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In vitro assessment of anti-HCV, antioxidant, cytotoxic and hypolipidemic activities of glycoprotein isolated from *Spirulina platensis*

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ABSTRACT

Objective: To evaluate the glycoprotein isolated from *Spirulina platensis* (*S. platensis*) as anti-HCV, cytotoxicity, antioxidant and hypolypidemic activities.
Method: Cold and hot aqueous extraction methods (SCEM and SHEM) of *S. platensis* were performed and their physico-chemical characterizations were studied. Further, monosaccharides and amino acids composition of SCEM and SHEM were studied using GLC and amino acid analyzer, respectively. Both glycoproteins SCEM and SHEM were evaluated *in vitro* for anti-HCV replicon, cytotoxicity, antioxidant and hypolipidemic activities. SCEM was fractionated and their physico-chemical characterization and anti-HCV replicon were studied.
Results: The yield of SCEM and SHEM was 4.45% and 3.37% of dried algal sample, respectively. The physico-chemical characterizations of SCEM and SHEM revealed the presence of ash (13.33% and 10.42% w/w), sulfur (1.22% and 0.71% w/w), nitrogen (7.14% and 5.59% w/w) and sugar (67.29% and 64.66% w/w) contents. The physico-chemical characterizations confirmed that SCEM and SHEM were polysaccharide bounded protein (glycoprotein). Twelve and eleven sugars could be identified in SCEM and SHEM polysaccharide of *S. platensis* using gas chromatography analysis, respectively. Glucose, galactose and mannose are predominant sugars in both extracts. Further, amino acid analysis of SCEM and SHEM revealed the presence 16 amino acids. Aspartic acid and alanine were detected as predominant non-essential amino acids in SCEM while glutamic and aspartic acids were existed as dominant amino acids in glycoprotein SHEM. Whereas leucine, phenylalanine and valine were presented as mean essential amino acids. Evaluation of both glycoproteins of SCEM and SHEM for anti-HCV, cytotoxic, antioxidant, and hypolipidemic activities revealed that SCEM reduced the HCV (genotype 4 replicon) to 50% at non-toxic dose (522 μg/mL). In addition, SCEM inhibitd enzyme activity, β-hydroxy-β- methyl glutaryl coA reductase, to 80% and had scavenging efficacy against nitric oxide 67.57%–62.16% at the concentration of 100–500 μg/mL. While, SHEM exhibited cytotoxic activity against Hep G2 human cell line with IC_{50} of 69.49 μg/mL.
Conclusions: Polysaccharide bounded protein (glycoprotein) isolated from cold water extract of *S. platensis* might become increasingly important in drug development for treatment hepatic disease.

1. Introduction

Spirulina has been consumed as nutritional supplement for both human and animal due to its alimentary value[1]. It possesses anti-inflammatory, immunosuppressive[2], antioxidant, radioprotective, renoprotective properties in addition to neovascularization[3]. Furthermore, *Spirulina* species showed various applications such as lowering hyperlipidaemia, hyperglycemia, and hypertension, probiotic effects, obesity, radiation protection effect, scavenging agents, to protect against renal failure, chemo-preventers and coronary heart diseases as well as suppress agents and enhance growth of intestinal *Lactobacillus*[4-7].

Furthermore, the water extract of *Spirulina platensis* (*S. platensis*) showed antiviral activities against both hepatitis A and hepatitis C viruses[8,9]. On the other hand, the aqueous extract of *S. maxima* showed potential activity against several tumoral cell lines such as human stomach, liver, lung and breast cancer cells[10].

The polysaccharides isolated from *Spirulina* species demonstrated

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anticancer, antioxidant, and antiviral activities[11]. Among these polysaccharides, calcium spirulan (Ca-SP) exhibited strong inhibitory effect against some enveloped viruses[12], beside it could suppress the replication of herpes simplex, mumps, measles, influenza A viruses and cytomegalovirus[13]. In addition to inhibit metastasis of mouse B16 melanoma cells[14], the polysaccharide from *Spirulina* species acts as hepatoprotectant against malignant cell[15]. Hence, the aims of this study are the isolation of polysaccharide from *S. platensis* and evaluation of its activity as anti-HCV, antioxidant, cytotoxic and hypolipidemic *in vitro*.

