



## Production and Assessment of Antioxidant Activity of Exopolysaccharide from Marine *Streptomyces globisporus* BU2018

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**M**ICROBIAL polysaccharides are involved in a wide range of biotechnological applications. Three streptomycetes isolates isolated from marine sediment from Sharm El-Sheikh were screened for their ability to produce exopolysaccharides (EPSs) as well as the antioxidant activity. *Streptomyces globisporus* BU2018 producing EPS which had the highest antioxidant activity was identified based on morphological, biochemical characteristics as well as molecular analysis of 16S rRNA gene. *S. globisporus* BU2018 produced 8.5g/L of EPS which was fractionated to give EPSSH3 as a major fraction and subjected to determine its antioxidant activities. Maximum antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was  $\sim 92.9 \pm 1.3\%$  at 1500 $\mu$ g/ml after 120min and the half maximal inhibitory concentration (IC<sub>50</sub>) value was 500 $\mu$ g/ml after 60min. Therefore, the most extreme activity against H<sub>2</sub>O<sub>2</sub> radical was  $68.5 \pm 1.48\%$  at 1500 $\mu$ g/ml after 60min with IC<sub>50</sub> value of about 1000 $\mu$ g/ml after 60min. Moreover, the maximum reducing power activity of exopolysaccharide SH3 (EPSSH3) was at concentration 1000 $\mu$ g/ml and the most extreme metal chelating activity was 90.2% at 1000 $\mu$ g/ml. The EPSSH3 fraction contained uronic acid (65.13%) and no sulfate. The monosaccharide composition was glucuronic acid: galacturonic acid: rhamnose: fructose through molar ratio 4.0: 3.0: 1.0: 1.0, respectively. These indicate that the fraction is acidic heteropolysaccharide. The EPSSH3 had an overall average molecular weight (*M<sub>w</sub>*) of  $8.15 \times 10^5$ g/mol and number average molecular weight (*M<sub>n</sub>*) of  $6.36 \times 10^5$ g/mol. Otherwise, the fourier transform infrared spectrometry analysis proposed that it belongs to a  $\beta$ -type heteropolysaccharide.

**Keywords:** Exopolysaccharides, *Streptomyces globisporus*, Marine habitat, Antioxidant.

### Introduction

One of the burdens that confront scientists is the oxidative stress arising from reactive oxygen species (ROS) accumulation inside cells. ROSs have an important role in cell signaling, homeostasis and killing pathogens. However, accumulation of ROS in the cells is also implicated in many serious diseases such as cancer, autoimmune diseases (e.g. arthritis), neurodegenerative disorders, diabetes, atherosclerosis and cardiovascular diseases. Due to the high reactivity of ROSs, they can react with macromolecules such as nucleotides, proteins and lipids which in turn could result in mutation in gene sequences, render a protein disrupt cellular or

organelles membranes (Emerit et al., 2004; Firuzi et al., 2011).

During the past several decades, great attention has been drawn to microbial polysaccharides, due to their various biological and pharmacological activities. Microbial exopolysaccharides (EPSs) also had biological properties which have been successfully used in many industrial fields such as food, agriculture, textile, cosmetics and pharmaceuticals (Ates, 2015). They have been used in medical applications as antioxidant (Adesulu-Dahunsi et al., 2018; Luo & Fang, 2008), antitumor (Di et al., 2017), antiviral (Wang et al., 2007), anticoagulant (Yoon et al., 2003),

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anti-inflammatory and immunostimulatory (Xu et al., 2009). Moreover, they have been used in intelligent drug delivery system for many drugs such as anticancer, anti-inflammatory, antibiotics, anticoagulant and antihypertensive drugs (D'Arrigo et al., 2014). Researchers tried them as gene delivery vectors to treat some diseases *in vivo* (Han et al., 2015). Also, they could be useful in vaccination as antigen carrier, antigen itself or an adjuvant plus antigen specific cancer immunotherapy (Li et al., 2014). Furthermore, application in wound healing, skin repair and tissue engineering were reported (Fu et al., 2013).

