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Antiviral, cytotoxic, antioxidant and anticholinesterase activities of polysaccharides isolated from microalgae *Spirulina platensis*, *Scenedesmus obliquus*, and *Dunaliella salina*

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ABSTRACT

Quantitative estimation of vegetative and stress forms of *Spirulina platensis* and *Scenedesmus obliquus*, as well as a vegetative form of *Dunaliella salina*, revealed that *S. obliquus* constituted the highest polysaccharide content than other tested microalgae. The isolated polysaccharides characterized as heterogeneous polysaccharides bounded protein by FT-IR, GLC, and Elemental Microanalysis. These polysaccharides constituted of 47-66% of sugar and 14.88-41.06% of protein contents whereas galactose, mannose, glucose, and rhamnose were represented as predominant sugar in isolated polysaccharides. The isolated polysaccharides were evaluated *in vitro* as antiviral, cytotoxic, antioxidant and anti-cholinesterase properties. The non-toxic dose of isolated polysaccharides on Huh 7.5, MA104, BGM, and Vero cell lines were determined. The *S. platensis* (CEM and HEM) polysaccharides have promising antiviral, which reduced replication up to 50 – 87.6% of HCV genotype 4a replicon, coxsackievirus B4, rotavirus and herpes simplex type 1 virus at nontoxic doses 1.8 and 1.5 mg/ml, respectively. Furthermore, the isolated polysaccharides were assessed for *in-vitro* cytotoxic activity against MCF-7, HepG2, and HCT116 cell lines. The cytotoxic activity revealed that *D. salina* HEM polysaccharide show potent cytotoxic activity against HCT 116 cell line with IC₅₀ 64.2 µg/mL. Additionally, the isolated polysaccharides showed DPPH• scavenging activity in a dose-dependent relationship and *D. salina* HEM and *S. obliquus* CEM showed the significantly highest activity (308.16 and 308.69%, respectively) at 100 µg/mL. Furthermore, *S. obliquus* CEM and HEM polysaccharides exhibited the significant highest cholinesterase % inhibition activity. Microalgal polysaccharides have great therapeutically potential in drug development used as antiviral, antitumor, antioxidant and anticholinesterase agents in near future.

Keywords: *Microalgae, polysaccharides, antiviral activity, cytotoxic activity, antioxidant, and anticholinesterase.*

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1. INTRODUCTION

Microalgae are still paid attention as a valuable source of various bioproducts in spite of their required for growth only inorganic compounds and light as energy sources [1]. Among these bioproducts were polysaccharides which were diverse, abundant and exhibited numerous biological properties as well as they had great potential applications for pharmaceutical and medicine industries [1]. Microalgae are easy to grow and cultivate economically and enable the production of polysaccharides without depending on the climate or season [1]. The interest in microalgal polysaccharides is growing increasingly; especially they possess several biological applications with various health benefits such as antiviral agents, antioxidants, anti-inflammatory, immunomodulatory and lubricants for bone joints [2]. Intracellular polysaccharides and exopolysaccharides were isolated from different *Spirulina species*, which showed broad-spectrum antiviral activity [2]. *S. platensis* produced a polysaccharide known as calcium spirulan that exhibited antiviral and anticoagulant effects and prevented pulmonary metastasis in addition treated spinal cord injuries [3, 4]. In addition, a water-soluble polysaccharide isolated from *S. platensis* reduced Hepatitis C replicon to 50% and displayed antioxidant activity as well as cytotoxic activity against hepatocarcinoma [5]. Ishaq et al. (2016) reported that water-soluble polysaccharide from *S. obliquus* had oxidative stability [6]. The crude polysaccharide isolated from *D. salina* containing glucose, galactose, xylose, mannose, and rhamnose [7]. Furthermore, Dai et al., (2010) identified acidic heteropolysaccharide, glucans, sulfated polysaccharides and polysaccharide linked with nucleic acids by covalent bonds in fractions yielded from crude polysaccharide isolated *D. salina* [8]. Whereas, Zhang et al. 2009 found that sulfated polysaccharides fraction inhibit influenza

virus FM1 and strengthen immune function more than de-sulfated polysaccharides [9]. On the other hand, extracellular polysaccharides isolated from *D. salina* possess cytotoxic and immunomodulatory activities [10]. In addition, polysaccharides derived from *Spirulina platensis*, showed protective effects against neuronal damage [11]. Considering the importance of inflammation and oxidative stress in Parkinson's disease (PD), *Spirulina* had a neuroprotective potential recommending its use as an alternative treatment for Parkinson's disease [12].

Therefore, our study aims to investigate the chemical characterization of the polysaccharides isolated from microalgae *Spirulina platensis*, *Scenedesmus obliquus*, and *Dunaliella salina* and evaluates their antiviral activity against (HCV, Rotavirus, Herpes simplex & Coxsackievirus) as well as cytotoxic activity on liver, breast and colon cell lines. In addition, DPPH' scavenging efficacy and cholinesterase property of isolated polysaccharides were evaluated.

2. MATERIAL AND METHODS

2.1. Algae material

Algae strains were obtained from algal biotechnology unit, National Research Centre. The used strains were *Spirulina platensis* belonging to Cyanophyta, *Scenedesmus obliquus* and *Dunaliella salina* belonging to Chlorophyta. All of them were originally varied due to their salinity margin. *Spirulina platensis* was laboratory grown using Zarrouk medium [13] while *Scenedesmus obliquus* was laboratory grown using BG-II medium [14] and artificial seawater medium was used for growing *Dunaliella salina* [15]. Stress growth for each alga was achieved basically by increasing salinity concentration to 2.0% sodium chloride, 45 mM organic carbon as sodium acetate and 125 ppm iron as ferrous sulfate [16]. Vegetative and stress-growth was performed within a 200-L vertical

sheet photo bioreactor [17]. Growth conditions were varied based on the growth site (in and outdoor). Indoor cultivation was performed as early described by El-Sayed (2007) and El-Sayed *et al.*, (2015) [18, 17]. Fully transparent plexi-columns containing 2L growth medium for each alga separately were used. The light was provided from one side light bank (6x40 watt white cool lamps), free oil, compressed air supported aeration and turbulence from the lower end of the growth column. When growth reached the maximum, the obtained biomass was used for growth scaling up until the desired volume. Outdoor growth was performed as described by El-Sayed *et al.*, (2001) [19]. The microalgae have been harvested by settling and centrifugation at 3000 g in room temperature (Runne Heidelberg RSV-20, Germany) and were dried in an oven (Heraeus, Germany) at 45 °C overnight then ground to a fine powder [18]. Purification of the obtained biomass was performed by a series of precipitation of the microalgae and washings using distilled tap water and a cooling centrifuge.