2. Materials and methods

2.1. Algal material

S. platensis was aseptically grown in the algal biotechnology unit, National Research Centre, Dokki-Cairo, Egypt, using Zarrouk's growth medium[16]. *S. platensis* cells were harvested at 4 ˚C by centrifugation at 5 000 r/min for 10 min. For removing salts and debris, the sample was rinsed with bi-distilled water for several times, dried in oven at 50 ˚C, milled using a grinder and then passed through a sieve No. 45.

2.2. Culture cells for in vitro antiviral

Human hepatocyte cell line (Huh 7.5) was obtained from the Lab. of Prof. Dr. Charles Rice (the Rockefeller University, USA) and cultured using specific growth media (10% Foetal calf serum) and will be kept in CO_2 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) CO_2. After 24 h incubation, the medium was discarded from confluent monolayer of Huh 7.5 cell.

2.3. In vitro hypolipidemic activity

DL-3-Hydroxy-3-methyl-glutaryl coenzyme A sodium salt (HMG-CoA) (Sigma–Aldrich, St. Louis, USA), NADPH (MP Biomedicals, Strasbourg, France), EDTA (El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt), dithiothreitol (Sigma–Aldrich, St. Louis, USA), bovine serum albumin (Sigma–Aldrich, St. Louis, USA), potassium dihydrogen phosphate (El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt) and dipotassium hydrogen phosphate (Sigma–Aldrich, St. Louis, USA) were used in the present study.

2.4. Extraction of water soluble polysaccharide

The crude polysaccharide isolated from cold extract (SCEM) and hot extract (SHEM) was extracted as described in Matloub *et al.*[17], then kept in refrigerator for chemical and biological evaluations. These polysaccharides were tested for the phenolic content using the ferric chloride color method and tested for non bounded protein content using different concentrations of trichloroacetic acid[18].

2.5. Physico-chemical characterization of polysaccharides (SCEM and SHEM) and fractions obtained from SCEM

Phenol-sulfuric method was used for quantification of total polysaccharide and sugar contents in dried algal sample and isolated polysaccharides, respectively[19]. The content of carbon, hydrogen, nitrogen and sulfur were determined in the isolated polysaccharides and fractions by Elemental Microanalysis (Elementary Vario EL) [20]. Moisture and ash contents were determined as mentioned in Matloub *et al.*[20]. Protein content and the degree of substitution (DS) were calculated as mentioned in Matloub *et al.*[17]. The Fourier transform IR spectra ranging between 400 and 4 000 cm^{-1} were recorded with a FT/IR-6100 (JASCO, Japan) using KBr pellets. Gel permeation chromatography (GPC) was used to determine the molar mass distribution using Agilent 1100 series (Germany), ASTRA 1.4 software (Wyatt, USA). Monosaccharide composition was analyzed by gas liquid chromatography (GLC) using arabinose, fructose, fucose, glucose, galactose, galacturonic acid, glucuronic acid, mannose, manitol, rhamnose, ribose, sorbitol and xylose as authentic sugars. The amino acid composition was analyzed using an LC 3000 amino acid analyzer (Eppendorf-Biotronik, Maintal, Germany) as described in Matloub *et al.*[20].

2.6. Fractionation of polysaccharide

The polysaccharide of SCEM was subjected to fractionation by stepwise ethanol-precipitation from 20% to 80%[21]. The chemical characterization of fractions was investigated using GLC, Fourier transform IR (FT-IR) and elemental microanalysis.

2.7. Biological activity

2.7.1. Antiviral activity

2.7.1.1. Determination of the non toxic dose on Huh 7.5 human cell lines

Each glycoprotein SCEM and SHEM (52.2 and 50.3 mg, respectively) was dissolved in bi-distilled water and decontaminated by adding 12 μL of 100× mixture of antibiotic-antimycotic [penicillin G sodium (10 000 IU), streptomycin sulfate (10 000 μg) and amphotericin B (250 μg)]. To evaluate the non toxic dose of SCEM, SHEM and SCEM fractions, tenfold serial dilution of decontaminated samples were inoculated in Huh 7.5 cells. The inverted light microscopy and trypan blue dye exclusion method were used for evaluating cell morphology and cell viability, respectively[17].

2.7.1.2. Determination of antiviral effect on HCV genotype 4a

HCV genotype 4a [ED-43/SG-Feo (VYG) replicon] was obtained from the Lab. of Prof. Dr. Charles Rice (the Rockefeller University, USA). The infectious HCV was treated with tenfold serial dilution of tested samples. HCV RNA in replicon cells was quantified after treatment with the samples as initial titers according to Saeed *et al.*[22].