Marine microorganisms were known to produce many beneficial compounds including secondary metabolites and biopolymers. To overcome harsh environmental conditions (e.g. hyper salinity, pH and predation), they produce these compounds for protection including extracellular polysaccharides. Marine Actinobacteria dominates the world of novel drug discovery for different purposes. They are responsible for almost two thirds of the commercial antibiotics. *Streptomyces* are Gram-positive bacteria characterized by a complex life cycle. They produce different types of secondary metabolites that having diverse structures and functions like antimicrobial, anticancer, immunosuppressive drugs herbicides (Williams et al., 1983). Approximately between 60-75% of the known compounds that had been produced by actinobacteria, are used in medicine and agriculture, respectively (Tanaka & Mura, 1993). Therefore, *Streptomyces* are considered as the most familiar bacteria in fermentation manufacturing of active pharmaceutical compounds.

The present study aimed to produce a bioactive exopolysaccharide (EPS) from an *Streptomyces* strain as well as schedule and recognize that strain. While, the objectives were analyzing the *in vitro* antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), H<sub>2</sub>O<sub>2</sub>, reducing power and metal chelating and characterization of the chemical composition of that EPS.

## Materials and Methods

### Isolation of *Streptomyces* species

Marine sediment was collected from Sharm El-Sheikh during summer 2016 in sterilized

plastic bottles. Streptomycetes were isolated using serial dilution method Hayakawa & Nonomura (1987) on marine media contain: Starch, 10g/L; K<sub>2</sub>HPO<sub>4</sub>, 1.0g/L; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5g/L; NaCl, 0.5g/L; KNO<sub>3</sub>, 2.0g/L; CaCO<sub>3</sub>, 2.0g/L; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01g/L; agar, 20.0g/L; dissolved in 750ml sea water completed to 1L with distilled water at pH 7 (Waksman, 1961) and incubated at 28°C for 14 days.

### Production of EPSs

Streptomycete isolates were screened for the production of EPSs in a liquid production medium. The pure isolates were inoculated into a 250ml flask containing 50ml of screening production medium containing glucose, 10.0g/L; tryptone, 5.0g/L; yeast extract, 5.0g/L; K<sub>2</sub>HPO<sub>4</sub>, 3.0g/L; NaCl, 3.0g/L; KH<sub>2</sub>PO<sub>4</sub>, 1.0g/L; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5g/L; CaCO<sub>3</sub>, 0.5g/L dissolved in 750ml sea water completed to 1L by distilled water at pH 7 (Manivasagan et al., 2013) and incubated at 28°C for 5 days at 120 rpm. After incubation, the culture medium was centrifuged at 5000rpm for 30min, the supernatant was mixed with Trichloroacetic acid (TCA) (10%) and left overnight at 4°C then centrifuged at 5000rpm for 20min to remove protein. The pH of the clear solution was adjusted to 7 with NaOH solution (Liu et al., 2010). The supernatant was mixed with four volumes with ethanol (95%) and left overnight at 4°C. The precipitated EPSs were separated by centrifugation at 5000rpm for 20min washed twice with acetone and dehydrated by ether (Shene et al., 2008).

### Antioxidant activity

The antioxidant activity of different concentrations of isolated EPSs (5, 10 and 15mg/ml) was measured against 1,1-diphenyl-2-picrylhydrazyl (DPPH) (20mg was dissolved in small volume of ethanol then completed to 1L distilled water) at 30, 60, 90 and 120min. The free radical scavenging activity was assessed spectrophotometrically at 517nm by turning the deep violet color solution of DPPH into colorless or pale yellow color (Brand-Williams et al., 1995).