2.2. Extraction and purification of water-soluble polysaccharide

The dried powder of three microalgae *S. platensis*, *S. obliquus*, and *D. salina* was subjected to cold and hot water extraction methods after defatting using petroleum ether 40-60 °C and chloroform. From defatted dried powder, the isolation of polysaccharides was performed as described in Matloub *et al.*, 2015 [20] and then kept in the refrigerator for chemical and biological evaluations.

2.3. Chemical characterization

The phenol-sulfuric method was used for quantification of total polysaccharide and sugar content in dried algal samples and isolated polysaccharides, respectively [21]. The content of carbon, hydrogen, nitrogen, and sulfur was

determined in the isolated polysaccharides and fractions by Elemental Microanalysis (Elementary Vario EL) [22]. Protein content and the degree of substitution (DS) were calculated as mentioned in Matloub *et al.*, 2015 [20]. IR spectra (using KBr pellets) ranging between 400 and 4000 cm^{-1} were recorded with an FT/IR-6100 (JASCO, Japan). The polysaccharide extracts were hydrolyzed with 4N hydrochloric acid and the hydrolysate was analyzed by GLC analyses (HP 6890, USA), after derivatization using the trimethylsilylation reagent (Merk), under the following condition: ZB-1701 capillary column, 30 m in length, 0.25mm i.d; 0.25 μm 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 (arabinose, fructose, fucose, glucose, galactose, mannose, mannitol, rhamnose, ribose, sorbitol, and xylose).

2.4. Biological studies

2.4.1. Culture cells for *in vitro* antiviral

Human hepatocyte (Huh 7.5), MA104, BGM and Vero cell lines (obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt) were used for growth HCV genotype 4a[ED-43/SG-Feo (VYG) replicon], rotavirus Wa, coxsackievirus B4, and HSV1, respectively. They were cultured using specific growth media Dulbecco's Modified Eagle Medium (DMEM) and will be kept in a CO₂ incubator. The cells were seeded in 96-well tissue culture plates (Greiner Bio-One, Germany) and incubated at 37 °C in a humidified atmosphere of 5% (v/v) CO₂. After 24 h incubation, the medium was discarded from confluent cells monolayers and replenished with 100 μL of bi-fold dilutions of different samples tested prepared in DMEM (GIBCO BRL). For cell controls, 100 μL of DMEM without samples were added [23, 24].

2.4.2. Determination of the nontoxic dose of Huh 7.5, MA104, Vero, and BGM cell lines

Each sample of CEM and HEM polysaccharide extract (50 mg) was dissolved in bi-fold distilled water and decontaminated by adding 24 μ L of 100 \times mixture of antibiotic-antimycotic [penicillin G sodium (10000 IU), streptomycin sulfate (10000 μ g) and amphotericin B (250 μ g)]. To evaluate the nontoxic dose of the samples, tenfold serial dilution of each decontaminated sample was inoculated in Huh 7.5, MA104, Vero and BGM cells. The inverted light microscopy and trypan blue dye exclusion method were used for examining cell morphology and cell viability, respectively [20].

2.4.3. Determination of antiviral effect on HCV genotype 4a, rotavirus Wa, Coxsackievirus B4, and HSV1 strains

HCV RNA in replicon cells was quantified after treatment with the samples as initial titers according to Saeed et al, 2015 [25] while rotavirus Wa strain, Coxsackievirus B4 and Herpes simplex virus type 1 in cultured cells were quantified according to Schmidtke et al, 1998 [26].

2.4.4. Cytotoxic activity on hepatocarcinoma, adenocarcinoma, and colon carcinoma human cell lines

Cytotoxic effect of water-soluble polysaccharides was evaluated on HepG2, MCF7, and HCT116 human cell lines. 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 [27].

2.4.5. The antioxidant study using DPPH free radical scavenging activity

Quantitative measurement of free radical scavenging properties of CEM and HEM

polysaccharides isolated from *S. platensis*, *S. obliquus* and *D. salina* was carried out according to the method of McCue et al. (2003) [28] which stated that 0.1mM solution of 1, 1- diphenyl-2-picryl-hydrazyl (DPPH \cdot) was prepared in 100 ml absolute methanol and 1 mL of this solution was added to 1 mL of each polysaccharide sample and ascorbic acid (reference drug) at three concentrations (1, 10 and 100 μ g/mL). Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. The scavenging ability of DPPH \cdot was calculated using the following equation: Scavenging effect (%) = $A_0 - A_1 / A_0 \times 100$

Where A_0 is the absorbance of DPPH \cdot solution (without the tested polysaccharides) and A_1 is the absorbance of the tested polysaccharides with DPPH \cdot solution.

2.4.6. Assay of acetyl cholinesterase (AChE) enzyme activity by the spectrophotometric method

AChE activity was measured by using spectrophotometer based on Ellman's method [29]. The enzyme hydrolyzes the substrate acetylthiocholine resulting in the product thiocholine which reacted with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. In test tube 1710 μ L of 50 mM Tris-HCl buffer pH 8.0 and 250 μ L of polysaccharide samples of tested microalgae and standard drug at three concentrations of 1, 10 and 100 μ g/ mL, 10 μ L 6.67 U/mL-1 AChE and 20 μ L of 10 mM of DTNB (5,5'-dithio-bis[2-nitrobenzoic acid]) in buffer were added. Positive control namely galanthamine was prepared in serial concentration as same as tested samples by dissolving in 50 mM Tris-HCl buffer pH 8.0. The mixture was incubated for 15 min at 37 $^{\circ}$ C.

Then, 10 μ L of acetylthiocholine iodide (200 mM) in buffer was added to the mixture and the absorbance was measured at 412 nm every 10 sec for 3 min. For a blank, the buffer instead of enzyme solution was used. The enzyme inhibition (%) was calculated from the rate of absorbance change with time ($V = \text{Abs}/\Delta t$) according to calculation as follows:

Inhibition (%) = $100 - \frac{\text{Change of sample absorbance}}{\text{Change of blank absorbance}} \times 100$

The experiment was done in triplicate for each concentration of the tested samples that inhibit the hydrolysis of the substrate (acetylcholine). The percent of acetylcholinesterase inhibition was calculated as follows: % Inhibition = $100 - \left[\frac{\text{Absorbance of the test polysaccharides}}{\text{Absorbance of the control}} \right] \times 100$.

2.4.7. Statistical analysis

Data of cytotoxic activity were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 14 (IBM software, NY, USA). The difference was considered significant where $P < 0.05$. In addition, a probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program. While statistical analysis for antioxidant and anticholinestrace is carried out using two ways ANOVA coupled with CO-state computer program.