2.7.2. Cytotoxic activity on hepatocelluar carcinoma human cell line

Cytotoxic effect was accomplished on hepatocelluar carcinoma human cell line (Hep G2) obtained from the American Type Culture Collection (University Boulevard, Manassas, USA). Cell viability test was depended on reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to purple formazan in the mitochondria of active cells[23]. Data were subjected to paired-samples SPSS Statistical Software Package (version 8.0). $P < 0.005$ was regarded as significant. Also, IC_{50} and IC_{90} were determined by probit analysis using SPSS 11 program.

2.7.3. Antioxidant activity

2.7.3.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging efficacy

The DPPH$^{\bullet}$ scavenging efficacy was assessed according to McCue

et al.[24]. Serial concentrations (100–500 μg/mL) of the isolated glycoproteins and ascorbic acid as reference drug were evaluated. The DPPH• scavenging efficacy was performed according to the following equation:

Scavenging efficacy (%) = $[(A_0 - A_1)/A_0] \times 100$ Eq. 1

where A_1 = Absorbance of the tested glycoproteins, A_0 = Absorbance of blank (DPPH• solution).

2.7.3.2 Radical-scavenging efficacy of nitrite

Radical-scavenging efficacy of nitrite was assessed as studied by Menaga *et al.*[25]. Serial concentrations (100–500 μg/mL) of the isolated glycoproteins and Na_2NO_2 as a reference drug were evaluated.

The percentage of radicals scavenging efficacy of nitrite was done using the following equation:

Scavenging efficacy (%) = $[(A_0 - A_1)/A_0] \times 100$ Eq. 2

where A_1 = Absorbance of the tested glycoproteins, A_0 = Absorbance of blank (DPPH• solution). The experiment was repeated in triplicate. Statistical analysis was carried out using paired *t*-test, and SPSS combined with co-state computer program, where different letters are significant at $P \leqslant 0.05$.

2.7.4. Hypolipidemic activity

The hypolipidemic activity of polysaccharide extracts was evaluated by the colorimetric method as mentioned in Matloub *et al.*[17] using fluvastatin as reference drug. The inhibitory activity of HMG-CoA reductase was determined at 340 nm absorbance[26].

3. Results

3.1. Chemical characterization of isolated polysaccharides

Phenol-sulfuric method revealed that *S. platensis* constituted 13.66%, 2.57% and 11.09% w/w of total carbohydrate, free sugar and polysaccharide contents, respectively. The yields of polysaccharides extracted from *S. platensis* by SCEM and SHEM were 4.45% and 3.37%, respectively. The physico-chemical characterizations of SCEM and SHEM were compiled in Table 1. The average of ash content of SCEM and SHEM were found 13.33% and 10.42%, respectively.

Elemental analysis revealed that SCEM and SHEM contained 1.22% and 0.71% of sulfur and degree of sulfation was calculated as 0.09 and 0.04, respectively.

Table 1
Physico-chemical characterization of SCEM and SHEM polysaccharides isolated from *S. platensis*.

Characters	SCEM	SHEM
Yield[a] (Mean ± SE) (%)	4.45 ± 1.12	3.37 ± 0.87
Appearance (visually)	Fine powder	Fine powder
Color (visually)	Off white	Off white
Moisture content (Mean ± SE) (%)	6.85 ± 0.25	5.20 ± 0.07
Ash content (Mean ± SE) (%)	13.33 ± 0.30	10.42 ± 0.19
Carbon (%)	29.53	37.60
Hydrogen (%)	5.92	6.89
Nitrogen (%)	7.14	5.59
Sulfur (%)	1.22	0.71
Total carbohydrate (Mean ± SE) (%)	67.29 ± 0.40	64.66 ± 0.30
Protein (%)	44.63	34.94
Sulfation degree	0.09	0.04
Molecular weight Mw (kDa)	182	82

[a]: % dried algal sample. Mean ± SE: Mean of three replicates ± standard error.

The GPC of isolated polysaccharides revealed one peak for each polysaccharide SCEM and SHEM and their characterization was compiled in Table 2. Both polysaccharides SCEM and SHEM had high degree of polymerization and narrow polydispersity D (2.950 and 1.670, respectively). The weight-average molecular weight (Mw) of SCEM and SHEM was 182 kDa and 82 kDa, respectively.