$$\text{Scavenging ability (\%)} = \left( \frac{A_{517} \text{ of control} - A_{517} \text{ of sample}}{A_{517} \text{ of control}} \right) \times 100.$$

where, A= The absorbance

*Identification of streptomycete isolate*

*Streptomycete* isolate produced the highest amount of EPSs with high antioxidant activity was identified according to its morphological, physiological and biochemical characteristics (Tresner & Backus, 1963; Shirling & Gottlieb, 1966; Szabó et al., 1975). Transmission Electron Microscopy (HR-TEM-2100, JEOL, Japan) was done at National research Center, Egypt. A Polymerase Chain Reaction was performed using the forward primer 5' GAGTTTGATCCTGGCTCAG 3' and the reverse primer 5' GGTTACCTTGTTACGACTT 3' (Gardes & Bruns, 1993). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Bio-systems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). Data were submitted to GenBank database. The DNA sequence was compared to the GenBank database (<https://www.ncbi.nlm.nih.gov/>) using the BLAST program. The 16S rRNA gene sequences of the bacteria were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers MH810296.

*Production and fractionation of EPSs*

The promising isolate which showed the highest antioxidant activity was selected to produce EPSs. The fermentation culture which contains glucose, 10.0g/L; tryptone, 5.0g/L; yeast extract, 5.0g/L; K<sub>2</sub>HPO<sub>4</sub>, 3.0g/L; NaCl, 3.0g/L; KH<sub>2</sub>PO<sub>4</sub>, 1.0g/L; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5g/L; CaCO<sub>3</sub>, 0.5g/L was incubated at 28°C with shaking at 120 rpm for 5 days. The fermented broth was centrifuged at 5000rpm at 4°C for 30min to remove bacterial cells. The biomass was obtained by washing the resulting precipitated cells with distilled water repeatedly and drying at 80°C at a constant weight. TCA (10%) was added to supernatant and left over night at 4°C and centrifuged at 5000rpm for 20min to remove protein. The pH of the supernatant was adjusted to 7 with NaOH solution (Liu et al., 2010). Four volumes of cold absolute ethanol were added to the supernatant and the precipitate was collected by centrifugation. The precipitate was dissolved in deionized water followed by dialysis against distilled water for 72hr with exchange each 6hr. After dialysis process 1, 2, 3, and 4 volumes of cold absolute ethanol were added to precipitate different fractions of EPSs varied in their molecular weight. The yield of the major fraction

obtained by one volumes of absolute ethanol was washed by acetone, diethyl ether, dried at 40°C and coded as EPSSH3.

*Analysis of EPSSH3**Molecular weight determination*

The weight-average molecular weight (*M<sub>w</sub>*) and number-average molecular weight (*M<sub>n</sub>*) of EPSSH3 were determined on an Agilent 1100GPC system equipped with a RI Detector. The EPSSH3 was dissolved in 2ml of solvent and then it was filtrated through a 0.45µm filter prior to injection. The polydispersity index (PI) was calculated using the *M<sub>w</sub>/M<sub>n</sub>* ratio (Jun et al., 2009; You et al., 2013).

*Chemical analysis*

EPSSH3 was hydrolyzed with formic acid then the hydrolyzed EPSSH3 was subjected to determine uronic acid at 525nm by the *m*-hydroxybiphenyl colorimetric method (Filisetti-Cozzi & Carpita, 1991). As well as, sulfate was determined using the turbidity method (Dodgson & Price, 1962). The monosaccharide composition was determined by (Agilate Pack, series1, 200), equipped with Aminex carbohydrate HP-87C column (300×7.8mm) with deionized water as the mobile phase at 0.5ml/min (Randall et al., 1989).

*Fourier-transform infrared spectroscopy (FTIR)*

The FTIR spectra of EPSSH3 was performed with potassium bromide (KBr) pellets, (2.0mg sample and 200mg KBr using the FTIR-UNIT Bruker Vector 22 Spectrophotometer), according to Brock Neely (1957).

