

ORIGINAL ARTICLES

Assessment of Anti-hyperlipidemic Effect and Physico-chemical Characterization of Water Soluble Polysaccharides from *Ulva Fasciata* Delile

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ABSTRACT

Hyperlipidemia is a major risk factor for heart disease that represents a major problem and public health burden. As a result, this lead us to assess the potential of polysaccharides isolated from *Ulva fasciata* as hypocholesterimic source on rats fed hypercholesterolemic diet using fluvastatin as reference drug. Also, the physico-chemical characterizations of isolated polysaccharides were investigated. The cold and hot polysaccharide extracts consisted of ash (9.80 and 5.71% w/w), sulphate (14.6 and 19.3% w/w), uronic acid (2.9 and 2.0% w/w), sugar (41.27 and 46.00 %w/w) and protein (15.63 and 36.25% w/w). The cold water extract composed mainly rhamnose, xylose, galactose, arabinose while fucose, xylose, rhamnose, glucose, arabinose, were found as the major sugars of hot water extract. Fourteen and fifteen amino acids could be identified in cold and hot polysaccharide extracts, respectively, aspartic and glutamic acids were found to be the predominant amino acids in both polysaccharides. The isolated polysaccharides could significantly reduce concentrations of total cholesterol, triglycerides and low density lipoprotein in the serum of hyperlipidemic rats while, significantly increase the level of high density lipoprotein. The polysaccharides isolated from *Ulva fasciata* act as natural hypocholesterimic source.

Key words: *Ulva fasciata*- sulphated polysaccharides - hypolipidemic effect - total cholesterol - lipoproteins- cholesterol - triglycerides

Introduction

In recent years, much attention has been focused on polysaccharides isolated from seaweed sources which possess broad spectrum therapeutic properties. The cell wall of green seaweeds contains mainly water soluble, sulfated polysaccharides (SPS), as they referred to Ulvan (Cho *et al.*, 2010) which consisted mainly of sulfate, rhamnose, xylose, iduronic and glucuronic acids (Lahaye and Ray, 1996). The polysaccharides isolated from *Ulva* species such as *Ulva pertusa* and *Ulva lactuca* showed antihyperlipidemic activity (Pengzhan *et al.*, 2003; Qi *et al.*, 2012; Sathivel *et al.*, 2008).

Ulva fasciata known as Lime palahalaha and Sea lettuce, which is used in soups and salads (Priya and Ali, 2011), and its polysaccharides have been reported to possess diverse biological activity, such as anticoagulant (Shanmugam *et al.*, 2001), antioxidant, antibacterial (Priya and Ali, 2011), antiviral (Mendes *et al.*, 2010) and antitumour (Govindan 2012) activities. The present study aims to assess the potential of the polysaccharides isolated from green seaweed *Ulva fasciata* as an alternative natural source for anti-hyperlipidemia.

Materials And Methods

Algal sample:

Ulva fasciata Delile (family Ulvaceae) was collected in June 2010 from Mediterranean Sea. Voucher specimen of the alga has been deposited under no (A01) at pharmacognosy Dept., NRC, Egypt. The collected sample of alga was cleaned by washing thoroughly in tap water, air dried and milled coarsely powdered. Herbarium specimens of the alga was identified by Dr. S. A. Shaalan, Professor of Phycology, Faculty of Science, Alexandria University.

Estimation of total polysaccharide of dried powdered *Ulva fasciata*:

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Quantitative estimation of total polysaccharides content in dried algal sample was determined with the phenol-sulfuric method (Dubois *et al.*, 1956).

Extraction of water soluble polysaccharide:

Air-dried alga 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 under reduced pressure, and precipitated by the addition of 4-fold volume of 95% (v/v) ethanol (Doummar & Sons Co., Syria), centrifuge at 3000 rpm for ten min. The algal residue was soaked in distilled water and heated at 100°C for 3h and hot water extract was obtained following the same procedure used for the cold water extract. The precipitate was washed twice with absolute ethanol, then dried by freeze dryer (Virtis, Gardiner, USA), to obtain a crude polysaccharide cold (CPE) and hot (HPE) extracts then kept in refrigerator for chemical and hypolipidemic effect evaluation.

Physico-Chemical Characterization of the Isolated Polysaccharides:

Molecular weight distribution:

The peak molar masses were estimated by gel permeation chromatography (GPC) with Agilent 1100 series (Germany) at room temperature using two columns, PL aquagel-OH (7.5mm id, 30µm pore size, 8 µm particle size) and PL aquagel-OH (7.5mm id, 50µm pore size, 8 µm particle size) using Refractive index detector, flow of 1.0 mL min⁻¹, 0.5% polysaccharide concentration. 0.01 N NaN₃ and water were used as running solvents and polyethylene oxide/ glycol were used as standard.

Viscosity:

The viscosity of isolated polysaccharides (CPE & HPE) was measured in duplicate by using a Brookfield viscometer at 25 °C in 2.5% SPS using # 1 and #2 spindle.

X-ray diffraction investigations:

Identification of the crystalline phases of polysaccharide samples were carried out with X-ray diffraction analysis. 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, was used. The X-ray diffraction patterns were recorded in a 2θ range of 4-70°.

Measurement of the microstructure:

The cold and hot polysaccharide extracts were examined by the transmission electron microscopy (TEM) type JEOL JSM-T20 (Japan).