The FT-IR spectra of SCEM and SHEM showed characteristic bands for polysaccharide bounded protein complexes; bands around 1 649.8 and 1 648.8 cm^{-1}, respectively, indicated carbonyl or amide group (C=O stretching, N-H bending) and bands around 1 546.6 and 1 545.7 cm^{-1} assigned to the secondary–CONH–group. In addition, absorption bands at 1 242.9 and 1 247.7 cm^{-1} were corresponded to S=O stretching vibration indicating the presence of esterified sulfate. Moreover, the band at 874.6 cm^{-1} was indicative of β-glycosidic linkages or assigned to sulfate groups in the axial position of C-6, C-4 and C-2. The absorption bands at 1 414.5 and 1 419.4 cm^{-1} were due to the symmetric stretch vibration and the stretch vibration of COO- and C-O within COOH, respectively. While, the C-O-C bridge of glucosides vibrations were recorded at wavenumbers 1 041.4 and 1 038.5 cm^{-1} (Table 3).

The GLC analysis of SCEM and SHEM hydrolysates revealed the presence of 12 and 11 monosaccharides, respectively (Table 4). Both polysaccharides SCEM and SHEM were found to be enriched in neutral sugars, representing 71.45% and 80.12% of the total monosaccharide content, respectively. Glucose (21.80% & 24.08%), galactose (9.42% & 12.12%) and mannose (8.88% & 12.28%) were the predominant neutral sugars. Also, other neutral sugars xylose, rhamnose, fucose, arabinose, ribose, mannitol and fructose were detected in both glycoproteins. Glucuronic and galacturonic acids were found in traces amount of in SCEM (0.13% & 0.08%), while glucuronic acid was only found in SHEM (0.15%).

The amino acid analyzer revealed the presence of 16 amino acids in both glycoproteins SCEM and SHEM. Glutamic acid and alanine (non-essential amino acid) were found as predominant amino acids in glycoprotein SCEM while glutamic and aspartic acids were existed as dominant amino acids in glycoprotein SHEM (Table 5).

3.2. Chemical characterization of SCEM fractions

As the most bioactive glycoprotein, SCEM was subjected to fractionation by ethanol stepwise precipitation and afforded 7 fractions. The yields percent, elemental microanalysis, protein contents and sulfation degree of these fractions were compiled in Table 6. The carbohydrate content of fractions was ranged 46.56%–65.50% of polysaccharides. The elemental microanalysis of SCEM fractions revealed that the fractions contained high protein content (30.75%–59.87%) and low sulphur content (0.47%–1.92%). From Table 3, FT-IR spectrum data of SCEM fractions showed bands nearly similar to their native polysaccharide which revealed the characteristic absorption bands of polysaccharides attributed to the hydroxyl, alkyl group, secondary amides (amide I) & secondary–CONH–groups of protein, symmetric stretch vibration of COO- and C-O the within COOH. Whereas, FT-IR spectrum data of SCEM fractions showed bands assigned to the glycosidic linkage, esterified sulfate, β-configuration of glycosidic linkage.

The result of GPC of SCEM fractions were recorded in Table 2. The weight-average molecular weights (Mw) of fractions (I–VI) between 585.1–27.9 kDa. While the fraction VII showed interestingly high molecular weight (1305 kDa) than other fractions. The GLC analysis of SCEM fractions hydrolysate led

Table 2

The gel permeation chromatography of SCEM and SHEM polysaccharides and SCEM fractions isolated from *S. platensis.*

Extracts	Peaks	Integration (min)	Mn	Mw	Mz	Mp	D	A
			g/mol					
Cold polysaccharide extract (SCEM)	1	5.160–7.990	6.180×10^4	1.820×10^5	4.580×10^5	5.050×10^4	2.950	1.780×10^4
Hot polysaccharide extract (SHEM)	1	5.930–7.760	4.920×10^4	8.200×10^4	1.210×10^5	6.690×10^4	1.670	8.850×10^3
Fraction I	2	5.180–5.977	5.050×10^5	5.851×10^5	6.747×10^5	5.102×10^5	1.150	1.570×10^3
		5.977–8.401	3.190×10^4	7.023×10^4	1.108×10^5	6.259×10^4	2.200	1.010×10^4
Fraction II	1	6.101–8.283	3.293×10^4	6.371×10^4	9.099×10^4	7.857×10^4	1.934	5.019×10^3
Fraction III	1	6.563–7.657	2.713×10^4	3.211×10^4	3.894×10^4	2.845×10^4	1.183	9.335×10^2
Fraction IV	1	6.465–7.768	2.762×10^4	3.398×10^4	4.273×10^4	3.041×10^4	1.230	3.610×10^2
Fraction V	1	6.582–7.704	2.790×10^4	3.345×10^4	4.043×10^4	3.001×10^4	1.194	4.874×10^2
Fraction VI	1	6.626–8.400	1.800×10^4	2.790×10^4	3.690×10^4	2.660×10^4	1.540	1.570×10^3
Fraction VII	2	4.263–5.774	9.040×10^5	1.305×10^6	1.897×10^6	8.145×10^5	1.380	1.380×10^3
		5.825–7.026	1.323×10^5	1.661×10^5	2.030×10^5	1.305×10^5	1.250	1.305×10^3