*Antioxidant activities of EPSSH3**Assay of scavenging activity*

EPSSH3 with different concentrations 100, 300, 500, 1000 and 1500µg/ml were used for assessment of antioxidant activity with DPPH assay whereas 2ml of DPPH solution (20mg/L) were mixed with EPSSH3. The mixture was shaken vigorously and left to react for 30, 60, 90 and 120min in the dark and the absorbance (A) was measured at 517nm and the scavenging activity was calculated as follows:

$$\text{Scavenging ability (\%)} = \frac{(A_{517} \text{ of control} - A_{517} \text{ of sample})}{A_{517} \text{ of control}} \times 100.$$

#### *Hydrogen peroxide scavenging activity*

The ability of EPSSH3 to scavenge hydrogen peroxide can be estimated according to Ruch et al. (1989). Different concentrations of EPSSH3 (200, 400, 600, 800, 1000 and 1500 µg/ml) dissolved in distilled water were added to H<sub>2</sub>O<sub>2</sub> and absorbance was determined at 230nm. The percentage of H<sub>2</sub>O<sub>2</sub> scavenging is calculated as follows:

$$\text{Scavenging ability (\%)} = \frac{(A_{230} \text{ of control} - A_{230} \text{ of sample})}{A_{230} \text{ of control}} \times 100.$$

#### *Reducing power activity*

According to the method which was described by Oyaizu (1986), 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of K<sub>3</sub>Fe (CN)<sub>6</sub> (1% w/v) were added to 1.0ml of EPSSH3 different concentrations (400, 600, 800 and 1000 µg/ml) dissolved in distilled water. The resulting mixture was incubated at 50°C for 20min, followed by the addition of 2.5ml of trichloroacetic acid (TCA) (10% w/v). The mixture is centrifuged at 3000rpm for 10min to collect the upper layer of the solution (2.5ml), mixed with distilled water (2.5ml) and 0.5ml of FeCl<sub>3</sub> (0.1%). The absorbance was then measured at 700nm against blank sample.

#### *Metal chelating activity*

The chelation of ferrous ions was estimated using the method described by Dinis et al. (1994). 0.1ml of the EPSSH3 of different concentrations (400, 600, 800 and 1000 µg/ml) were added to solution of 0.5ml FeCl<sub>2</sub> (0.2mM) and the reaction is started by adding 0.2ml of ferrozine (5mM) to the solution and incubated at room temperature for 10min and then the absorbance was measured at 562nm.

#### *Statistical analysis*

All results were analyzed using the Graph Pad InStat version 2 software. All graphs were plotted using the Graph Pad Prism version 8 software. Results were considered statistically significant with P values < 0.05.

## **Results and Discussion**

#### *Screening of streptomycetes for production of EPSs and its antioxidant activity*

Three Streptomycetes isolates were isolated from marine sources according to their special morphological characteristics on starch nitrate

agar media to produce EPSs for screening of their antioxidant activity so, streptomycetes were cultivated for 7 days. Table 1 showed that the cell dry weight and EPSs productivity of the three isolates. The percentage of DPPH radical scavenging activity for the isolated EPSs was demonstrated in Table 2. Marine microorganisms frequently produce bioactive compounds with diverse functions and structures as a result of harsh environmental conditions (Fenical, 1989). EPSs from marine microorganisms are important to new medication revelation (Han et al., 2005; Miranda et al., 2008; Xu et al., 2009). Manivasagan et al. (2013) used DPPH radical-scavenging activity for detection of antioxidant activity of an extracellular polysaccharide (EPS) which was isolated from a marine *Streptomyces violaceus* MM72. As well as He et al. (2010) isolated an EPS from *Streptomyces virginia* H03 which could inhibit food spoilage and food poisoning microorganisms as well as its antioxidant activity against DPPH.

#### *Identification of the promising streptomycete isolate*

The isolate which produced the EPS having the highest radical scavenging activity (SH3) was subjected to morphological, physiological and biochemical characteristics. Table 3 showed that the isolate characterized by rectiflexibiles spore chains and smooth spore surface, melanin pigments are generally produced at tyrosine agar, the color of spore mass is pale yellow and diffusible pigments are not produced. Also, the isolate had a varied utilization of different sugars. Figures 1 and 2 showed the flexuous sporephores hyphae and smooth surface ornamentation of the spores.

