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ORIGINAL ARTICLE

Crude sulfated polysaccharides extracted from marine cyanobacterium *Oscillatoria simplicissima* with evaluation antioxidant and cytotoxic activities

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ABSTRACT

Background and Objectives: Microalgae have been widely used as a novel source of bioactive substances. These substances exhibit various biological actions including, antioxidant and antitumor effects material. The present work is carried out to evaluate potential applications of cyanobacterium *Oscillatoria simplicissima* containing mainly polysaccharides.
Materials and Methods: Crude polysaccharides from marine cyanobacteria *Oscillatoria simplicissima* and *Oscillatoria acutissima* were extracted and characterized according to their chemical content and cytotoxic activities. The isolated polysaccharides characterized by the Fourier transmittance infrared spectrum (FT-IR).
Results: These polysaccharides constituted 34.68 mg/g of sugar, 0.011 mg/g of protein, and 28.92 mg/g of sulfate contents. The antioxidant property of the methanol extracts of these green microalgae was evaluated by measuring the free radical scavenging activity by the DPPH assay method. The algal extracts were then evaluated for their suppressive effect on tumor cell growth (A-549, MDA-MB-231, PC-3, HT-29, HepG2, and HeLa) by using the SRB assay. At a concentration of 10 mg/mL, *Oscillatoria simplicissima* exhibits an antioxidant activity of 45.97%. The cytotoxic activity revealed that *Oscillatoria simplicissima* polysaccharide shows potent cytotoxic activity against lung cancer (A-549) cell line 49.465 µg/mL.
Conclusion: Microalgal polysaccharides have great therapeutically potential in drug development used as antitumor and antioxidant agents in near future.

Keywords: Marine microalgae; Polysaccharides; Antioxidant; Cytotoxic activities

INTRODUCTION

Microalgae are wealthy in numerous treasured compounds like carotenoids, phycocyanin, phenolic, amino acids, polyunsaturated fatty acids, and sulfate polysaccharides which are used as raw material for the production of biodiesel, animal feed, meals additives, cosmetics, and medicine (1). These compounds offer wonderful numerous organic sports, which include antioxidant, antimicrobial, antiviral, antitumor, anti-inflammatory, and anti-allergic outcomes (2). Many varieties of biologically lively additives had been recognized in marine algae and utilized in medicinal applications. For example, sulfate polysaccharides (SP) had been discovered enriched with inside the molecular partitions of brown marine algae (3). Polysaccharides showcase a great variety of systems due to their range in glycosidic composition, substitutions, and linkages. Polysaccharides

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remoted from plants, fungi, yeasts, and algae have attracted enormous interest for his or her organic sports in biochemistry and medicine (4). They showcase an extensive variety of organic sports inclusive of anti-inflammatory, antioxidant, antitumor, anticoagulant, antithrombotic, antimetastatic, antiviral, antimicrobial, and immunostimulatory (5). Microalgae may be used as herbal reasserts of antioxidants. The antioxidant interest of microalgae is limited, especially with regard to the connection between its phenolic content material and its antioxidant capacity. Therefore, it becomes applicable to pick out a few wealthy reasserts of antioxidants from a big institution inclusive of microalgae to assess and validate the connection among those parameters (6). The anti-tumor interest becomes one of the maximum essential sports in drugs, coming from marine ecosystems. A big species of algae and its metabolites had been proven to have amazing cytotoxicity. These metabolites have performed an essential function in the synthesis of new pharmaceutical compounds from algae for antitumor drugs (7). Centered on water-soluble antitumor materials from numerous marine algae, however, maximum anticancer dealers have now no longer been used clinically due to their unwanted facet outcomes on regular cells (8). Microalgae, being microscopic, diverse, and having developed their very own protection mechanisms via way of means of synthesizing secondary metabolites, which might be explored in anticancer studies (9). Herein, we report the extraction of water-soluble polysaccharides in cyanobacterium *Oscillatoria simplicissima*, the evaluation of its cytotoxicity against a cancer cell line. This study also presents the antioxidant activities of these polysaccharide extracts.