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

Table 3

FT-IR analysis of SCEM and SHEM polysaccharides and SCEM fractions.

Fractions	Assignment wave number (cm^{-1})											
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
SCEM	3370.00	2962.1		1649.8	1546.6	1414.5	-	1242.9	1041.4	874.7	-	603.6
SHEM	3423.99	2930.3		1648.8	1545.7	1454.1 1419.4	1319.1	1247.7	1038.5	874.6		604.6
SC1	3425.9	2961.5	-	1655.6	-	1413.6	1323.9	-	1042.3	873.6	-	661.5
SC2	3427.9	2927.4	1723.1	1659.5	1595.8	1436.7	-	-	1045.2		685.6	600.7
SC3	3385.4	2959.2	-	1657.5 1638.2	1595.8	1410.7	1321.0	1247.7	1087.7 1043.3		777.2	639.3 595.8
SC4	3401.8	2937.1	-	1638.2	1595.8	1409.7	-	1236.2	1063.6	897.7 825.4	709.7	649.9
SC5	3393.1	2936.1	-	1635.3	1595.8	1410.7	1323.9	1249.9	1033.7	887.1	780.1 705.8	655.7 634.5
SC6	3409.5	2942.8	1756.8	1630.5		1409.7 1380.8	-	-	1041.4	811.9		648.9
SC7	3463.5	2940.9	-	1623.8	-	1398.1		-	1039.4	-	685.6	606.5

(1): The assignment for stretch vibration of O-H (hydroxyl groups); (2): The assignment for stretch vibration of C-H: (3): The assignment for C=O stretching (esters); (4): The assignment for C=O stretching in secondary amides (amide I); (5): The assignment for Amide II (N-H bending of amino acid group); (6): The assignment for symmetric stretch vibration of –COO- (uronic acids); (7, 8): The assignment for stretching vibration of S=O (esterified sulfate); (9): The assignment for C-O-C bending mode in glycosidic linkages; (10): The assignment for bending vibration of C-O-S of the sulfate in axial position; (11): The assignment for bending vibration of C-O-S of the sulfate in equatorial position; (12): The assignment for asymmetric deformation of O-S-O groups.

to identification of 7–9 monosaccharides (Table 7). Glucose, galactose, xylose and mannose are detected as the major neutral sugars. In addition, rhamnose and fucose were found in low content in hydrolysate of SCEM. Moreover, glucuronic acid was only detected in small amount in fraction III.

Table 4

Monosaccharides composition of SCEM & SHEM glycoproteins isolated from *S. platensis*.

Sugar	RRT	% Sugar component	
		Cold	Hot
Arabinose	0.681	4.55	5.66
Xylose	0.685	6.08	8.65
Ribose	0.713	4.05	0.98
Rhamnose	0.762	5.75	7.81
Fucose	0.768	7.43	5.99
Mannitol	0.884	3.06	1.82
Fructose	0.914	0.22	0.58
Galactose	0.980	9.42	12.12
Mannose	0.985	8.88	12.28
Glucose	1.00	21.80	24.08
Galacturonic acid	1.101	0.13	-
Glucuronic acid	1.319	0.08	0.15
Total identified		71.45	80.12

RRT: Relative retention time.

Table 5

Amino acids composition of SCEM & SHEM glycoproteins isolated from *S. platensis*.