Chemical analysis:

Moisture content of polysaccharide samples was determined after heating for 24 h at 105 °C. Ash content was quantified gravimetrically after heating for 12 h at 550 °C and further 4h at 900°C. Carbon, hydrogen, nitrogen and sulphur were determined by elemental microanalysis (elementar vario EL). Total sugar content of isolated polysaccharide was determined by the phenol-sulfuric method (Dubois *et al.*, 1956) using glucose as standard whereas total uronic acids was determined by *m*-phenyl phenol colorimetric method (Thibault *et al.*, 1979) using glucuronic acid as standard. The polysaccharide extracts were hydrolyzed with 4N hydrochloric acid according to Chrums & Stephen (1973) and the hydrolysate was analysed by GLC analyses (HP 6890, USA), after derivatization using the trimethylsilylation reagent (Merk) according to Ronald and Ronald (1991), under the following condition: ZB-1701 capillary column, 30 m in length, 0.25mm i.d; 0.25µm film thickness, carrier gas, helium at a flow rate at 1.2 ml/min, temperature programmed 150-200°C at a rate of 7°C/min, flame ionization detector. Sugar identification was done by comparison with reference sugars. Protein content of isolated polysaccharide was estimated as N Kjeldhal × 6.25. The amino acid content was determined as described by Moore *et al.*(1958) using an LC 3000 amino acid analyzer (Eppendorf-Biotronik, Maintal, Germany). Sulfate content was determined after hydrolysis (2 N HCl at 100°C for 2hr) of the polysaccharides, according to the gelatin- barium method, using potassium sulfate as the standard (Craigie *et al.*, 1984). 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 (Liu *et al.*, 2010):

$$DS = \frac{162 \times \frac{S\%}{32}}{100 - \left(\frac{162}{32} \times S\% \right)}$$

Fourier transform IR spectra of the polysaccharide extracts were recorded in frequency range of 400-4000 cm^{-1} using of KBr pellets on FT/IR-6100 (JASCO, Japan).

Assay of antihyperlipidemic activity:

One hundred and five male Wister rats ($120 \pm 10\text{gm}$), were used for the evaluation of anti-hypercholesterolemic effect of sulphated polysaccharides (SPS) from *Ulva fasciata* were provided by the Animal house of the National Research Center (NRC), and housed in a temperature-controlled environment ($26-29^\circ\text{C}$) with a fixed light/dark cycle for two weeks as an adaptation period to acclimatize under normal combination with free access to water and food *ad libitum*. The present study is approved by the Ethical Committee of the National Research Center (NRC), Egypt, provided that the animals will not suffer at any stage of the experiment.

Rats were fed with high fat diet for twelve consecutive weeks (orally administration) according to Adaramoye *et al.* (2008) to obtain hyperlipidemic rats model (HC rats). The lipid profile of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) was determined for select hypercholesterolemic animal.

Normal diet composed of Yellow corn, soybean meal, Extruded soybean seed, Corn gluten, Limestone powder, mono-calcium phosphate, L- Lysine hydrochloride, salt, sodium bicarbonate, a mixture of vitamins and minerals, D & L-Methionine and Choline chloride. Whereas, high fat diet was composed of lard and normal diet in ratio (1:5).

Doses given to normal and HC-induced rats:

The dose of 100 mg/kg b.w. of polysaccharide extracts, was used for evaluating hypocholesterolemic activity and calculated from the therapeutic dose for rats (Pengzhan *et al.*, 2003).

The dose of 0.09 mg/kg b.w. of Fluvastatin, was used as a reference drug for evaluating hypocholesterolemic activity, was calculated from the therapeutic dose (10 mg/day) for human beings (Koter *et al.*, 2002) using the conversion table of Paget and Barnes (1964). Polysaccharide extracts and Fluvastatin are dissolved in distilled water.

Experimental design:

Animals were randomly divided into seven groups of 15 rats each as follows:

- Group 1:** Served as a negative control (given normal diet and distilled water).
- Group 2:** Normal rats given orally cold polysaccharide extract.
- Group 3:** Normal rats given orally hot polysaccharide extract.
- Group 4:** Served as a group of hypercholesterolemic positive control rats.
- Group 5:** Hypercholesterolemic rats received an oral dose of cold polysaccharide extract.
- Group 6:** Hypercholesterolemic rats received an oral dose of hot polysaccharide extract.
- Group 7:** Hypercholesterolemic rats received an oral dose of Fluvastatin.

Normal groups were continued to be provided with the common commercial rat chow. All treatments were given orally 5 times/week and for 4 weeks.

At the end of the experiment, the animals were withheld food for at least 12h and venous blood samples were collected by cutting sublingual vein into sterilized tubes for serum separation and immediately centrifuged at 3000 rpm for 15 min at 4°C , the clear, non hemolysed supernatant sera were quickly removed and frozen at -20°C until biochemical investigations.

The serum was used to determine the level of TC, TG, LDL-C, HDL-C and total lipids using colorimetric methods (Allain *et al.*, 1974; Fassati and Prencipe, 1982; Friedewald *et al.*, 1972; Burstein *et al.*, 1970; Lopes-Virella, 1977; Zollner and Kirsch, 1962). Results were expressed as mean \pm SD and the differences between groups were compared statistically using Student *t* test and Co -state statistical computer program, where unshared letter is significant at $p \leq 0.05$.