3. RESULTS AND DISCUSSION

3.1. Chemical characterization of isolated polysaccharides from *Spirulina platensis*, *Scenedesmus obliquus*, and *Dunaliella salina*:

The phenol-sulfuric estimation of the carbohydrate content of the vegetative and stress forms of *S. platensis* and *S. obliquus* as well as the vegetative form of *D. salina* revealed that total carbohydrate of the vegetative forms of *S.*

platensis, *S. obliquus* and *D salina* were 15.67, 25.33 and 22% w/w, respectively. Whilst the stress forms of *S. platensis* and *S. obliquus* constituted of 11.67 and 27.7% w/w of total carbohydrate content. Also, the phenol-sulfuric study showed that the vegetative forms of *S. platensis*, *S. obliquus* and *D salina* constituted of 8.67, 13.33 and 13% w/w whereas the stress forms of *S. platensis* and *S. obliquus* contained 7.67 and 10% w/w of free sugars, respectively. Consequently, total polysaccharide content of the vegetative form of *S. platensis*, *S. obliquus* and *D salina* was 7, 12 and 9% w/w while, the stress form constituted of 4 and 17.7% w/w, respectively. The obtained results revealed that both forms of *S. obliquus* contained the highest total carbohydrate, free sugars, and polysaccharides contents than that of the other tested microalgae. On the other hand, the stress form of *S. platensis* constituted of less content of carbohydrate, free sugars and polysaccharides than that of vegetative form and this result was in agreement with Lee et al, 2011 [30] which revealed that increase salinity (NaCl concentration $> 0.75 \text{ mol L}^{-1}$) led to a decrease in carbohydrate production. In contrast, increasing salinity, organic carbon, and iron concentrations in growth media of *S. obliquus* led to stimulating polysaccharide production. Liu et al, 2011 observed that the total polysaccharide content of *S. obliquus* increased with increased glyoxylate concentrations as a carbon source [31]. While, Angelaalincy et al, 2017 reported that high salinity and acidic PH had a positive effect on polysaccharide production in *S. obliquus* rather than nitrogen and heavy metal stress [32].

The chemical characterizations of water-soluble polysaccharides (CEM and HEM) isolated from vegetative and stress forms of tested microalgae were compiled in **Table (1)**. The sugar content of isolated polysaccharide was ranged from 47-66%. HEM polysaccharide

isolated from *D. salina* stood the highest sugar content whilst the lowest content was found in CEM polysaccharide of *D. salina*. Interestingly, the sugar content of obtained polysaccharides of stress form was found higher than that of vegetative form. The nitrogen content of isolated polysaccharides was ranged from 2.38 to 6.57% corresponding to 14.88-41.06 % of protein. Being

higher in the polysaccharide extracts of vegetative form of *S. platensis* (41.06% and 36.94% protein in HEM and CEM, respectively) than other polysaccharide extracts and this result was agreement with the study of Matloub et al. 2017 [5]. From **Table 2**, the degree of substitution for sulfate (DS sulfate) was found to be very low in all isolated polysaccharides.

Table 1. Chemical characterization of polysaccharides isolated from vegetative and stress forms of *Spirulina platensis*, *Scenedesmus obliquus* and vegetative form of *Dunaliella salina*

Characters	% of isolated polysaccharide									
	<i>Spirulina platensis</i>				<i>Scenedesmus obliquus</i>				<i>Dunaliella salina</i>	
	Vegetative form		Stress form		Vegetative form		Stress form		Vegetative form	
	CEM	HEM	CEM	HEM	CEM	HEM	CEM	HEM	CEM	HEM
Sugar content	61.0	56.0	59.0	57.0	53.0	56.0	62.0	65.0	47.0	66.0
Carbon (%)	31.79	33.05	29.93	27.38	17.73	20.85	23.89	24.85	19.96	26.99
Hydrogen (%)	2.29	2.44	1.84	1.78	1.27	1.51	1.68	2.06	2.75	2.90
Nitrogen (%)	5.91	6.57	5.18	4.23	3.11	3.22	2.65	2.38	4.18	4.76
Sulfur (%)	1.11	0.59	0.63	0.68	0.74	0.60	0.43	0.27	1.11	0.79
Protein (%)	36.94	41.06	32.38	26.44	19.44	20.13	16.56	14.88	26.13	29.75
Sulfation degree	0.08	0.04	0.05	0.06	0.09	0.06	0.04	0.02	0.13	0.07

CEM: Polysaccharides isolated from cold water extract, HEM: Polysaccharides isolated from hot water extract

The IR spectrum data of vegetative and stress forms of *S. platensis*, *S. obliquus*, and *D. salina* were illustrated in **Figs. (1-3)** showed the most pronounced functional groups for polysaccharides. The major absorption bands around 3405.67 - 3445.21 cm^{-1} and bands around 2924.52 - 2961.16 cm^{-1} attributed to O-H stretching of hydroxyls and CH stretching peak of CH_2 groups, respectively. Meanwhile, the absorption bands around 1641.13-1681.62 cm^{-1} and 1525.42 -1553.38 cm^{-1} assigned for CO stretching in secondary amides (amide I) and N-H deformation and C-N stretching in -CO-NH- of protein (amide-II), respectively. This result in addition to microelement analysis confirmed the fact that polysaccharide extracts bounded with protein. The absorption band around 1410.67-1456.96 cm^{-1} assigned 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 around 1229.40 - 1251.58 cm^{-1} and around 1307.5-1321 cm^{-1} were assigned as S=O stretching vibration indicating the presence of esterified sulfate. Whereas, bands around 873.59-881.30 cm^{-1} attributed to β -configuration of glycosidic linkage. The bands around 709.67-789.70 cm^{-1} attributed to the bending vibration of C-O-S of sulfate in the equatorial position. Further, 552.50-698.10 cm^{-1} corresponded to the asymmetric deformation of O-S-O groups. While the vibration of the C-O-C bridge of glucosides was recorded at wave numbers 1035.59-1070.30 cm^{-1} .

The GLC analysis of CEM and HEM hydrolysates of stress form of *S. platensis* and *S. obliquus* as well as a vegetative form of *D. salina* were compiled in **Table (2)**. Eight monosaccharides were detected in CEM and HEM of *S. platensis* and *S. obliquus*, as well as ten and seven monosaccharides, were identified in CEM & HEM of *D. salina*, respectively.

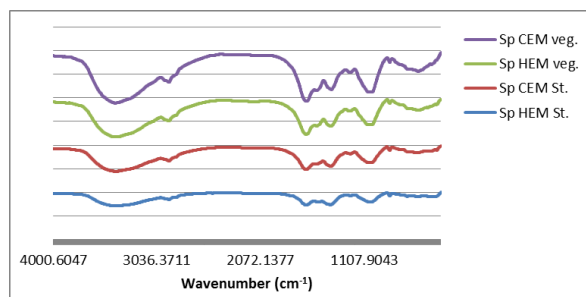


Fig.1. IR spectra of polysaccharides isolated from *Spirulina platensis*

Sp CEM veg = Polysaccharides isolated from cold water extract of vegetative form of *S.platensis*.