Amino acids		mg/100 mg of isolated polysaccharide	
		Cold	Hot
Essential amino acids	Threonine	1.44	1.28
	Valine	2.44	2.06
	Isoleucine	1.49	1.38
	Leucine	4.31	2.85
	Phenylalanine	3.65	2.50
	Lysine	2.41	1.40
	Methionine	1.10	1.17
	Total	16.84	12.64
Non-essential amino acids	Aspartic acid	3.45	3.45
	Glutamic acid	5.99	4.28
	Serine	1.83	1.25
	Glycine	0.92	1.00
	Histidine	1.75	1.01
	Arginine	3.06	2.18
	Alanine	3.97	2.69
	Proline	1.75	0.76
	Tyrosine	1.98	1.39
	Total	24.70	18.01
Total contents of amino acids		41.54	30.65

Table 6
Chemical characterization of SCEM fractions.

Fractions	Ethanol	Percentage of isolated polysaccharide (%)							Degree of sulfation
		Fraction	Carbohydrate	Carbon	Hydrogen	Nitrogen	Protein	Sulfur	
I	0%	49.00	54.82	24.15	4.77	4.92	30.75	0.47	0.04
II	20%	10.13	46.56	33.73	5.62	8.61	53.81	0.70	0.04
III	30%	11.00	53.06	27.78	5.49	9.58	59.87	1.39	0.11
IV	40%	12.26	65.50	33.26	4.87	5.75	35.93	1.19	0.08
V	50%	6.56	52.92	27.72	6.30	5.76	36.00	1.92	0.15
VI	60%	8.10	56.68	33.34	5.02	5.68	35.50	1.05	0.07
VII	80%	2.90	53.62	28.03	4.86	7.30	45.62	1.56	0.12

Table 7
Monosaccharide composition of the fractions obtained from bioactive SCEM.

Sugar	RRT	% Sugar component					
		I	II	III	IV	V	VI
Arabinose	0.681	12.02	-	-	-	-	6.37
Xylose	0.685	-	13.26	12.35	15.84	14.91	7.01
Ribose	0.713	4.24	5.32	5.01	2.47	-	0.35
Rhamnose	0.762	7.88	8.66	7.46	6.38	4.35	7.93
Fucose	0.768	4.80	4.07	5.26	8.22	8.42	7.46
Mannitol	0.884	1.22	0.96	1.61	1.48	2.45	1.91
Fructose	0.914	-	-	-	-	-	-
Galactose	0.980	12.42	11.69	15.98	15.15	12.34	15.08
Mannose	0.985	10.83	8.94	10.15	10.79	11.11	8.61
glucose	1.000	27.24	25.19	20.94	21.40	26.89	21.34
Galacturonic acid	1.101	-	-	-	-	-	-
Glucouronic acid	1.319	-	-	2.88	-	-	-
Total identified		80.65	78.09	81.64	81.73	80.47	76.06

RRT: Relative retention time related to glucose.

3.2. Biological activity

The non toxic dose of the glycoproteins SCEM and SHEM were 522 and 503 μg/mL, respectively, was determined on Huh 7.5 cell line (Table 8). The antiviral activity of SCEM and SHEM was evaluated against HCV replicon and is compiled in Table 8. The SCEM has promising anti-HCVcc genotype 4a replicon which reduced to 50% at the non toxic concentration 522 μg/mL. SHEM didn't exhibit antiviral activity on HCV. This study gives a great importance to evaluate natural products as antiviral candidate compounds because of no antiviral drug against enteric viruses worldwide used as antiviral reference drug. The current result led us to fractionate glycoprotein SCEM and appraised the non toxic dose against Huh 7.5 cell line as well as HCV activity (Table 9).

Table 8
Cytotoxicity on Huh 7.5 cell line and HCVcc reduction of different doses of SCEM and SHEM glycoproteins obtained from *S. platensis*.

Extract	Tested concentration	Cytotoxicity (%)	HCVcc reduction (%)
SCEM	52.2 mg/mL	50	100
	5.22 mg/mL	10	70
	522 μg/mL	0	50
	52.2 μg/mL	0	0
	5.22 μg/mL	0	0
SHEM	50.3 mg/mL	60	80
	5.03 mg/mL	20	10
	503 μg/mL	0	0
	50.3 μg/mL	0	0
	5.03 μg/mL	0	0

Table 9
Cytotoxicity on Huh 7.5 cell line and HCVcc reduction of different doses of SCEM fractions.