MATERIALS AND METHODS

Algal isolation. The algal species used in this study were isolated from Gulf of Aqaba of Red Sea coast of Alexandria. Samples were grown in F/2 medium (10). The medium was then autoclaved at 120°C for 30 minutes. The culture was incubated at temperature 30 ± 1°C, pH of 8 and light intensity 3000 lux. The algae were kept under optimum conditions. The isolated strain was identified according to Cronberg et al. (11).

Measurements of algal growth. Determination of algal growth as achlorophyll (a) according to the method described by Khaleghi et al. (12). The pigment content in filtered extract was determined by the absorbance at 663 and 645 nm in a 1cm quartz cell against a blank of 80% aqueous acetone by spectrophotometer using the following equation:

Chlorophyll a=12.7. E663-2.69. E645

Determination of the polysaccharides of cyanobacteria. Polysaccharides were extracted and determined as the method described by Shi Y. et al. (13).

Total sugar, proteins and sulfate measurement. Total sugars were measured by the phenol-H_2SO_4 reaction using D-glucose as a standard (14). Briefly, a mixture of 0.5 mL of sample and 0.5 mL of 5% aqueous phenol solution was treated with 2.5 mL of concentrated sulfuric acid. The mixture was stirred for 30 min. The absorption was measured at 490 nm and glucose was used as standard. Sulfate content was determined using barium chloride/gelatin method with some modifications (15). Concisely, the aqueous extracts (0.2 mL) were treated with trichloroacetic acid (3.8 mL) followed by the addition of 1.0 mL of barium chloride/gelatin. The mixture was stirred for 20 min. The absorbance was read at 360 nm and potassium sulfate was used as standard. Protein contents were measured following the Pothiraj method (16).

Antioxidant activity: DPPH assay. The antioxidant activities of the polysaccharide were assayed according to the methods adopted by Sagar (17). In this method, the algal extracts were dissolved in methanol, where the metabolic 2,2 diphenyl-1- picryl hydrazyl (DPPH) was used as a control. A mixture of 5 mL DPPH solution (10, 20, and 40 mg/mL) was used, and the absorbance was measured spectrophotometrically at 517 nm after 30 min. The free radical scavenging activities of the algal extracts were indicated by the degree of decolonization of DPPH. The percentage of decolonization was calculated using the following equation: Free radical scavenging % = (Ac–As)/Ac × 100 where Ac is Absorbance of control and As is Absorbance of the sample. Ascorbic acid was used as a reference free radical scavenger.

Total antioxidant capacity (TAC). Total antioxidant capacity (TAC) was measured using different solvents according to the protocol explained by Pierre B et al. (18). TAC reagent was prepared through a

mixture of sulfuric acid (0.6 M), sodium phosphate (28 mm), and ammonium molybdate (4 mm) with distilled water. Then, 3 ml of polysaccharide extract was added to 3 ml of TAC reagent and incubated at 95°C for 90 min. T70 spectrophotometer (PG Instruments, UK) was utilized for measuring the absorbance at 695 nm. Total antioxidant capacity was determined as a percentage with a standard that is represented with ascorbic acid. Total antioxidant capacity (TAC) % = [(Ac-As) /Ac] ×100, where Ac is the control absorbance and As is the sample absorbance.

Cell culture. Lung cancer (A-549), breast cancer (MDA-MB-231), prostate cancer (PC-3), colorectal cancer (HT-29), hepatocellular carcinoma (HepG2) and cervical cancer (HeLa) cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units /mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO_2 atmosphere at 37°C (19).

Cytotoxic activity. Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension ($5 \times 10^{\times 3}$ cells) were in 96-well plates and incubated incomplete media for 24 h. Cells were treated with another aliquot of 100 µL media containing drugs at various concentrations ranging from (10 ug/ml, 100 ug/ml). After 72 h of drug exposure, cells were fixed by replacing media with 150 µL of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 n musing a BMGLABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany) (20).

Fourier transform infrared spectroscopy (FTIR). The polysaccharides extracted from two cyanobacteria were prepared in thin pellets using potassium bromide (KBr) for FTIR analysis. IR spectra were obtained from PERKIN Elmer model at the resolution of 1 cm^{-1} in the range of 4000 to 450 cm^{-1}.

Statistical analysis. The experiments were performed in triplicate and presented with the ± SD. To evaluate the statistical significance of the cell viability reduction, a comparison between exposed and control probes was performed by Student's t-test. p-values lower than 0.05 were considered statistically significant.