Results And Discussion

The total carbohydrate, free sugar and polysaccharide contents of *Ulva fasciata* were 20.30 ± 0.01 , 2.37 ± 0.006 and 17.93 % w/w of the seaweed dried weight, respectively. Chemical analyses data of polysaccharides obtained from cold (CPE) and hot (HPE) extracts of *U. fasciata* are presented in Table 1. The yields of (CPE) and (HPE) were 11.71 and 5.16 % w/w of the seaweed dried weight, respectively. Ash content of (CPE) and (HPE) were found 9.80 and 5.71% of the isolated polysaccharide, respectively, which is low in comparison to reported data (Siddhanta *et al.*, 2001). Elemental analysis showed that the proportion of carbon, hydrogen, nitrogen and sulfur in CPE and HPE was about 1: 2.8: 0.18: 0.13 and 1: 1.46: 0.16: 0.13, respectively. The sulfate content of CPE and HPE was 14.6 and 19.3 % of isolated polysaccharide and the degree of substitution was 0.23 and 0.83 , respectively. The uronic acid content of CPE and HPE was 2.9 and 2.0 % of isolated polysaccharide which is low in comparison to reported data (Siddhanta *et al.*, 2001).

Table 1: Physico-Chemical Characterization of the Isolated Polysaccharides

Characters	Cold polysaccharide extract	Hot polysaccharide extract
Yield (% dried algal sample) (M \pm S.E)	11.71 ± 1.32	5.16 ± 0.97
Appearance(visually)	Fine powder	Fine powder
Color (visually)	Off white	white
Moisture content (M \pm S.E)	6.32 ± 0.002	2.34 ± 0.001
Ash content	9.80 ± 0.001	5.71 ± 0.002
C	12.3	31.3
H	2.8	3.8
N	2.50	5.8
S	10.8	4.00
Total carbohydrate(M \pm S.E)	41.27 ± 0.008	46.00 ± 0.006
Protein(M \pm S.E)	15.63 ± 0.78	36.25 ± 0.67
Sulphate content(M \pm S.E)	14.6 ± 1.01	19.3 ± 1.2
Substitution degree	0.23	0.83
Uronic Acid content(M \pm S.E)	2.9 ± 0.82	2.0 ± 0.91

(M \pm S.E) mean of three replicates \pm standard error

Total sugar content of CPE & HPE were 41.27 & 46.00% w/w of isolated polysaccharides. The GC analysis of hydrolysate of CPE & HPE revealed the identification of 11 and 12 sugars, respectively, which are summarized in Table 2. Rhamnose, xylose, galactose were the predominant component sugars in the hydrolysates of CPE with molar ratio $145.31: 59: 21.07$. Whereas hot extract polysaccharide was mainly composed of fucose, xylose, rhamnose with molar ratio $139.98: 52.59: 39.19$, respectively. Fucose (34.37% of total hydrolysate) was also detected in polysaccharides isolated from *Ulva lactuca* (Nam *et al.*, 2007), *Ulva conglobata* (Mao *et al.*, 2013) and *Enteromorpha linza* (Zhang *et al.*, 2013). Furthermore, we cannot identify the iduronic acid, which is characteristic for *Ulva* polysaccharide because of the difficulty in precisely quantifying this sugar due to the lack of easily accessible standard. These results are different from other reported data (Siddhanta *et al.*, 2001) which depend on locality and period of collection and method of extraction.

Ulvan has been shown to dissolve only in water due to its charged and highly hydrophilic nature but it is not transparent, indicating the formation of microaggregates of polymeric material not fully dispersed in water and the large presence of methyl groups provided by the rhamnose repeating unit has been considered responsible for the unusual hydrophobic behavior of this highly charged polysaccharide (Robic *et al.*, 2009).

Table 2: Results of GC analysis of trimethylsilyl derivative of hydrolysate of cold and hot water polysaccharide extract

Sugars	RRt	Cold polysaccharide extract		Hot polysaccharide extract	
		% Sugar	Molar ratio	% Sugar	Molar ratio
Arabinose	0.681	3.68	13.61	4.05	24.90
Xylose	0.685	14.16	52.22	8.53	52.59
Ribose	0.713	2.76	10.16	0.65	3.98
Rhamnose	0.762	38.14	129.05	6.95	39.16
Fucose	0.768	1.38	4.66	34.37	193.79
Unknown	0.780	8.84	-	12.36	-
Mannitol	0.884	1.57	4.77	3.74	18.98
Sorbitol	0.897	0.30	0.88	0.42	2.13
Fructose	0.914	-	-	0.20	1.02
Galactose	0.980	6.07	18.66	4.25	21.79
Mannose	0.985	2.13	6.55	2.59	13.24
glucose	1.00	1.57	4.83	5.10	26.20
Unknown	1.031	5.54	-	-	-
Galacturonic acid	1.101	0.35	1	0.21	1
Glucouronic acid	1.319	-	-	-	-
Total identified % sugars		72.29		71.06	

Each value are duplicated

Ulvan structure shows great complexity and variability as evidenced by the numerous oligosaccharide repeating structural units identified in native and chemically modified ulvan preparations (Lahaye *et al.*, 2007). The main repeating disaccharide units reported are ulvanobiouronic acid 3-sulfate types containing either glucuronic or iduronic acid. Whereas, minor repeat units contain sulfated xylose replacing the uronic acid or glucuronic acid as a branch on O-2 of the rhamnose-3-sulfate (Lahaye & Ray, 1996; Lahaye *et al.*, 1997). Ulvan is present in close association with proteins and was found in CPE and HPE 15.63 and 36.25% of isolated polysaccharides, respectively, which is a characteristic of *Ulva* polysaccharides (Dave & Parekh, 1978). Amino acid analysis are presented in Table 3 and revealed that fourteen and fifteen amino acids could be identified in protein of (CPE) and (HPE) of *U. fasciata* which were characterized by high contents of non essential amino acids aspartic and glutamic acid. Alves *et al.* (2010) reported that the conventional methods of extraction and purification was not completely effective in the removal of the protein fraction even after a specific deproteinization protocol.