Fractions	Tested concentration	Cytotoxicity (%)	HCVcc reduction (%)
SCEM Fraction I	5.7 mg/mL	20	70
	570 μg/mL	0	40
	57 μg/mL	0	0
	5.7 μg/mL	0	0
SCEM Fraction II	5.5 mg/mL	20	30
	550 μg/mL	0	10
	55 μg/mL	0	0
	5.5 μg/mL	0	0
SCEM Fraction III	5.5 mg/mL	20	70
	550 μg/mL	0	30
	55 μg/mL	0	0
	5.5 μg/mL	0	0
SCEM Fraction IV	5.5 mg/mL	20	20
	550 μg/mL	0	0
	55 μg/mL	0	0
	5.5 μg/mL	0	0
SCEM Fraction V	5.3 mg/mL	20	10
	530 μg/mL	0	10
	53 μg/mL	0	0
	5.3 μg/mL	0	0
SCEM Fraction VI	5.5 mg/mL	25	20
	550 μg/mL	10	0
	55 μg/mL	0	0
	5.5 μg/mL	0	0
SCEM Fraction VII	4.4 mg/mL	10	0
	440 μg/mL	0	0
	44 μg/mL	0	0
	4.4 μg/mL	0	0

The fractions I, II, III and V showed reduction of HCV replicon into 40%, 10%, 30% and 10%, respectively at non toxic dose. While fractions IV, VI and VII didn't show any antiviral activity at non toxic dose.

Concerning to cytotoxic study, the SCEM and SHEM were evaluated *in vitro* on Hep G2 cultured. The percentages of growth inhibition are shown in Figure 1. The glycoprotein SHEM exhibited cytotoxic activity against Hep G2 *in vitro* with the ED_{50} of 69.49 μg/mL. However, SCEM didn't exhibit cytotoxic activity against Hep G2 *in vitro* compared to doxorubicin as a reference drug.

The antioxidant activity of SCEM and SHEM (100–500 μg/mL) can be expressed as their abilities to scavenge either DPPH and/or nitrate. The DPPH$^{\bullet}$ and nitric oxide scavenging % were calculated according to Eq. 1 and Eq. 2, respectively. Both glycoproteins SCEM and SHEM had DPPH$^{\bullet}$ scavenging capacities in dose-dependent

Table 10

DPPH• and nitrite scavenging efficacy of glycoproteins SCEM and SHEM isolated from *S. platensis*.

Concentration (μg/mL)	Scavenging effect (%)			Nitric oxide inhibition (%)		
	SCEM	SHEM	Ascorbic acid	SCEM	SHEM	Sodium nitrite
100	41.82 ± 2.10[a]	29.10 ± 1.12[b]	53.85 ± 2.11[c]	67.57 ± 2.66[a]	70.27 ± 1.33[b]	76.89 ± 3.23[d]
200	41.80 ± 1.19[a]	30.91 ± 2.23[b]	63.64 ± 3.00[d]	64.86 ± 3.47[a]	67.56 ± 2.33[a]	70.27 ± 4.00[b]
300	43.64 ± 3.21[a]	30.00 ± 1.36[b]	61.82 ± 3.67[d]	62.16 ± 2.87[a]	64.86 ± 3.25[a]	67.57 ± 2.55[a]
400	47.27 ± 1.34[c]	32.73 ± 1.45[b]	67.27 ± 4.15[d]	62.10 ± 1.98[a]	64.06 ± 3.54[a]	64.86 ± 3.63[a]
500	49.09 ± 2.65[c]	40.00 ± 1.76[a]	67.90 ± 2.55[d]	40.54 ± 2.82[b]	24.32 ± 3.83[c]	64.86 ± 2.44[a]

Each value represents mean ± standard error of mean of three replicates. Statistical analysis was carried out using SPSS computer program coupled by Co-state computer program version 8, where different letters are significant at $P \leqslant 0.05$.

fashion but less than that of ascorbic acid (Table 10). On the other hand, both glycoproteins reduced the nitrite production which led to suppressing the released NO, but scavenging efficacy was in inverse to concentration (Table 10).

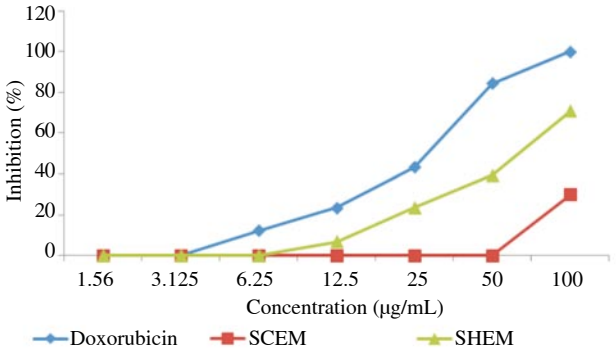

Figure 1. Cytotoxic activity of the isolated glycoproteins SCEM and SHEM against Hep G2 human cell line *in vitro*.