Sp HEM veg= Polysaccharides isolated from hot water extract of vegetative form of *S. platensis*.

Sp CEM st = Polysaccharides isolated from cold water extract of stress form of *S. platensis*.

Sp HEM st= Polysaccharides isolated from hot water extract of stress form of *S. platensis*

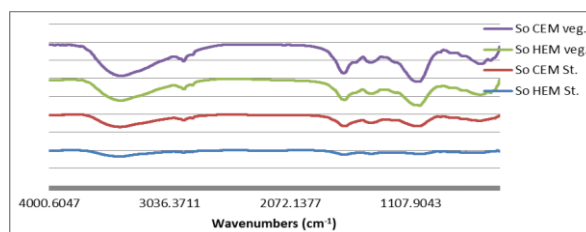


Fig. 2. IR spectra of polysaccharides isolated from *Scenedesmus obliquus*

So CEM veg = Polysaccharides isolated from cold water extract of vegetative form of *S. obliquus*

So HEM veg = Polysaccharides isolated from hot water extract of vegetative form of *S. obliquus*.

So CEM st = Polysaccharides isolated from cold water extract of stress form of *S. obliquus*.

So HEM st = Polysaccharides isolated from hot water extract of stress form of *S. obliquus*.

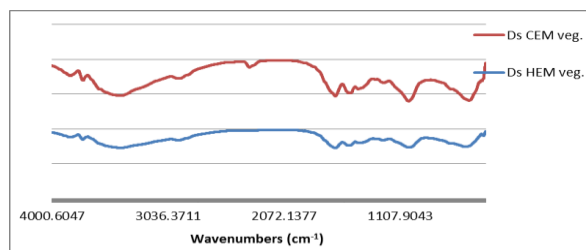


Fig. 3. IR spectra of polysaccharides isolated from *Dunaliella salina*

Ds CEM veg = Polysaccharides isolated from cold water extract of vegetative form of *D. salina*.

Ds HEM veg = Polysaccharides isolated from hot water extract of vegetative form of *D. salina*

Table 2. Comparative GLC analysis of the isolated polysaccharide hydrolysates of *Spirulina platensis*, *Scenedesmus obliquus*, and *Dunaliella salina*

Sugar	RRt*	Relative%**					
		<i>Spirulina platensis</i>		<i>Scenedesmus obliquus</i>		<i>Dunaliella salina</i>	
		CEM	HEM	CEM	HEM	CEM	HEM
Arabinose	0.58	6.56	6.42	10.52	11.67	9.39	9.59
Xylose	0.59	9.69	10.29	7.23	9.67	7.10	10.89
Ribose	0.62	2.58	2.40	3.05	2.22	5.49	6.13
Rhamnose	0.67	10.07	11.88	15.78	19.84	9.29	21.83
Fucose	0.68	4.14	5.85	3.17	3.23	6.95	6.63
Mannitol	0.82	-	-	-	-	4.28	-
Sorbitol	0.84	-	-	-	-	6.33	-
Fructose	0.89	-	-	-	-	10.54	-
Galactose	0.99	26.99	26.01	27.81	28.76	22.75	31.75
Mannose	1.02	27.37	24.77	17.76	12.95	-	-
Glucose	1.05	12.60	12.08	14.67	11.66	17.46	13.18

*RRt: relative retention time

** Relative% = the relative area% of each sugar to the total identified sugars.

CEM: Polysaccharides isolated from cold-water extract

HEM: Polysaccharides isolated from hot water extract

Table 3. Antiviral activity of nontoxic doses of the isolated polysaccharides from microalgae *Spirulina platensis*, *Scenedesmus obliquus*, and *Dunaliella salina*

Isolated polysaccharide	Non-toxic dose (mg/ml)	Mean % of reduction			
		HCV genotype 4	rotavirus Wa strain	Herpes Simplex Virus	Coxsackievirus B4
<i>S. platensis</i> CEM	1.8	86.7	70	50	73.3
<i>S. platensis</i> HEM	1.4	86.7	70	50	70
<i>S. obliquus</i> CEM	1.5	40	30	10	40
<i>S. obliquus</i> HEM	1.1	30	30	20	30
<i>D. salina</i> CEM	1.4	20	30	30	36.7
<i>D. salina</i> HEM	1.3	20	30	30	30

S. platensis CEM = Polysaccharides isolated from cold water extract of the stress form of *S. platensis*.

S. platensis HEM = Polysaccharides isolated from hot water extract of stress form of *S. platensis*.

S. obliquus CEM = Polysaccharides isolated from cold water extract of stress form of *S. obliquus*.

S. obliquus HEM = Polysaccharides isolated from hot water extract of stress form of *S. obliquus*.

D. salina CEM = Polysaccharides isolated from cold water extract of vegetative form of *D. salina*.

D. salina HEM = Polysaccharides isolated from hot water extract of vegetative form of *D. salina*.

Galactose, mannose, glucose, rhamnose, and xylose were found as predominant sugars in stress form of *S. platensis* CEM and HEM polysaccharides with molar ratio (2.14: 2.17: 1.00: 0.80: 0.76) and (2.15: 2.05: 1.00: 0.98: 0.85), respectively, in addition fucose, arabinose and ribose. Whereas, stress form of *S. obliquus* CEM and HEM polysaccharides composed of galactose, mannose, glucose, rhamnose and arabinose with molar ratio (1.90: 1.21: 1.00: 1.08: 0.72) and (2.46: 1.11: 1.00: 1.70: 1.00), respectively, in addition fucose, xylose and ribose were found as trace sugars. Furthermore GC

analysis of CEM and HEM hydrolysates of vegetative form of *D. salina* revealed that CEM contained mainly galactose, glucose, fructose, arabinose and rhamnose with molar ratio (1.30: 1.00: 0.60: 0.54: 0.53) while HEM hydrolysates composed mainly of galactose, rhamnose, glucose and xylose with molar ratio (2.40: 1.65: 1.00: 0.83). In addition, mannitol and sorbitol were found only in CEM of *D. salina*.

The integration of GC, FT-IR and microelement analysis revealed that the isolated polysaccharides from tested microalgae were heterogeneous and bounded with protein.