RESULTS

Microalgae isolated. The algal strains were identified as *Oscillatoria simplicissima* and *Oscillatoria acutissima*. The algal strains were harvested at their exponential phase of their growth which was 14th day under 30 ± 2°C, pH 8 and 3000 Lux (Fig. 1).

Total sugar, proteins, and sulfate composition. Fig. 2 shows the composition in sugars, proteins and sulfate of the microalgae aqueous extracts. The total sugar content of *Oscillatoria simplicissima* and *Oscillatoria acutissima* were 34.02512 mg/g (34.68% of total dried matter) and 12.59856 mg/g (30.51% of total dried matter), respectively. The sulfate groups represented respectively 28.92 mg/g and 14.1mg/g of *Oscillatoria simplicissima* and *Oscillatoria acutissima* extracts. The percentage of proteins in the microalgae aqueous extracts were respectively 0.011 mg/g and 0.023 mg/g for *Oscillatoria simplicissima* and *Oscillatoria acutissima*. Carbohydrates represented around 54% of dry matter of *Oscillatoria simplicissima*.

Antioxidant activity. Table 1 shows the scavenging power of DPPH radicals by cyanobacterium. *Oscillatoria simplicissima* and *Oscillatoria acutissima* showed the significantly highest percent of inhibition (45.97and 42%, respectively) at 1 mg /ml. The algal methanol extracts indicated a total antioxidant capacity of *O. simplicissima* and *O. acutissima*, 55.186 and 47.4 mg/g AsA equivalent dw, respectively as shown in (Table 1).

Cytotoxic activity of *O. simplicissima* polysaccharides. *O. simplicissima* polysaccharide was selected to test the anticancer activity on A-549, MDA-MB-231, PC-3, HT-29, HepG2 and HeLa cell lines because of its high antioxidant activity. The cells were treated with 10, or 100 µg /mL of polysaccharide for 72 h. It is interesting to remark that the effect of treatment is different from one cell line to anoth-

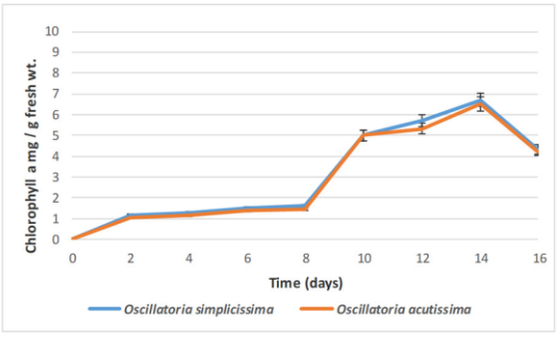

Fig. 1. The growth of *Oscillatoria simplicissima* and *Oscillatoria acutissima* measured as chlorophyll (a) mg/g fresh wt.

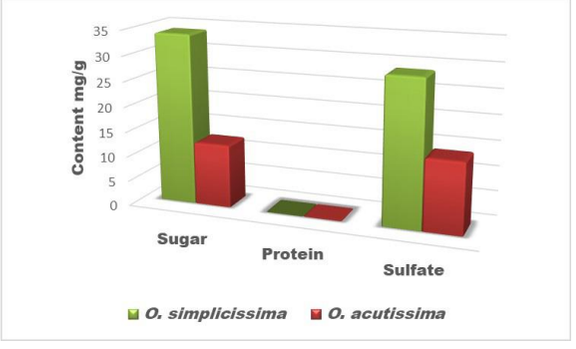

Fig. 2. Total sugar, proteins and sulfate content in water-soluble polysaccharide extracts of *Oscillatoria simplicissima* and *Oscillatoria acutissima*. All assays were carried out in triplicate.

Table 1. Antioxidant activity of polysaccharide extract from *Oscillatoria simplicissima* and *Oscillatoria acutissima*

Polysaccharide extract	DPPH%	TAC (mg/g ASA)
O. simplicissima	45.97	55.186 ± 0.12
O. acutissima	42	47.4 ± 0.34

*Data are means of three measurements ± S.E.

er. The lowest effect was measured on breast cancer (MDA-MB-231) cell line (non cancer cell line). The extracts induced concentration-dependent cytotoxic effects in Lung Cancer (A-549) cell line 100 µg/ml of cell viability (49.465%) and also showed higher dead counts when compared to control (Fig. 3).

