of adjacent groups as well as with the number of substitution on the same molecule. This leads to the shifting in the stretching vibrations of function groups [31].

Antiproliferative Activity (APA) of *Ulva*-SPS: Potential antiproliferative activity of *Ul*-SPS extracted with two polar systems (hot water (HW) and ethanol) from of *Ulva* grown in NSW and ASW was determined *in vitro* against two culture human cancer cell lines including, breast adenocarcinoma (MCF-7) and hepatocellular-carcinoma (HepG2). As shown in Table 3 and Fig. 2, all *Ulva* SPS extracts exhibited a potential APA on MCF-7 cells (with an IC_{50} values 0.54 to 0.91 µg/ml). The most potent APA (indicated by lower IC_{50} value) was found for *Ul*-SPS extracted with HW of *Ulva* grown in either NSW

Table 6: Anticoagulant activity of *Ulva lactuca* polysaccharides extracts

		Inhibition of clot % Time of clotting	
Treatments	Polysaccharides extracts Concentration 100 µg	10 min	15 min
Ulva lactuca grown in NSW	Hot water extract	100	100
	Ethanolic extract	80	80
Ulva lactuca grown in ASW	Hot water extract	100	100
	Ethanolic extract	100	100
Heparin		100	100

Table 7: Antiviral activity of *Ulva lactuca* polysaccharides extracts against HSV-1

		Viral Inhibition %		
Treatments	Polysaccharides extracts	100 µg/ml	200 µg/ml	IC_{50} µg
Ulva lactuca grown in NSW	Hot water extract	25.62	65.62	152.4
	Ethanolic extract	35.33	79.21	126.25
Ulva lactuca grown in ASW	Hot water extract	33.14	79.15	126.34
	Ethanolic extract	47.32	84.29	118.63

(IC_{50} 0.54 µg/ml) or AWS (IC_{50} 0.74 µg/ml) media, following by *Ul*-SPS extracted by ethanol of *Ulva* grown in NSW (IC_{50} 0.85 µg/ml) and AWS (IC_{50} 0.91 µg/ml) media. On HepG2 cells, the IC_{50} values induced by *Ul*-SPS extracted by HW and ethanol of *Ulva* grown in NSW (in parentheses) were 7.85 (9.22) and 5.70 µg/ml (7.32 µg/ml), respectively. Thus, treatments with various *Ul*-SPS on MCF-7 and HepG2 cells displayed a high antiproliferation effect in a concentration-depended manner. Moreover, the results clearly demonstrated that the HepG2 cancer cells are more susceptible to all *Ul*-SPS extracts than that of MCF-7 cells and *Ul*-SPS extracted by HW exhibited relatively higher antiproliferative activity than that was extracted with ethanol. These results may be apparently related to the chemical constituents of algal polysaccharides, in particular, for sulphate contents in *Ul*-SPS. The ability of algal polysaccharides to inhibit the proliferation of many cultured of cancer cells has been well documented [32]. Moreover, a marine alga contains large amounts of characterized polysaccharides such as fucoidan and carrageenan, etc., exhibited anti-tumor, anti-cancer and-anti-metastatic properties and they also reduced cell proliferation [33]. However, their activity may be correlated with the presence of sulphate and uronic groups in their compounds [32,34,35].

Antioxidant Activity of *Ul*-SPS: Free radical scavenging activity (FRSA) of *Ulva* polysaccharides extracted (*Ul*-SPS) by hot water and 95% ethanol of cells grown either in ASW or NSW and standard antioxidant compounds (includes BHA, BHT and α-tecopherol) towards DPPH and APTS are shown Table 4 and 5. All the *Ul*-PSE extracts exhibited significant FRSA values (scavenging activity % ranged 61.74 to 93.1%) at all tested concentrations and in a concentration-depended manner. The *Ul*-PSE extracted by hot water extractions of *Ulva* algae grown in ASW and NSW media exhibited remarkable antioxidant activity, with IC_{50} of 0.57 and 0.41 mg/ml and their activity was approximately to those of the commercial antioxidant BHA, BHT and α-tecopherol (IC_{50} were 0.29, 0.23 and 0.35 mg/ml, respectively). The IC_{50} values recorded for their ethanolic extracts were 0.81 and 0.78 mg/ml, respectively. Consequently, DPPH scavenging activity of various tested samples was in increasing order *Ul*-SPS of NSW< *Ul*-SPS of ASW< α-TOC< BHA < BHT.

ABTS assay was also used to evaluate the antioxidant activity. This assay is usually employed in order to determine efficacy of antioxidant activity (the values of the results were expressed as trolox equivalents) and their assay was superior over DPPH [12]. The various *Ul*-SPS extracts exhibited appreciable radical cation scavenging activity (RCSA) ranged 13.54 to 19.55 µmol trolox equivalents and a significant difference was observed among all tested samples (Table 5). The order of RCSA of *U. lactuca* grown in different media was HWE (of ASW) > HWE (of NSW) > EE (of ASW) > EE (of NSW). The scavenging activity of *Ul*-SPS toward DPPH and $ABTS^{+}$ radicals were comparable to previous finding for SPS extracted from different marine macroalgae

[32]. Moreover, *Ul*-SPS showed potential antioxidant activities compatible to those of synthetic antioxidants (BHA and BHT). However, the natural antioxidant is compared with synthetic antioxidants to minimize adverse effects on humankind [36].