Table 3: Amino acids composition of isolation polysaccharides obtained from *Ulva fasciata*

Amino acids		% Amino Acid in Isolated Polysaccharide	
		Cold	Hot
Essential Amino Acids	Threonine	0.67	1.62
	Valine	0.93	1.69
	Isoleucine	0.52	0.29
	Leucine	2.01	1.13
	Phenylalanine	1.25	1.41
	Lysine	0.91	0.58
	Total	6.29	6.72
Non-Essential Amino Acids	Aspartic acid	2.47	4.55
	Glutamic acid	2.27	5.11
	Serine	0.86	1.85
	Glycine	0.56	1.17
	Histidine	0.70	2.40
	Arginine	1.39	1.08
	Alanine	1.93	3.98
	Proline	-	1.29
	Tyrosine	1.47	2.90
	Total	9.18	24.33
Total Contents of Amino Acids		15.47	31.05

The IR spectrum of CPE and HPE Fig.1 exhibited absorption bands at 3386.39 and 3415.31 cm⁻¹, respectively, (O-H stretching), 1641.13 and 1653.66 cm⁻¹ (COO⁻ stretching) and 1547.59 cm⁻¹ and 1550.48 cm⁻¹ (shoulder) were assigned to the amide-II band and this result confirmed the fact that both polysaccharide extracts contain protein (Guezennec *et al.*, 1994). The IR spectrum of HPE showed an absorption band at 1239 cm⁻¹ (S=O stretching) and 1321 cm⁻¹, indicating the presence of esterified sulfate. In addition, IR spectrum showed an absorption band at 841.77 cm⁻¹ due to sulfate groups at the axial C-4 position (Patankar *et al.*, 1993) and 597.825 cm⁻¹ attributed to the asymmetric deformation of O-S-O groups (Matsuhiro, 1996). While, the vibration of the C-O-C bridge of glucosides were recorded at wave numbers 1096.33 and 1094.4 cm⁻¹ in CPE and HPE, respectively. Beside, no absorption band over 1700 cm⁻¹ indicates the absence or trace of uronic acids in the samples. This amalgamation indicates that the isolated polysaccharides are heterogeneous, with high sulfate and protein and the cold polysaccharide extract refer to ulvan in nature while hot polysaccharide extract refer to fucoidan in nature.

The gel permeation chromatography (GPC) profile of CPE and HPE revealed three and two major peaks, respectively, and their characterizations were summarized in Table 4. The average molecular weights of CPE were 1.73×10⁶, 3.48×10⁴, 3.01×10³ g mol⁻¹ while the average molecular weights of HPE were 1.4916×10⁶ and 3.0512×10³ g mol⁻¹ by GPC. The viscosity of both polysaccharide extracts CPE and HPE was too low to be measured.

Table 4: The gel permeation chromatography of cold polysaccharide extract (a) and hot polysaccharide extract (b) isolated from *Ulva fasciata*

Fraction	Peaks	Integration (min)	Mn	Mw	Mz	Mp	D	A (ml*V)
			g mol ⁻¹					
Cold polysaccharide extract	peak 1	8.14-11.11	3.60×10 ⁵	7.35×10 ⁵	1.73×10 ⁶	3.09×10 ⁵	2.04	1.38×10 ³
	peak 2	11.11-13.91	2.44×10 ⁴	2.89×10 ⁴	3.48×10 ⁴	2.74×10 ⁴	1.18	6.42×10 ³
	peak 3	14.50-18.02	1.77×10 ³	2.33×10 ³	3.01×10 ³	2.23×10 ³	1.32	9.43×10 ³
Hot polysaccharide extract	peak 1	7.86-14.21	1.00×10 ⁵	4.41×10 ⁵	1.49×10 ⁶	2.65×10 ⁵	4.41	1.52×10 ⁴
	peak 2	14.59-17.57	1.92×10 ³	2.47×10 ³	3.05×10 ³	1.98×10 ³	1.29	3.32×10 ³

Mw: The weight-average molecular weights, Mn: The number-average molecular weights, D: Polydispersity 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.