FTIR spectra of polysaccharides extracted from *O. simplicissima*. The calculated FTIR spectra are shown in Fig. 4 and Table 2. The IR spectra within

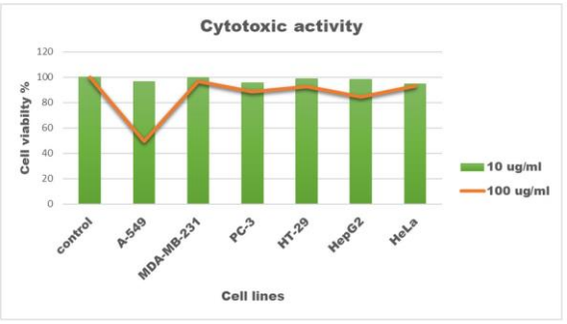

Fig. 3. Cytotoxicity of *O. simplicissima* crude polysaccharides against A-549, MDA-MB-231, PC-3, HT-29, HepG2 and HeLa cells using SRB assay.

the 450 cm^{-1} to 4,000 cm^{-1} wave number region. The major absorption bands around 3660.76-3555.95 and 3225.17 cm^{-1} attributed to O-H stretching of hydroxyls. The peak at 2351.21 and 2328.72 cm^{-1} are originated from the asymmetric stretching vibrations of the carboxylate O—C—O bond. Meanwhile, the absorption bands around 1633.47-1644.16 and1650.95 cm-1 assigned for CO stretching in secondary amides (amide I).

DISCUSSION

The most studies on the biochemical production of algal and their analysis were carried out in stationary phase of growth period (21). Many studies reported that chemical composition as carbohydrates, proteins and lipids in *N. oculata* and *I. galbana* was dependent on the environmental growing conditions like salinity, light intensity, nitrogen content, photoperiod, and stage of harvest (22). Elisabete (23) showed that *N. oculata* is composed of 35% of proteins and 7.8% of carbohydrates. However, Picardo et al. reported that carbohydrates represent 29.4% when grown at 25°C (24). The DPPH free-radical scavenging efficiency demonstrated that the isolated crude polysaccharides had a moderate impact on preventing the formation of these radicals. These results are in agreement with those of Custódio et al. (25) indicated that organic extracts from *N. oculata* had also antioxidant properties with *IC50* values between 4.93 and 7.31%. Moreover, Balavigneswaran et al. (26) reported that an ethanol soluble polysaccharides extract from *I. galbana* was active against DPPH (almost 40%) at 10 mg/mL. The reducing properties are generally associated with the presence of reductions which have