3.2. Biological activity

3.2.1. Antiviral activities

The determination of nontoxic dose of the isolated polysaccharide of stress form of *S. platensis* and *S. obliquus* as well as vegetative form of *D. salina* against Huh 7.5, MA104, BGM and Vero cell lines showed the same toxicity for each isolated polysaccharide and their nontoxic concentration was ranged from 1.1 to 1.8 mg/mL (**Table 3**). The antiviral activity of the isolated polysaccharide against HCV, rotavirus, coxsackievirus and HSV1 was compiled in **Table (3)**. The polysaccharides (CEM and HEM) isolated from stress form of *S. platensis* have promising antiviral activity against HCV genotype 4a replicon, coxsackievirus B4, rotavirus and herpes simplex virus 1 which reduced replication of tested virus about 50–87.6% at nontoxic doses 1.8 and 1.5 mg/mL, respectively. Both polysaccharides isolated from *S. platensis* exhibited the highest antiviral activity on HCV genotype 4a replicon > coxsackievirus B4 > rotavirus > herpes simplex virus 1 than other isolated polysaccharides. While, the polysaccharide CEM and HEM isolated from stress form of *Scenedesmus obliquus* and vegetative form of *D. salina* showed a considerable antiviral activity; inhibit HCV genotype 4a replicon, coxsackievirus B4, rotavirus and herpes simplex virus 1 replication about 10-40% at nontoxic concentrations. Several studies reported that the polysaccharides isolated from different *Spirulina species* showed broad spectrum antiviral activity against vaccinia virus (VACV and VACV-GFP), ectromelia virus (ECTV), herpes simplex virus types 1 and 2 (HSV-1 & 2), human cytomegalovirus (HCMV), measles virus, mumps virus, the human immunodeficiency virus type 1 (HIV-1) and influenza virus type A (Flu-A) [2, 33].

3.2.2. Cytotoxic activity

Assessment of cytotoxic activity of the CEM and HEM of stress form of *S. platensis* and *S. obliquus* as well as a vegetative form of *D. salina* *in vitro* on HepG2, MCF7 and HCT116 human cell lines comparing with doxorubicin as reference drug was illustrated in **Figs. 4-6**. The cytotoxic activity revealed that the polysaccharide *D. salina* HEM with an IC₅₀ value 64.2 µg/mL exhibited significantly a potent cytotoxicity effect on HCT116 human cell line than other tested isolated polysaccharides. The percentage inhibition of different tested cell lines at the maximum concentration tested (100 µg/mL) were compiled in **Table 4**. The IC₅₀ values of other polysaccharides could not be determined even at the maximum concentration (100 µg/mL). **Table 4** showed that the *S. obliquus* CEM inhibited 50.4% of HepG2 cell line at the maximum concentration tested. Matloub et al, (2017) found that glycoprotein isolated by hot aqueous extraction method exhibited cytotoxic activity against Hep G2 *in vitro* with the IC₅₀ of 69.49 µg/mL [5].

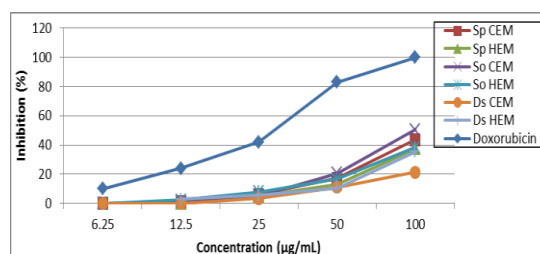


Fig. 4. Cytotoxic activity of the isolated polysaccharides against HepG2 human cell line *in vitro*

Sp CEM = Polysaccharides isolated from cold water extract of the stress form of *Spirulina platensis*.

Sp HEM = Polysaccharides isolated from hot water extract of stress form of *Spirulina platensis*.

So CEM = Polysaccharides isolated from cold water extract of stress form of *scenedesmus obliquus*.

So HEM = Polysaccharides isolated from hot water extract of stress form of *scenedesmus obliquus*.

Ds CEM = Polysaccharides isolated from cold water extract of vegetative form of *Dunaliella salina*.

Ds HEM = Polysaccharides isolated from hot water extract of vegetative form of *Dunaliella salina*

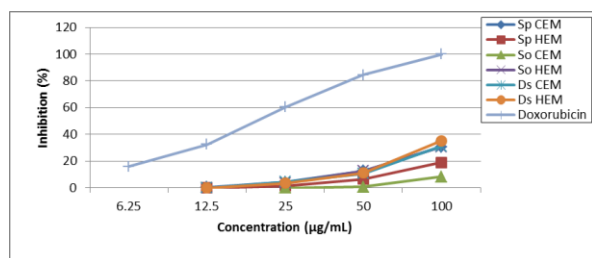


Fig 5. Cytotoxic activity of the isolated polysaccharides against MCF7 human cell line *in vitro*

Sp CEM = Polysaccharides isolated from cold water extract of the stress form of *Spirulina platensis*.

Sp HEM = Polysaccharides isolated from hot water extract of stress form of *Spirulina platensis*.

So CEM = Polysaccharides isolated from cold water extract of stress form of *scenedesmus obliquus*.

So HEM = Polysaccharides isolated from hot water extract of stress form of *scenedesmus obliquus*.

Ds CEM = Polysaccharides isolated from cold water extract of vegetative form of *Dunaliella salina*.

Ds HEM = Polysaccharides isolated from hot water extract of vegetative form of *Dunaliella salina*

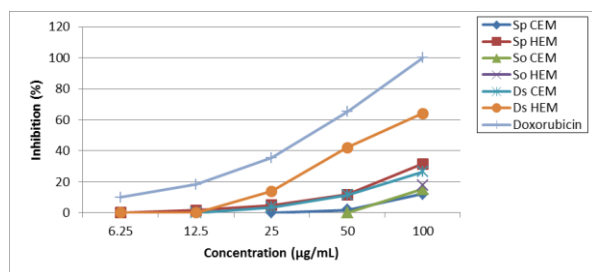


Fig 6. Cytotoxic activity of the isolated polysaccharides against HCT116 human cell line *in vitro*

Sp CEM = Polysaccharides isolated from cold water extract of the stress form of *Spirulina platensis*.

Sp HEM = Polysaccharides isolated from hot water extract of stress form of *Spirulina platensis*.

So CEM = Polysaccharides isolated from cold water extract of stress form of *scenedesmus obliquus*.

So HEM = Polysaccharides isolated from hot water extract of stress form of *scenedesmus obliquus*.

Ds CEM = Polysaccharides isolated from cold water extract of vegetative form of *Dunaliella salina*.

Ds HEM = Polysaccharides isolated from hot water extract of vegetative form of *Dunaliella salina*

3.2.3. DPPH free radical scavenging activity

From **Table (5)** it can be noticed that the increase in the % of inhibition is dose dependent relationship i.e. increase in the percent of inhibition as an increase in the concentration of isolated polysaccharides compared to standard

ascorbic acid. Additionally, *D. salina* CEM, HEM and *S. obliquus* CEM showed the significantly highest percent of inhibition (308.16, 308.16, and 308.69%, respectively) at 100 µg/mL, followed by *S. platensis* HEM and *S. obliquus* CEM polysaccharides as they recorded % inhibition 206.12 and 200.20%, respectively at 100 µg/mL. Matloub et al. (2017) found that *Spirulina* cold water extract SCEM had scavenging efficacy against nitric oxide [5].