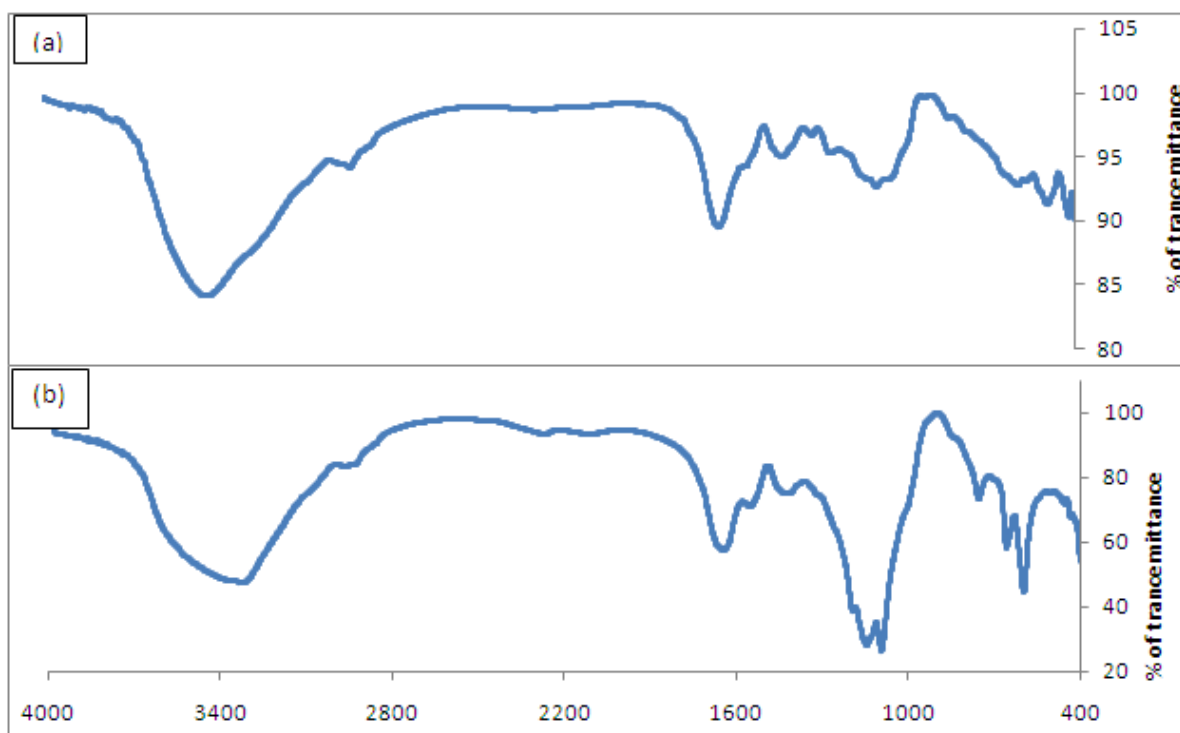


Fig. 1: IR spectrum of cold polysaccharide extract (a) and hot polysaccharide extract (b) isolated from *Ulva fasciata*

The X-ray diffraction (XRD) analysis was applied to determine the crystallinity degree of the isolated ulvans. The XRD patterns, presented in Fig. 2 and 3 and represent the fingerprint of CPE and HPE, respectively. The XRD pattern of CPE is a typical for crystalline polymer, whereas, the XRD pattern of HEP revealed that the structure is poorly crystalline.

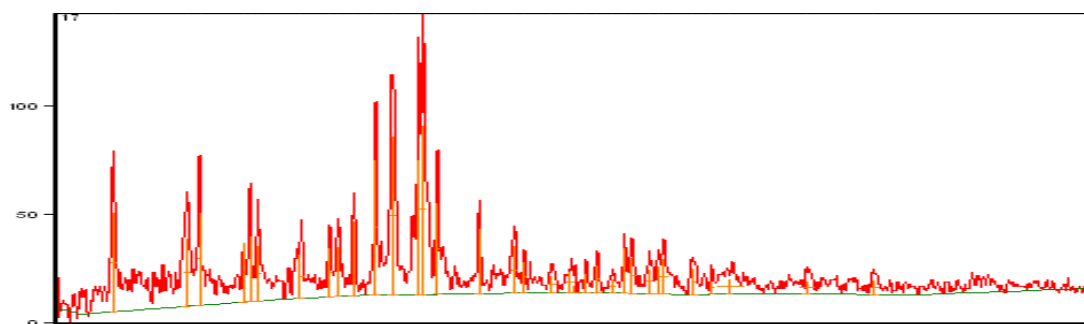


Fig. 2: X-ray diffraction of cold polysaccharide extract

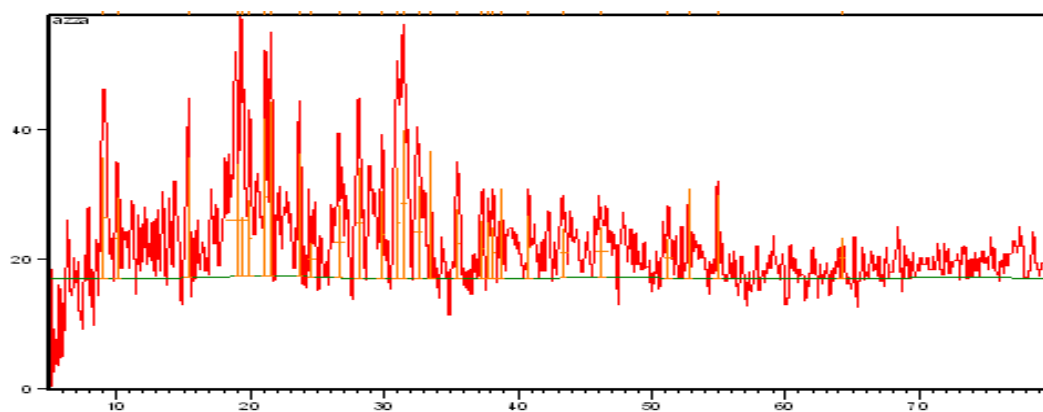


Fig. 3: X-ray diffraction of hot polysaccharide extract

Polysaccharides crystals obtained from *U. fasciata* have been showed by TEM (Fig.4) to be of various sizes which tend to self-aggregates.