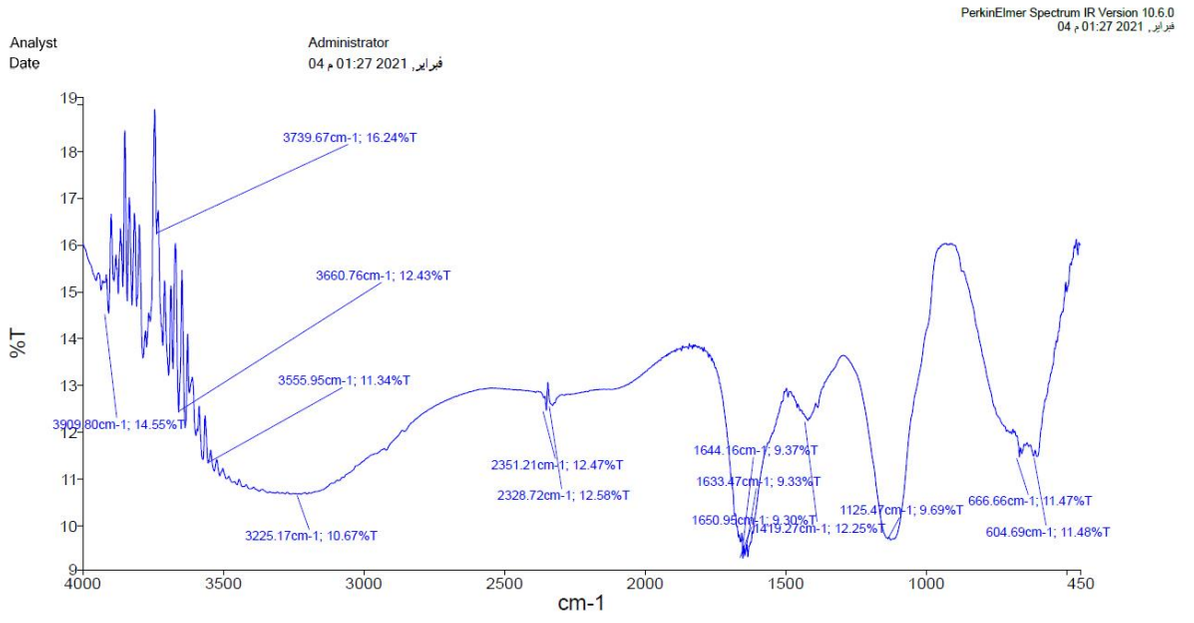

Fig. 4. Fourier transform infrared analysis of the crude polysaccharide fractions extracted from marine cyanobacterium *O. simplicissima*

Table 2. A list of infrared (IR) vibrational modes characteristic to polysaccharides

IR absorption wave number (cm^{-1})	Signal characteristics
3660.76-3555.95 and 3225.17	The broad peak signifies the stretching vibrations of the OH group
2351.21 and2328.72	O=C=O (stretch carbonic dioxide)
1633.47-1644.16 and1650.95	C=O stretching anhydride
1125.47	S=O stretching
666.66 and 604.69	C-Br or C-Cl stretching (halo compound)

been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are reported to react with some precursors of peroxide, thus preventing peroxide formation (27). Carboxyl groups may play an important role in scavenging radicals, possibly due to the higher hydrogen donation ability of carboxyl groups than hydroxyl groups, proteins and sulfate groups (28). The antioxidant activities depend on polysaccharides molecular weight, degree of ramification, monosaccharide composition, sulfate content and configuration (29). The influence of sulfate content on the antioxidant activity depends rather on the origin of polysaccharides. For example, the polysaccharides from *Ulva fasciata* and other macro and microalgae with low sulfate content demonstrated a strong antioxidative power, while the antioxidant activity observed in polysaccharides from *Enteromorpha linza* showed to be dependent on sulfate content. Furthermore, highly sulfated polysaccharides were shown to have an enhanced scavenging power, this property being also dependent on the sulfate distribution pattern (30). Marine algae are focused on as the main target for effective antioxidants against oxidative stress in the human body because of the presence of various natural products that have unique structures probably resulting from the extreme marine environment. A lot of metabolites isolated from marine algae have been proven to have bioactive effects (31). Polysaccharides play an important role in many biological processes. Recently, some authors demonstrated that polysaccharides had a broad spectrum of biological effects including anticancer activities (32). However, the attempts to establish a relationship between the structures of PS and their bioactivities/ actions had been a challenge due to the complexity of this type of

polymers. The IR spectra of isolated polysaccharides showed an absorption band around 1125.47 cm^{-1} was assigned as S=O stretching vibration indicating the presence of esterified sulfate (33). These results are in agreement with Alves et al. (34). Moreover, algal polysaccharides have been reported to have antioxidant activity (35) and effective antitumor activity by attacking the cancer cell directly (36) or enhancing the host immune function (37). This finding, which agrees with many studies, suggests that the dynamic substances cooperate with particular cancer-related receptors or cancer cell special molecule, thus triggering some mechanisms that cause cancer death (38). However, the role of algal polysaccharide structure in antioxidative mechanisms has not been elucidated. Therefore, it is necessary to explore new algal polysaccharides with antioxidant and anticancer activities and also to elucidate their mode of action (39).

CONCLUSION

Oscillatoria simplicissima had the highest antioxidant activity. The effect of *Oscillatoria simplicissima* polysaccharide had cytotoxic effects on A-549, HepG2 and PC-3 cell lines. It could be concluded that polysaccharide extracted from *Oscillatoria simplicissima* was effective as anticancer agents. Further studies could be done to explore new compounds from microalgae to develop alternative therapeutic drugs.

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