3.2.4. Acetylcholinesterase

Table (6) illustrated cholinesterase % inhibition activity of *D. salina*, *S. obliquus*, and *S. platensis* and revealed dose dependant relationship of both *D. salina* and *S. obliquus*, While *S. platensis* showed inverse relation i.e. decrease in % inhibition with increase in concentration, so it recorded the highest significant % inhibition at 1 µg/mL and *S. platensis* HEM showed significant increase in % inhibition activity compared to *S. platensis* CEM. On the other hand, *S. obliquus* CEM and HEM polysaccharides exhibited the significant highest cholinesterase % inhibition activity as they recorded 48.69 and 42.20%, followed by *D. salina* CEM and HEM polysaccharides which exhibited 32.00 and 39.36%, respectively, at concentration of inhibitor 100 µg/mL compared to galanthamine standard (78.00%).

Polysaccharides have still gained interest in the biomedical and pharmaceutical industries because of their non-toxicity, safety, biodegradability, and biocompatibility.

Several studies evaluated the antiviral activity of polysaccharides isolated from microalgae particular *Spirulina platensis* against the pathogenic human virus. These studied revealed that the polysaccharides had broad antiviral spectrum against enveloped viruses such as the herpes simplex virus type 1 (HSV-1), the human immunodeficiency virus type 1 (HIV-1) or

influenza virus type A (IFV-A). The mode of antiviral action of polysaccharides is still not recognized but it may be attributed to inhibition

viral adsorption, the penetration, or the replication in the host cells [33-35].

Table 4. Cytotoxic activity of the isolated polysaccharides from *S. platensis*, *S. obliquus* and *D. salina* at maximum concentration (100 µg/mL)

Tested polysaccharides	% of inhibition± SD		
	HepG2	MCF7	HCT116
<i>S. platensis</i> CEM	43.5±1.93 ^a	30.7±1.32 ^j	12±0.74 ⁿ
<i>S. platensis</i> HEM	37.4±2.08 ^b	18.9±1.63 ^h	31.4±1.56 ^p
<i>S. obliquus</i> CEM	50.4±1.37 ^c	8.4±1.15 ^t	15.1±2.84 ^q
<i>S. obliquus</i> HEM	38.4±2.54 ^b	30.7±0.95 ^j	17.6±2.69 ^q
<i>D. salina</i> CEM	21±2.71 ^e	31.1±1.1 ^j	26.5±1.07 ^r
<i>D. salina</i> HEM	35±2.79 ^t	35.1±2.51 ^k	64.2±2.36 ^s
Doxorubicin (reference drug)	100±1.37 ^g	100±1.63 ^m	100±1.07 ^t

Data are represented by the mean ± SD of three replicate. Statistical analysis is carried out using one way ANOVA, where similar letters are insignificant and different letters are significant at $P < 0.05$.

S. platensis CEM = Polysaccharides isolated from cold water extract of the stress form of *S. platensis*. *S. platensis* HEM = Polysaccharides isolated from hot water extract of stress form of *S. platensis*. *S. obliquus* CEM = Polysaccharides isolated from cold water extract of stress form of *S. obliquus*. *S. obliquus* HEM = Polysaccharides isolated from hot water extract of stress form of *S. obliquus*. *D. salina* CEM = Polysaccharides isolated from cold water extract of vegetative form of *D. salina*. *D. salina* HEM = Polysaccharides isolated from hot water extract of vegetative form of *D. salina*.

In the current study noted that both polysaccharides isolated from *S. platensis* were bounded with protein and had nearly the same chemical composition, constituted highest Gal/Glu and Man/Glu ratios in double folds (i.e galactose and mannose contents are 2 folds to glucose content) than other tested polysaccharides. On the other hand, these polysaccharides exhibited potent antiviral activity

than other tested polysaccharides. this result was agreed with Matloub et al., 2017 [5] where the polysaccharides isolated from a vegetative form of *S. platensis* reduced replication of HCV genotype 4a replicon to 50% which composed of Gal/Glu and Man/Glu ratios in 0.5 fold (i.e galactose and mannose 0.5 fold to glucose). While the polysaccharides isolated from stress form reduced replication of HCV genotype 4a

replicon to 85%. Galctose and mannose to glucose ratios may be played a key role in their antiviral activity.

Chakraborty et al., (2012) reported that stress factors through growth microalgae contributed to

the production of polysaccharides and influence the changes in the structure and functional properties of these polysaccharides [36].

Table 5. % inhibition of polysaccharides isolated from *Spirulina platensis*, *Scenedesmusobliquus* and *Dunaliella salina* with ascorbic acid using DPPH

Concentration Samples	% of inhibition \pm SD		
	1 $\mu\text{g}/\text{mL}$	10 $\mu\text{g}/\text{mL}$	100 $\mu\text{g}/\text{mL}$
control	0.98 \pm 0.05 ^a	1.01 \pm 0.02 ^a	0.98 \pm 0.005 ^a
<i>S. platensis</i> CEM	10.20 \pm 0.95 ^e	24.49 \pm 1.10 ^m	206.12 \pm 6.09 ^s
<i>S. platensis</i> HEM	20.2 \pm 1.76 ^b	34.45 \pm 2.23 ⁿ	171.43 \pm 11.45 ^q
<i>S. obliquus</i> CEM	195.92 \pm 10.22 ^d	308.16 \pm 14.11 ^k	308.69 \pm 14.90 ^p
<i>S. obliquus</i> HEM	2.14 \pm 0.056 ^e	146.94 \pm 9.01 ^l	308.16 \pm 15.87 ^q
<i>D. salina</i> CEM	10.2 \pm 0.80 ^b	24.48 \pm 2.12 ⁱ	200.00 \pm 12.66 ^q
<i>D. salina</i> HEM	22.45 \pm 1.22 ^c	97.95 \pm 12.98 ^j	308.16 \pm 20.00 ^p
Ascorbic Acid	29.32 \pm 1.23 ^h	80.00 \pm 10.22 ^o	94.82 \pm 1.10 ^j

Data are represented by the mean \pm SD of three replicate. Statistical analysis is carried out using two ways ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at $P < 0.05$.