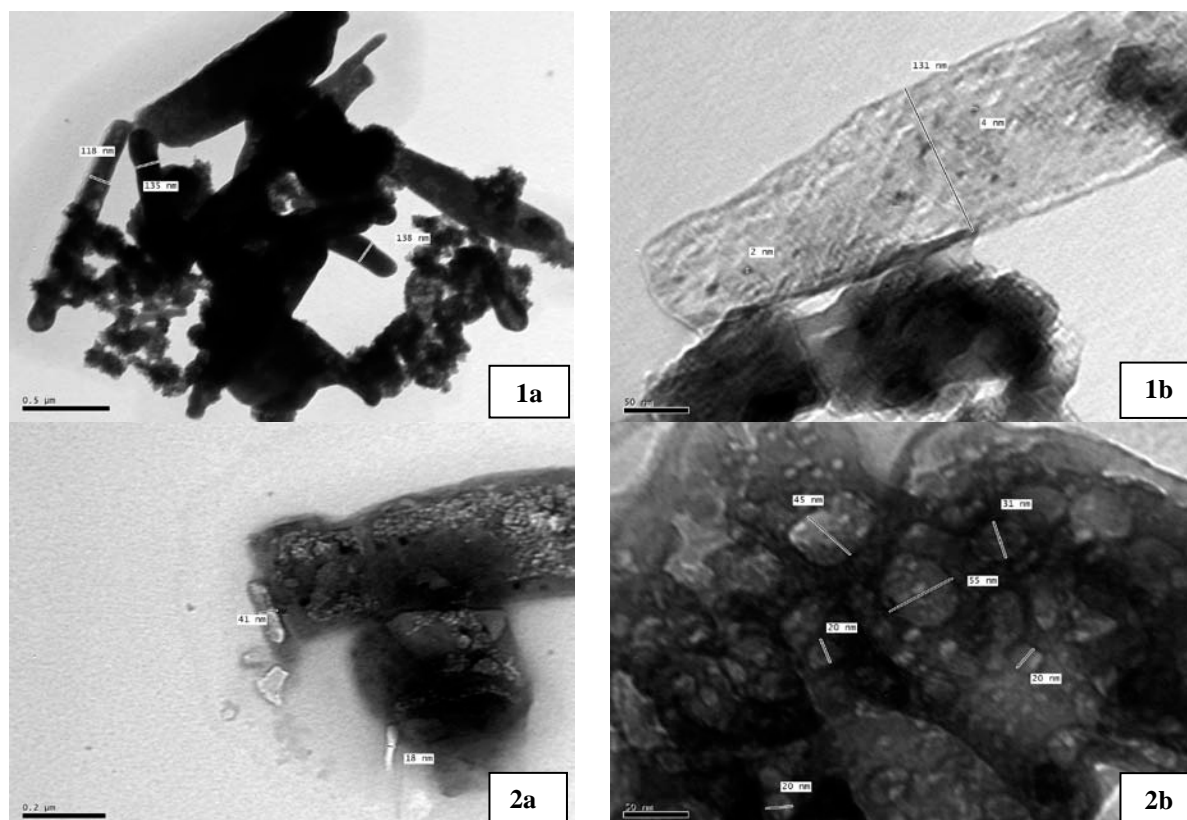


Fig. 4: TEM photographs of cold polysaccharide extract 1a (at 0.5μm) and 1b(at 50nm) and hot polysaccharide extract 2a (at 0.2 μm) and 2b(at 50 nm)

Hypercholesterolemic evaluation:

In the present study, hypercholesterolemia-induced diet feeding for 12 weeks is chosen as experimental model of early phase of atherogenesis. In comparing feeding rats with cholesterol-enriched diet to negative control rats (Table 6), the results declared that, significant elevation of serum total cholesterol (107.49%), total lipids (63.74%) and triglycerides (176.30%). Concerning lipoproteins and atherogenic index (AI), it was noticed that, serum HDL-C level was significantly decreased in the HC- rats (85.74%), whereas serum LDL-C, VLDL-C levels and LDL/HDL ratios of HC-rats were significantly increased as compared to normal control group with percentages amounting to 567.17, 175.21 and 4076.36%, respectively. In a parallel results Shehata and Yousef (2010) found that cholesterol-enriched diet resulted in a significant increase in total cholesterol, total lipids, phospholipids and triacylglycerol in serum and liver and these elevated parameters associated with increased serum LDL-C level and decreased circulating HDL-C, thus providing a model for dietary hyperlipidemia. In addition, the increase of lipid parameters in hypercholesterolemia was shown to be a strong risk factor for coronary heart diseases in many populations. The high cholesterol level in liver and serum may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids. The metabolism of free and ester cholesterol are impaired in liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats (Shali *et al.*, 2001).