Concerning to *in vitro* hypolipidemic activity of cold and hot glycoprotein extracts, SCEM insignificantly reduced β-hydroxy-β-methyl glutaryl coA reductase activity to 3.21 ± 0.01 μmol/mg (80.02% of inhibition) in comparing to fluvastatin as reference drug 1.51 ± 0.16 μmol/mg (90.58% of inhibition). While, SHEM reduced β-hydroxy-β-methyl glutaryl coA reductase activity to 14.46 ± 0.29 μmol/mg (10.02% of inhibition).

4. Discussion

Cold and hot aqueous extraction methods *S. platensis* led to isolate SCEM and SHEM polysaccharides. These polysaccharides of SCEM and SHEM were rich in protein (44.63%) and (34.94%), respectively. No precipitation was detected when added 10%–50% trichloroacetic acid and this was in agreement with the result of Shekharam *et al.*, who found that cold and hot water soluble polysaccharides composed from 22.8% and 38.0% of protein, respectively[27]. The integration of chemical analysis revealed that these polysaccharides were heterogeneous and bounded with protein, referred as glycoprotein.

The weight-average molecular weight (Mw) of SCEM and SHEM were 182 kDa and 82 kDa, respectively. Different studies showed various Mw of polysaccharides isolated from *S. platensis*, *e.g.* Pugh *et al.* isolated immulina which is characterized by high Mw > ten million Daltons[28], while Majdoub *et al.* isolated sulphated polysaccharide with Mw 199 kDa[29]. Moreover, Hayashi *et al.* isolated antiviral polysaccharides whose Mw ranged between 250 and 300 kDa[13].

On the other hand, the fraction VII isolated from SCEM showed the highest molecular weight than other fractions. This may be attributed to high concentration of ethanol (80%) which could interact with proteins either by affecting backbone hydration led to aggregate protein by attractive electrostatic and dipole forces or by interacting with function groups of backbone and side chains[30].

The GLC analysis of SCEM and SHEM hydrolysates revealed that glucose was a major monosaccharide of polysaccharide isolated from *S. platensis* alongside to galactose, mannose, rhamnose and this result was in concordant with studies of Shekharam *et al.*[27] and Wang *et al.*[31] except for glucose which was found with a great disparity in the ratio among monosaccharide.

SCEM exhibited antiviral activity against HCV and this was in agreement with other study which showed the antiviral activities of water extract of *S. platensis* against hepatitis A and herpes simplex viruses[8] as well as the antiviral activity of calcium spirulan (Ca-SP) which exhibited a strong inhibitory against several enveloped viruses, by targeting the viral absorption/penetration and some replication stages after penetration into cells[12,32]. Moreover, a comparative clinically trial of *S. platensis* revealed that *Spirulina* decrease more significantly the HCV virus load at least 2-$\log_{10}$ and also significantly improved alanine aminotransferase[9]. The polysaccharide from *Spirulina* sp. proved that it can act as hepatoprotectant against malignant cell[15].

The excess production of NO free radical is accompanied with various diseases in mammalian cells which play key role in the regulation of several physiological processes. The sulfated polysaccharides were demonstrated stronger antioxidant capacities than de-sulfated polysaccharides[33]. Also, the high degree of sulfation and low molecular weight showed the best antioxidant capacities[34].

S. platensis is one of the edible microalgae, paid more attention because of its nutritional and medicinal applications. Whereas, polysaccharides have still attracted to scientist because of their special physicochemical properties and high biological activities.

Cold and hot water soluble polysaccharides as well as fractions of cold polysaccharide of *Spirulina* characterized by heteropolymers bounded protein refer as glycoproteins. They constituted mainly glucose alongside eight other monosaccharides in addition 16 amino acids and were rich in glutamic acid, luciene, alanine and aspartic acid. From our data, the SCEM biopolymers frequently show antiviral activity against HCV, radical scavenging and hyopolipidemic properties while, SHEM shows cytotoxicity on Hep G2 cell line. Therefore, the isolated glycoproteins have great therapeutically potential in drug development for counteracting HCV, prevention of hepatocarcinoma and could be used as hepatoprotective and hypolipidemic agent in near future.

Conflict of interest statement

We declare that we have no conflict of interest.

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