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

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**ARTICLE INFO**

**Article history:**
Received 11 Jul 2017
Received in revised form 16 Aug 2017
Accepted 4 Sep 2017
Available online 24 Oct 2017

**Keywords:**
*Spirulina platensis*
Glycoprotein
Anti-HCV
Hepatocarcinoma
Antioxidant
Hypolipidemic activity

**OBJECTIVE:** To evaluate the glycoprotein isolated from *Spirulina platensis* (*S. platensis*) as anti-HCV, cytotoxicity, antioxidant and hypolipidemic 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 of 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 inhibited 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\(^3\).

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Foundation Project: Supported by the National Research Centre (Grant No. 9080104).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

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...
antimicrobial, 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 5000 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 growth medium [16]. 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 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.,2017. 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.,2017. Protein content and the degree of substitution (DS) were calculated as mentioned in Matloub et al.,2017. The Fourier transform IR spectra ranging between 400 and 4000 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.,2017.

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 (10000 IU), streptomycin sulfate (10000 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.,2012.

2.7.2. Cytotoxic activity on hepatocellular carcinoma human cell line

Cytotoxic effect was accomplished on hepatocellular 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, IC₀⁰ and IC₉₀ 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 scavenging efficacy was assessed according to McCue et al.
Statistical analysis was carried out using paired t-test where A1 = Absorbance of the tested glycoproteins, A0 = 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 Na₂NO₂ as a reference drug were evaluated.

The percentage of radicals scavenging efficacy of nitrite was done using the following equation:
Scavenging efficacy (%) = \[\left(1 - \frac{A_t}{A_0}\right) \times 100\] Eq. 2
where \(A_t\) = Absorbance of the tested glycoproteins, \(A_0\) = Absorbance of blank (DPPH solution).

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.

| Characters | SCEM (Mean ± SE) (%) | SHEM (Mean ± SE) (%) |
|------------|----------------------|----------------------|
| Yield      | 4.43 ± 1.12          | 3.37 ± 0.87          |
| Appearance (visually) | Fine powder | Fine powder |
| Color (visually) | Off white | Off white |
| Moisture content | 6.85 ± 0.25 | 5.20 ± 0.07 |
| Ash content | 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 | 67.29 ± 0.40 | 64.66 ± 0.30 |
| Protein (%) | 46.63 | 34.94 |
| Sulfation degree | 0.09 | 0.04 |
| Molecular weight Mw (kDa) | 182 | 82 |

*% 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 1649.8 and 1648.8 cm⁻¹, respectively, indicated carbonyl or amide group (C=O stretching, N-H bending) and bands around 1546.6 and 1545.7 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, thamnose, fucose, arabinose, ribose, mannotol and fructose were detected in both glycoproteins. Glucuronic and galacturonic acids were found in traces amount of in SCEM (0.13% & 0.08%), while gluuronic 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...
The gel permeation chromatography of SCEM and SHEM polysaccharides and SCEM fractions isolated from S. platensis.

| Extracts | Peaks | Integration (min) | Mw | Mz | Mp | D | A |
|----------|-------|------------------|----|----|----|---|---|
| Cold polysaccharide extract (SCM) | 1 | 5.160–7.990 | 6.180×10^3 | 1.820×10^4 | 4.580×10^4 | 5.050×10^4 | 2.950 | 1.780×10^4 |
| Hot polysaccharide extract (SHEM) | 1 | 5.930–7.760 | 4.920×10^3 | 8.200×10^4 | 4.210×10^4 | 6.690×10^4 | 1.670 | 8.850×10^4 |
| Fraction I | 2 | 5.180–5.977 | 5.050×10^3 | 5.851×10^4 | 6.747×10^4 | 5.102×10^4 | 1.150 | 1.570×10^4 |
| Fraction II | 1 | 5.977–8.401 | 3.190×10^3 | 7.023×10^4 | 1.108×10^4 | 6.259×10^4 | 2.200 | 1.010×10^4 |
| Fraction III | 1 | 6.101–8.283 | 3.293×10^3 | 6.371×10^4 | 9.099×10^4 | 7.857×10^4 | 1.934 | 5.019×10^4 |
| Fraction IV | 1 | 6.563–7.657 | 2.713×10^3 | 3.211×10^4 | 3.894×10^4 | 2.845×10^4 | 1.183 | 9.335×10^4 |
| Fraction V | 1 | 6.465–7.766 | 2.762×10^3 | 3.989×10^4 | 4.273×10^4 | 3.041×10^4 | 1.230 | 3.610×10^4 |
| Fraction VI | 1 | 6.582–7.704 | 2.790×10^3 | 3.345×10^4 | 4.043×10^4 | 3.001×10^4 | 1.194 | 4.874×10^4 |
| Fraction VII | 1 | 6.626–8.400 | 1.800×10^3 | 2.790×10^4 | 3.690×10^4 | 2.660×10^4 | 1.540 | 1.570×10^4 |
| SC7 | 2 | 4.263–5.774 | 9.040×10^3 | 1.305×10^4 | 1.897×10^4 | 8.145×10^4 | 1.380 | 1.380×10^4 |
| SC5 | 3 | 4.253–5.296 | 1.323×10^3 | 1.661×10^4 | 2.030×10^4 | 1.305×10^4 | 1.250 | 1.305×10^4 |