The treatment with polysaccharides (CPE and HPE) isolated from *Ulva fasciata* attenuated the high serum lipid profile levels and AI (Table 5). This implies that, the treatment could be effective to alleviate atherosclerosis which then eventually prevents the occurrence of cardiovascular disease (CVD). Regarding to the recorded changes in lipid profile, cold and hot polysaccharide extracts showed improvement by 88.83, and 96.34%, respectively for TC; while it recorded 214.12 and 190.73%, respectively for triglycerides and 56.72 and 190.73%, respectively for total lipids. However, fluvastatin recorded the lowest percent of amelioration for TC, triglycerides and total lipid as compared to both polysaccharide extracts (77.81, 90.56 and 54.97%, respectively). The enhanced percentage of lipoproteins post cold, hot polysaccharide extracts and fluvastatin treatments showed that hot extract exhibited the highest percentages 540.67 %, 95.29 %, 4070.91%,

respectively for LDL, HDL and AI. While, the highest improvement level of VLDL was detected post cold extract treatment. Oral supplementation of fluvastatin demonstrated the lowest ameliorative percentage.

From the manipulated data, it clearly demonstrate the possibility using polysaccharide extracts of *U. fasciata* (CPE and HPE), as hypocholesterolemic agents. In addition, this study revealed that the hot polysaccharide extract of *U. fasciata* is more potent as an hypocholesterolemic agent than cold polysaccharide extract, and at the same time, both types of the algal extracts are more effective in reducing hypercholesterolemia than the standard drug; fluvastatin. In fact, dietary fiber are known to interfere with cholesterol absorption and enterohepatic bile circulation, resulted in depletion of hepatic cholesterol pools and reduce triacylglycerol levels by inhibition of hepatic lipogenesis (Venkateson *et al.*, 2003), and there is also another type of anti-hyperlipidemic mechanism: bile acid sequestrant mechanism (Qi *et al.*, 2012).

Polysaccharides can act as stimulators of bile acid synthesis. Most bile acids are reabsorbed in the small intestine and return to the liver so that the bile acid pool remains essentially constant. Bile acid sequestering resins act in the small intestine by interrupting the enterohepatic circulation and increasing the fecal excretion of bile acids so that fewer bile acids return to the liver. This increases the synthesis of bile acids, and the loss of bile acids is compensated for by oxidation of more hepatic cholesterol, the only precursor to bile acids, thereby decreasing the total blood cholesterol levels (Qi *et al.*, 2012). Significantly reductions of serum total and LDL-C concentrations and elevations of daily bile acid excretion in ulvan-fed rats suggested the mechanism of cholesterol breakdown into bile acid, by which ulvan effectively lowered serum cholesterol levels. When diet supplemented with ulvan was consumed, other nutritional components were digested and absorbed from the small intestine and ulvan became a major component in the gut lumen. LDL-C was removed from blood and converted into bile acids by the liver to replace the bile acids lost in the stool, consequently serum LDL-C was also decreased simultaneously (Dvir *et al.*, 2000).

The mechanism by which *U. fasciata* reduce serum lipoprotein levels in HC rats, may be through the inhibition of 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis (Bobek *et al.*, 1995). The extract produced a significantly lower serum triglyceride level, another well-established and recognized risk factor for developing atherosclerosis (Ademuyiwa *et al.*, 2007). Thus, the significantly lower serum total cholesterol levels produced by the extract, connotes the ability of the extract to protect against cardiovascular diseases. In addition, the decreased serum triglycerides concentration in HC-rats treated with isolated polysaccharide may be explained on the basis of increased clearance of triglycerides secondary to increase activity of lipoprotein lipase (LPL) (Nofer *et al.*, 2002). Thus, the sulphated polysaccharides of the CPE and HPE showed a high anti-hyperlipidemic activities which may be attributed to an important relationship between the anti-hyperlipidemic effect and the antioxidant activities of both extracts (Sathivel *et al.*, 2008).

Table 5: Lipid profile of normal and hypercholesterolemic rats after treatment with cold and hot polysaccharide extracts of *Ulva fasciata* and fluvastatin

Groups	TC (μ g/dl)	TG (μ g/dl)	Total lipids (mg/dl)	LDL-C (μ g/dl)	HDL-C (mg/dl)	VLDL-C	THR	AI
Control	48.63 \pm 10.85	23.72 \pm 8.96	1000 \pm 52.6	12.49 \pm 9.24	31.21 \pm 1.48	4.76 \pm 1.79	1.56 \pm 0.25	0.55 \pm 0.27
Negative Cold extract	54.03 \pm 5.45 ^{NS}	24.21 \pm 5.49	1105.26 \pm 52.65 ^{NS}	22.07 \pm 2.69 ^{NS}	27.25 \pm 4.77 ^{NS}	4.86 \pm 1.07 ^{NS}	2.01 \pm 0.20 ^{NS}	1.02 \pm 0.19 ^{NS}
Negative Hot extract	57.65 \pm 8.28 ^{NS}	24.84 \pm 5.19 ^{NS}	1105.26 \pm 105.25 ^{NS}	25.1 \pm 5.10 ^{NS}	27.74 \pm 3.09 ^{NS}	4.96 \pm 1.02 ^{NS}	2.06 \pm 0.151 ^{NS}	1.07 \pm 0.14 ^{NS}
HC Rats	100.9 \pm 11.24*	65.54 \pm 7.05*	1637.43 \pm 96.64*	83.33 \pm 8.68*	4.45 \pm 1.48*	13.10 \pm 1.40*	23.95 \pm 6.19*	22.97 \pm 6.23*
HC-Cold extract	57.7 \pm 6.23 ^{NS}	14.75 \pm 1.90 ^{NS}	1070.2 \pm 60.79 ^{NS}	31.96 \pm 12.05 ^{NS}	27.25 \pm 2.27 ^{NS}	4.95 \pm 0.38 ^{NS}	2.12 \pm 0.09 ^{NS}	1.11 \pm 0.08 ^{NS}
HC-Hot extract	54.05 \pm 5.43 ^{NS}	20.3 \pm 3.4 ^{NS}	1140.33 \pm 80.40 ^{NS}	15.80 \pm 3.30 ^{NS}	34.19 \pm 1.50 ^{NS}	4.06 \pm 0.65 ^{NS}	1.6 \pm 0.1 ^{NS}	0.58 \pm 0.09 ^{NS}
HC-Fluvastatin	63.06 \pm 8.30 ^{NS}	44.06 \pm 8.95 ^{NS}	1087.73 \pm 80.40 ^{NS}	24.18 \pm 7.99 ^{NS}	30.21 \pm 2.27 ^{NS}	5.83 \pm 1.79 ^{NS}	2.08 \pm 0.15 ^{NS}	1.076 \pm 0.13 ^{NS}