There findings lend weight to the content of sulphated polysaccharides in different *Ulva* extracts, which might explain their high antioxidant activities, a direct correlations between sulphated polysaccharides content of algal and its antioxidant activity is observed. Therefor, the all tested sample with scavenging activity had the same structure feature, all of them had many –OH and $–OSO_3H$ groups in the molecules. Their groups had a significant effect on scavenging free radicals. Polysaccharides promote antioxidant activity, which exhibited the greater eae of abstraction of anomaric hydrogen from the internal monosaccharides units [32]. Therefore, *Ul*-SPS exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [5].

Anticoagulant Activity of *Ulva*-SPS: Table (6) indicated the anticoagulant activity of different SPS extracted into hot-water and 58% ethanol from *Ulva* grown in ASW and NSW media the evaluation was carried out the classical coagulation (based on determination of clotting time (CT), of rat's plasma) assays, using heparin as a reference. The results showed that CT was effectively prolonged by treated with *Ulva*-sulphated polysaccharide and lack of prolongation effect of the some *Ul*-SPS extracts on CT was observed. Moreover, the clotting time was lengthened reached to 15 min, in the presence of *Ul*-SPS at high concentration level (100µg/ml). Generally, the different *Ul*-SPS exhibited remarkable anticoagulant activity and was found to be concentration-depended. Among them, *Ul*-SPS extracted with ethanol from *Ulva* grown in NSW media (at 100 µg/ml, inhibition of clotting was 80%, after 10 min,) showed much high anticoagulant activity than those other SPS extracts (inhibition of clotting was 80% after 1 h, at the same levels). However, the anticoagulant activity of *Ul*-SPS was weaker than that of commercial counterparts (heparin) and higher concentration of SPS were required to achieve the same effect as with heparin in CT assays. The marine green algae belong to Ulvales (*U. conglobate*, *U. lactuca* and *U. perusa*) represent an important source of sulphated polysaccharides with novel structures and these compounds have demonstrated a powerful anticoagulant and antithrombotic activity [29,32,37]. In addition, the ability of the sulfated polysaccharide extracted by ethanol from *Ulva* grown in ASW media to stimulate the inhibition effect of clotting process was different from that of other SPS extracts. The differences of clotting inhibition between them may be attributed to the variation in chemical constituted. However, the complex relationships existed between the structure and anticoagulant activity of the sulphated polysaccharides is well documented [38]. Many researcher have been reported a positive correlation between molecular size, charge density, sulphation position, type of sugar and linkage and molecular geometry and the differences of sulphation positions in the sulfated polysaccharides and anticoagulant activities [39,40,11]. Finally, the sulfated polysaccharide from *Ul. lactuca* could be considered a promising anticoagulant polysaccharide and could be used as a heparinoids active compound or can be developed as a model for the same purposes.

Antiviral Activity of *Ulva* SPS: All *Ul*-SPS extracted from *Ulva lactuca* were used to detect the antiviral activity (AVA) against HSV-1 (standard strain) as a model of DNA virus by plaque redaction assay (Table 7 and Fig. 3). The different *Ul*-SPS exhibited moderate AVA (less active down to 100 µg/ml) at against HSV-1 in a dose-depended manner. For instant, the hot water extracts of *Ulva* grown in either ASW and NSW at concentration levels 100 and 200 µg/ml (in parentheses) inhibited the viral plaques with 25.6 (65.62) and 31.4% (79.15%), respectively. Their IC_{50} values against HSV-1 were 152.4 and 126.34 µg/ml, respectively. On the other hand, there *Ul*-SPS of ethanol extraction showed lower AVA compared with the values of hot water extract with an average IC_{50} values (IC_{50} >100 µg/ml) of 118 and 126 µg/ml, respectively. These values indicated that *Ul*-SPS of hot water extraction of *U. lactuca* grown either in NSW or ASW had a higher AVA than that of *Ul*-SPS extracted in ethanol from algae grown on the same media. Thus, higher AVA activity of *Ul*-SPS may be due to the high acontent of sulphate contents. Therefore, the content of sulphated groups in the hot water extracts of *Ulva algal* grown in ASW might explain their high antiviral activity (Table 1). A large amounts of polysaccharides extracted from marine algae have been found to possess significant antiviral activity against both the herpes virus (containing DNA) and immunodeficiency virus (containing RNA), due to presence of sulphated groups and uronic units [41,34,13]. Furthermore, *in vivo* and *in vitro* studies demonstrated that marine algae contains large amounts of characteristic PSs such as carrageenan, fucoidans and ulvan have antiviral activity [33]. *Ulva lactuca* can be considered an important source of salphated polysaccharides, which

exhibited antioxidant, anticoagulant, antiproliferative and antiviral activity. Thus, its may be helpful in prevention of degenerative diseases as a cancer and heart diseases and is supporting its potential as functional food.

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