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) |
|-----------|--------------------------------|
| SC1       | 3425.9                        |
| SC2       | 3422.0                        |
| SC3       | 3385.4                        |
| SC4       | 3401.8                        |
| SC5       | 3393.1                        |
| SC6       | 3409.5                        |
| SC7       | 3463.5                        |
| SHEM      | 3423.9                        |

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 | 1.101 | 0.13              |      |
| Glucuronic   | 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 | ng/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 7
Monosaccharide composition of the fractions obtained from bioactive SCEM.

| Sugar          | RRT     | 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 | -   | -   | -   | -   | -   | -   |
| Glucuronic acid | 1.319 | -   | -   | -   | -   | -   | -   |
| Total identified | 80.65 | 78.09 | 81.64 | 81.73 | 80.47 | 76.06 |

RRT: Relative retention time related to glucose.

### 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                  |
|         | 52 µg/mL             | 0               | 50                  |
|         | 5.2 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                   |

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

| Fractions | Tested concentration | Cytotoxicity (%) | HCVcc reduction (%) |
|-----------|----------------------|-----------------|---------------------|
| SCEM I    | 5.7 mg/mL            | 20              | 70                  |
|           | 570 µg/mL            | 0               | 40                  |
|           | 57 µg/mL             | 0               | 0                   |
|           | 5.7 µg/mL            | 0               | 0                   |
| SCEM II   | 5.5 mg/mL            | 20              | 30                  |
|           | 550 µg/mL            | 0               | 0                   |
|           | 55 µg/mL             | 0               | 0                   |
|           | 5.5 µg/mL            | 0               | 0                   |
| SCEM III  | 5.5 mg/mL            | 20              | 70                  |
|           | 550 µg/mL            | 0               | 30                  |
|           | 55 µg/mL             | 0               | 0                   |
|           | 5.5 µg/mL            | 0               | 0                   |
| SCEM IV   | 5.5 mg/mL            | 20              | 20                  |
|           | 550 µg/mL            | 0               | 0                   |
|           | 55 µg/mL             | 0               | 0                   |
|           | 5.5 µg/mL            | 0               | 0                   |
| SCEM V    | 5.3 mg/mL            | 20              | 10                  |
|           | 530 µg/mL            | 0               | 10                  |
|           | 53 µg/mL             | 0               | 0                   |
|           | 5.3 µg/mL            | 0               | 0                   |
| SCEM VI   | 5.5 mg/mL            | 25              | 20                  |
|           | 550 µg/mL            | 10              | 0                   |
|           | 55 µg/mL             | 0               | 0                   |
|           | 5.5 µg/mL            | 0               | 0                   |
| SCEM 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 ED₅₀ 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• and nitric oxide scavenging % were calculated according to Eq. 1 and Eq. 2, respectively. Both glycoproteins SCEM and SHEM had DPPH• 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 (%) | Sodium nitrite |
|-----------------------|-----------------------|-----------------------------|----------------|
|                       | SCEM | SHEM | Ascorbic acid | SCEM | SHEM | Ascorbic acid | SCEM | SHEM | Ascorbic acid |
| 100                   | 41.82 ± 2.10a       | 29.10 ± 1.12a | 53.85 ± 2.11a | 67.57 ± 2.66a | 70.27 ± 1.33a | 76.89 ± 3.23a |
| 200                   | 41.80 ± 1.19a       | 30.91 ± 2.23a | 63.64 ± 3.00a | 64.86 ± 3.47a | 67.56 ± 2.33a | 70.27 ± 4.00a |
| 300                   | 43.64 ± 3.21a       | 30.00 ± 1.36a | 61.82 ± 3.67a | 62.16 ± 2.87a | 64.86 ± 3.25a | 67.57 ± 2.55a |
| 400                   | 47.27 ± 1.34a       | 32.73 ± 1.45a | 67.27 ± 4.15a | 62.10 ± 1.98a | 64.06 ± 3.54a | 64.86 ± 3.63a |
| 500                   | 49.09 ± 2.65a       | 40.00 ± 1.76a | 67.90 ± 2.55a | 40.54 ± 2.82a | 24.32 ± 3.83a | 64.86 ± 2.44a |

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 $P \leq 0.05$.

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% cold aqueous and this was in agreement with the result of Shekhharam *et al.*, 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 Shekham *et al.*[27] and Wang *et al.*[31] except for glucose which was found with a great disparity in the ratio among monosacchara.

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 clinical trial of *S. platensis* revealed that Spirulina decrease more significantly the HCV virus load at least 2-\text{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 along side 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 hypolipidemic 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.
Acknowledgments

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

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