(TC): Total cholesterol; (TG): Triglycerides; (LDL-C): low density lipoprotein cholesterol; (HDL-C): high-density lipoprotein cholesterol; (VLDL-C): very low density lipoprotein cholesterol; (THR): TC/HDL ratio; AI: Atherogenic index

Data presented as mean \pm SD, n=15 for each treatment group, * significant as compared to control, ** significant as compared to (HC), ^{NS} non significant as compared to either normal or HC-rats, P \leq 0.05.

Structure Activity Relationship (SAR):

The mechanism underlying the hypocholesterimic effect of polysaccharide is not completely understood. There are very few reports in the literature on the structure /activity relationship of the polysaccharides under analysis. The anti-hyperlipidemic activity results obtained in our study clearly established the hypolipidemic potency of the polysaccharides isolated from the cold and hot polysaccharide extracts of *U. fasciata*. The relationship between the structure of ulvan (alga polysaccharide) and hyoplipidemic mechanisms requires further studies.

Ulvan is known to resist degradation by human endogeneous enzymes (Taboada *et al.*, 2010) and the presence of ion charged groups along their structure has shown to improve this beneficial activity (Guillon and Champ, 2000).

Many factors are potentially contributing to the antihyperlipidemic action of ulvan polysaccharide such as the sulphated polysaccharide can enhance the negative charges of cell surface so as to affect the aggradation of cholesterol in blood, as a result decreasing the cholesterol in serum (Li *et al.*, 2008). In addition, the ionic groups of ulvan are thought to interact and bind effectively with bile acids due to its high content of negatively charged groups (Lahaye, 1991). Consequently, increase fecal bile acid excretion and sequestration in the intestinal lumen

thus promoting blood cholesterol attenuation (Yu *et al.*, 2003). This activity has been shown to be strongly dependent on the molecular weight of the polysaccharide because a decrease in the viscosity of these materials affected negatively the interaction with bile acids.

On the hand, The antioxidant activity of the sulfated polysaccharide was also playing a role for anti-hyperlipidemic activity. Some authors have observed a correlation between antioxidant and anti-hyperlipidemic activity; i.e: the antioxidant can scavenge superoxide radical, decrease lipid peroxidation, reduce the content of malondialdehyde in serum, and prevent low density lipoprotein peroxidation (Sathivel *et al.*, 2008 and Kanner, 1990). Ajisaka *et al.* (2009) showed antioxidant activity of various carbohydrate molecules, including mono-, di-, oligo-, and polysaccharides of various structures and that possessed either an amino, carboxyl, carbonyl, or sulfonyl group. Dianzani (1978) mentioned that the sulphur compounds in the extracts are capable of reducing the excessive accumulation of intracellular triglycerides. Furthermore, Yu *et al.*, (2003) found that ulvans with different molecular weights exhibited diverse effects on lipid metabolism and the high molecular weight ulvan was effective in serum total and LDL- cholesterol, whereas low molecular weight fractions were in TG and HDL- cholesterol. Chang-Hu (2001) mentioned that the LDL-Cholesterol was significantly reduced due to the antioxidant property of the polysaccharides which were capable of inhibiting the LDL-C peroxidation.

Conclusion

Sulphated polysaccharides are complex and heterogenous, and their biological activities are so attractive that much research is being done on their structures and bioactivities. These polymers are appropriate to reduce hyperlipidemia so they are promising substances in reducing coronary heart disease. This stands in contrast to the use of fluvastatin that have life-threatening side effects like aching or weakness of skeletal muscles. In conclusion, the results demonstrate that polysaccharides from *U. fasciata* is a potential natural product drug and functional food for the prevention and treatment of hyperlipidaemia. The effects of cold and hot polysaccharide extracts of *U. fasciata* were better than those of the fluvastatin and hot extract of *U. fasciata* is more effective in reducing hypercholesterolemia than cold one. Moreover, the acute toxicity test of the studying polysaccharides showed that the rats didn't exhibit toxicity when orally administrated dose up to 1500g/kg b. w. (unpublished data). However, serious further experiments must be carried out on human patients.

Acknowledgments

The financial support for this study by National Research Centre fund (No:9080104) is gratefully acknowledged.

Conflict Of Interest:

Conflict of interest declare none.

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