S. platensis CEM = Polysaccharides isolated from cold water extract of the stress form of *S. platensis*.

S. platensis HEM = Polysaccharides isolated from hot water extract of stress form of *S. platensis*.

S. obliquus CEM = Polysaccharides isolated from cold water extract of stress form of *S. obliquus*.

S. obliquus HEM = Polysaccharides isolated from hot water extract of stress form of *S. obliquus*.

D. salina CEM = Polysaccharides isolated from cold water extract of vegetative form of *D. salina*.

D. salina HEM = Polysaccharides isolated from hot water extract of vegetative form of *D. salina*.

The significant antioxidant activity observed in our study is agreed with Wu et al. (2017) [37] who showed that polysaccharides of *Spirulina platensis* which purified by DEAE Sephadex A-50 and high speed counter-current chromatography (HSCCC) had strong scavenging effects with a similar concentration dependence property on hydroxyl free radical and DPPH free radical. Additionally, concerning with the noticeable antioxidant activity of *Scenedesmus* polysaccharides, the present results are in concomitant with the study of Ishaq et al. (2016) who illustrated that *Scenedesmus* polysaccharides protected against oxidative stress [6]. Further, the significant antioxidant activity detected in our study was in accordance with Liu et al. (2011)

who declared that polysaccharide fractions separated from *Dunaliella salina* (PD1, PD2, and PD3) exhibited varies different antioxidant activity [38].

On the other hand, the anticholinesterase activity of *Scenedesmus* sp was estimated for the first time in the present study and demonstrated promising inhibiting activity at a high concentration of polysaccharides. In addition, marked anticholinesterase activity of *Dunaliella salina* was observed in the current study, which is run in parallel with the findings of Aly et al. (2016) who found that *D. salina* exhibited neuro-modulating effect against Alzheimer's disease in rats in comparison with Donepezil reference drug [39].

Table 6. Cholinesterase % inhibition of isolated polysaccharides from *Spirulina platensis*, *Scenedesmus obliquus* and *Dunaliella salina* with galanthamine

Concentration Samples	% of inhibition \pm SD		
	1 μ g/mL	10 μ g/mL	100 μ g/mL
control	2.02 \pm 0.12 ^a	1.81 \pm 0.34 ^a	1.98 \pm 0.02 ^a
<i>S. platensis</i> CEM	29.16 \pm 1.70 ^b	12.17 \pm 1.22 ^m	6.43 \pm 0.19 ^s
<i>S. platensis</i> HEM	31.20 \pm 1.06 ^t	16.45 \pm 1.03 ⁿ	9.12 \pm 0.85 ^q
<i>S. obliquus</i> CEM	26.29 \pm 2.00 ^d	39.16 \pm 3.10 ^k	48.69 \pm 4.20 ^p
<i>S. obliquus</i> HEM	24.00 \pm 1.55 ^e	35.94 \pm 9.01 ^l	42.20 \pm 15.87 ^q
<i>D. salina</i> CEM	31.90 \pm 3.20 ^b	32.00 \pm 2.40 ^b	32.00 \pm 2.06 ^b
<i>D. salina</i> HEM	40.15 \pm 3.92 ^c	39.00 \pm 2.90 ^c	39.36 \pm 2.00 ^c
Galanthamine standard (μ g/mL)	56.00 \pm 3.55 ^f	60.60 \pm 2.77 ^f	78.00 \pm 3.09 ^u

Data are represented by the mean \pm SD of three replicate. Statistical analysis is carried out using two ways ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at $P < 0.05$.

S. platensis CEM = Polysaccharides isolated from cold water extract of the stress form of *S. platensis*.

S. platensis HEM = Polysaccharides isolated from hot water extract of stress form of *S. platensis*.

S. obliquus CEM = Polysaccharides isolated from cold water extract of stress form of *S. obliquus*.

S. obliquus HEM = Polysaccharides isolated from hot water extract of stress form of *S. obliquus*.

D. salina CEM = Polysaccharides isolated from cold water extract of vegetative form of *D. salina*.

D. salina HEM = Polysaccharides isolated from hot water extract of vegetative form of *D. salina*.

4. CONCLUSION

Among edible microalgae, *S. platensis*, *S. obliquus*, and *D. salina* are paid attention because of their nutritional value for human and aquatic animals beside their medicinal applications. The polysaccharides isolated from microalgae have still attracted to scientist because of their special physicochemical properties and varied biological activities. They are crucial sources of structurally diverse bioactive polysaccharides and remain largely unexploited in nutraceutical and pharmaceutical areas. Fortunately, the possibility for optimization of these biopolymer productions by manipulating growth conditions is economically easy for biomedical and pharmaceutical industries. Our investigation emphasized these microalgal biopolymers have great therapeutically potential in drug development used as broad spectrum antiviral especially enveloped virus, antitumor, antioxidant and anticholinestras agents in near future.

Conflict of interest statement

The authors declare that they have no conflict of interest.

5. REFERENCES

1. Philippe M. Polysaccharides from Microalgae, What's Future? *Adv Biotech & Micro* 2018; 8(2): AIBM.MS.ID.555732.
2. Raposo MF, Costa de Morais RM, and Bernardo de Morais AM. Bioactivity and applications of Sulphated Polysaccharides from Marine Microalgae. *Mar. Drugs* 2013; 11: 233-252.
3. Morais MG, Stillings C, Dersch R, Rudisile M, Pranke P, Costa JA and Wendorff J. Preparation of nanofibers containing the microalga *Spirulina* (*Arthrospira*). *Bioresour. Technol* 2010; 101: 2872–2876.
4. Abd El Baky H, El Baz HK and EL-Latife SA. Induction of Sulfated Polysaccharides in *Spirulina platensis* as Response to Nitrogen Concentration. *Aquaculture Research and Development* 2014; 5: 206.
5. Matloub AA, El-Senousy WM, El-Sayed AB, and Aly HF, 2017, *In vitro* assessment of anti-HCV, antioxidant, cytotoxic and hypolipidemic activities of glycoprotein isolated from *Spirulina platensis*, *Asian Pac J Trop Dis* 2017; 7(11): 676-682.
6. Ishaq AG, Matias-Peralta HM and Basri H. Bioactive compounds from green microalga – *Scenedesmus* and its potential applications: A brief review. *Pertanika J. Trop. Agric. Sci.* 2016; 39 (1): 1 – 16.
7. Zheng SZ, Wang DY, Zhang Q, and Shen XW. Chemical studies on the polysaccharide from *Dunelinella salina* (I). *Journal of Northwest Normal University (Natural Science Edition)* 1997; 33(4): 93–95.
8. Dai J, Wu Y, Chen S, Zhu S, Yin H, Wang M and Tang J. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohydrate Polymers* 2010; 82(3):629-635.
9. Zhang TT, Yin HP, Sheng YQ, Wang M. Studies on antiviral and immune activities of polysaccharides from *Dunaliella salina*. *Pharmaceutical Biotechnology* 2009; 04. Website: http://en.cnki.com.cn/Article_en/CJFDTotat-YWSW200904009.htm
10. Goyal M and Baranwal M. Extracellular Polysaccharide Isolated from *Dunaliella salina* having Immunomodulatory and Cytotoxic Activity. *Indo Global Journal of Pharmaceutical Sciences* 2017; 7(1): 61.
11. Olasehinde TA, Olaniran AO and Okoh AI. Therapeutic Potentials of Microalgae in the Treatment of Alzheimer's disease. *Molecules* 2017; 22: 480. Doi: 10.3390/molecules22030480.

12. Lima FA, Joventino IP, Joventino FP, Cordeiro de Almeida A, Neves KR, Regina do Carmo M, Leal LK, Matos de Andrade G, and Viana GS. Neuroprotective Activities of *Spirulina platensis* in the 6-OHDA Model of Parkinson's disease are related to Its Anti-Inflammatory effects. *Neurochem Res* 2017; 42:3390–3400.
13. Zarrouk C. Contribution á l' etude d'une cyanophyceé . Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. Ph. D. Thesis, Université de Paris 1966; 22:100–108.
14. Stainer RY, Kunisawa R, Mandel MC. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev* 1971; 35:171-205.
15. Kester DR, Duedall IW, Connors DN, and Pytkowicz RM. Preparation of Artificial Seawater. *Limnology & Oceanography* 1967; 12: 176—179.
16. El-Sayed AB. Carotenoids accumulation in the green alga *Scenedesmus* sp. incubated with industrial citrate waste and different inductions stress. *Nature and Science* 2010; 8(10):34-40.
17. El-Sayed AB, Battah MG, and El-Sayed EW. Utilization efficiency of artificial carbon dioxide and corn steam liquor by *Chlorella vulgaris*. *Biolife* 2015; 3(2):391-403.
18. El-Sayed AB. Economizing of intensive outdoor mass production of the green alga *Scenedesmus* sp. *Egyptian J. of Phycology* 2007; 8: 85-96.
19. El-Sayed AB, Abdalla FE, and Abdel-Maguid AA. Use of Some Commercial Fertilizer Compounds for *Scenedesmus* Cultivation. *Egyptian J. of Phycology* 2001; 2: 9-16.
20. Matloub AA, El-souda SS, El-Senousy WM, Hamed M, Aly H, and Ali SA. In vitro antiviral, cytotoxic, antioxidant and hypolipidemic activities of polysaccharide isolated from marine algae. *Int J Pharm Phytochem* 2015; 7(5): 1099-111.
21. Albalasmeh AA, Berhe AA, Ghezzehei TA. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydr Polym* 2013; 97: 253-61.
22. Matloub AA, El-Sherbini M, Borai IB, Magda KE, Rizk MZ, and Aly HF. Assessment of anti-hyperlipidemic effect and physico-chemical characterization of water soluble polysaccharides of *Ulva fasciata* Delile. *Appl Sci Res* 2013; 9(4): 2983-93.
23. Simões, C., Amoros, M., & Girre, L. Mechanism of antiviral activity of triterpenoid saponins. *Phytother Res*, 1999; 21: 317–325.
24. Walum, E., Strenberg, K., & Jenssen, D. Understanding cell toxicology: Principles and practice. NewYork: Ellis Howood., 1990; pp. 97–111.
25. Saeed M, Troels K, Scheel H, Gottwein J M, Marukian S, Dustin LB, Bukh J, and Rice CM. Efficient Replication of Genotype 3a and 4a Hepatitis C Virus Replicons in Human Hepatoma Cells. *Antimicrob. Agents Chemother* 2012; 56(10): 5356-5373.
26. Schmidtke M, Knorre C, Blei L, Stelzner A, Birch-Hirschfeld E. Penetration and Antiviral Activity of Coxsackievirus B3 (Cvb3)-Specific Phosphorothioate Oligodeoxynucleotides (Ps-Odn). *Nucleosides and Nucleotides* 1998; 17: 1557-1566.
27. Mosmann T. Rapid colorimetric assays for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65:55-63.
28. McCue P, Horii A and Shetty K. Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by *Rhizopus oligosporus*: the

- role of carbohydrate-cleaving enzymes. *J Food Biochem*, 2003; 27(6): 501-514.
29. Ellman GL, Courtney KD, Andres VJ, and Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol* 1961; 7: 88-95.
 30. Lee MC, Chen YC, and Peng TC. A two-stage culture method for optimized polysaccharide production in *Spirulina platensis*. *J Sci Food Agric*. 2012; 92(7):1562-9.
 31. Liu Y, Wang W, Zhang M, Xing P, Yang Z. PSII-efficiency, polysaccharide production, and phenotypic plasticity of *Scenedesmus obliquus* in response to changes in metabolic carbon flux. *Biochemical Systematics and Ecology* 2010; 38(3): 292-299.
 32. Angelaalincy M, Senthilkumar N, Karpagam R, Kumar GG, Ashokkumar B, and Varalakshmi P. Enhanced Extracellular polysaccharide production and self-sustainable Electricity generation for PAMFCs by *Scenedesmus* sp.SB1. *American Chemical Society (ACS) Omega* 2017; 2:3754–3765.
 33. Mader J, Gallo A, Schommartz T, Handke W, Nagel CH and Gunther P. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. *J Allergy Clin Immunol* 2016; 137: 197-203.
 34. Falaise C, François C, Travers MA, Morga B, Haure J, Tremblay R, Turcotte F, Pasetto P, Gastineau R, Hardivillier Y, Leignel Vand Mouget JL. Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. *Mar. Drugs* 2016; 14: 159; doi:10.3390/md14090159.
 35. Hayashi T. Studies on the evaluation of natural products for antiviral effects and their applications. *Yakugaku Zasshi* 2008; 128(1): 61-79.
 36. Chakraborty T, Sen AK, and Pal R. Chemical characterization and the stress induced changes of the extracellular polysaccharide of the marine cyanobacterium, *Phormidium tenue*. *J. Algal Biomass Utln* 2012; 3: 11– 20.
 37. Wu X, Li R, Zhao Y, and Liu Y. Separation of polysaccharides from *Spirulina platensis* by HSCCC with ethanol-ammonium sulfate ATPS and their antioxidant activities. *Carbohydrate Polymers* 2017; 173: 465-472.
 38. Liu A, Luo J, Liu D, Zhan H and Zheng J. Extraction of the polysaccharides from *Dunaliella salina* by alkaline protease and its oxidative stability. *IEEE* 2011; 978(1):4244-5089.
 39. Aly H, EL-Baz FK, and Ali GH. Neuromodulating effect of *Dunaliella salina* extract in the regression of Alzheimer's disease in rats. *Int J Pharm Bio Sci* 2016; 7(3): (B) 921 – 931.