Phylogenetic analysis based on the 16S rRNA gene sequence of strain SH3 was compared to reference 16S rRNA quality arrangement accessible in the GenBank and EMBL database acquired from the National Centre of Biotechnology Data database utilizing BLAST search (<http://ncbi.nlm.nih.gov/BLAST/>) So, it was identified as *Streptomyces globisporus* BU2018 with accession number MH810296. Figure 3 showed phylogenetic tree of the partial sequence of 16S rRNA of the local isolate *Streptomyces globisporus* BU2018 respects to closely related sequences available in Gen Bank databases. The phylogenetic tree was constructed using neighbor-joining tree method using the software MEGA7.



**TABLE 1. Cell dry weight (g/L), EPSs dry weight (g/L) and productivity (%) of exopolysaccharides produced by the three streptomycete isolates**

Isolate code.	Dry weight (g/L)	EPS (g/L)	Productivity (%)
SH1	7.6	10.6	140.7
SH2	9.0	8.2	91.1
SH3	10.0	8.5	85.0

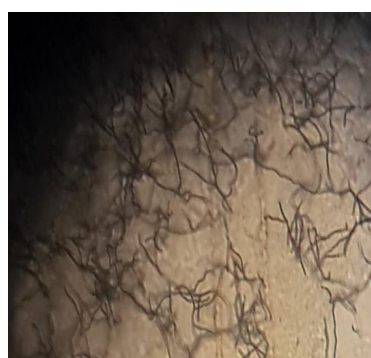
**TABLE 2. The radical scavenging activity (%) of the crude exopolysaccharides which were produced by the three isolates of streptomycetes**

Isolate no.	EPS conc.	DPPH free radical scavenging activity (%) with time			
		10min	30min	60min	90min
SH1	5mg/ml	30.00	37.87	44.26	46.89
	10mg/ml	44.59	56.89	64.75	64.75
	15mg/ml	61.54	62.29	70.66	70.99
SH2	5mg/ml	44.92	60.66	72.62	77.87
	10mg/ml	65.25	76.56	84.92	86.23
	15mg/ml	76.07	80.62	87.38	89.34
SH3	5mg/ml	59.34	79.34	86.23	92.62
	10mg/ml	73.44	88.19	88.19	92.62
	15mg/ml	78.69	89.34	89.34	92.63

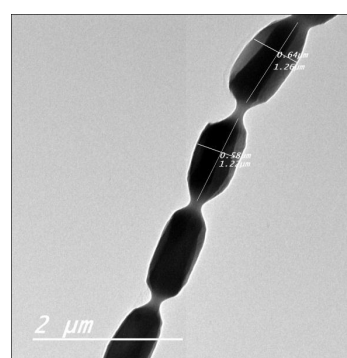
**TABLE 3. Different characteristics of high potent antioxidant activity of Streptomycete isolate**

Morphological and cultural characteristics								
Spore chain morphology		Spore surface ornamentation		Color of spore mass		Pigmentation of substrate mycelium		Diffusible pigment
Straight/Flexuous>40		Smooth		whitish yellow		Pale yellow		-ve
Physiological and biochemical characteristics								
Melanin pigment production			Degradation activities			Nitrate reduction	H <sub>2</sub> S production	
peptone iron	tyrosine	xanthine	Elastin	arbutin		+ve	+ve	
-ve	+ve	+ve	-ve	+ve				
Utilization of sugars								
D-fructose	Sucrose	Rhamnose	D-mannitol	D-xylose	Raffinose	I-inositol	Galactose	L-arabinose
-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve

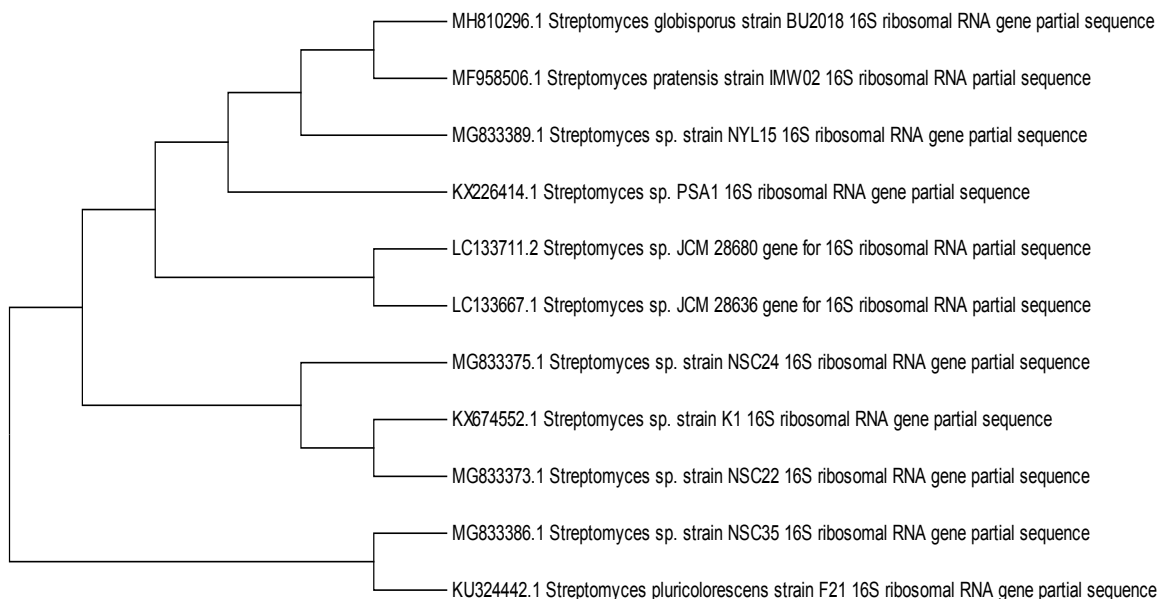
\* +ve: Positive result, -ve: Negative result.



**Fig. 1. Photomicrograph showing flexuous sporephores hyphae.**



**Fig. 2. TEM photomicrograph of SH3 showing smooth spore surface.**



**Fig. 3. Phylogenetic tree of the partial sequence of 16S rRNA of *Streptomyces globisporus* BU2018 and closely related strains from Genbank.**

#### *Isolation and characterization of EPSSH3*

*Streptomyces globisporus* BU2018 which have high antioxidant activity was exposed toward scaling up fermentation for 5 days at production medium. Extraction of EPSs by ethanol was done after removal of bacterial cells and protein yielding 8.5g of crude extract. The dialyzed solution was fractionated by precipitation using cold absolute ethanol. The major fraction obtained by one volume of ethanol (EPSSH3) was assessed to determine its activity as antioxidant. The EPSSH3 fraction was contained uronic acid (65.13%) and no sulfate. The monosaccharide composition of these fraction composed of glucuronic acid: galacturonic acid: rhamnose: fructose with a molar ratio 4.0: 3.0: 1.0: 1.0, respectively. These indicated that the fraction is acidic heteropolysaccharide. The weight average molecular weight ( $M_w$ ), number average of molecular weights ( $M_n$ ) and polydispersity ( $M_w/M_n$ ) of EPSSH3 were analyzed by GPC. The EPSSH3 in the GPC chromatogram were widely dispersed molecules polydispersity index ( $PI=1.28$ ) and had an overall average molecular weight ( $M_w$ ) of  $8.15 \times 10^5$ g/mol and number average molecular weight ( $M_n$ ) of  $6.36 \times 10^5$ g/mol. Figure 4 showed the FTIR spectrum of EPSSH fraction with many peaks ranging from  $3413.39$  to  $615.181$ cm<sup>-1</sup>. FTIR spectrum exhibited a significant broad characteristic peak at around at  $3413.39$ cm<sup>-1</sup> region was attributed to

the expansion vibration of O–H in the ingredient sugar residues (Kanmani et al., 2011). The band at  $2960.2$ cm<sup>-1</sup> was correlated with the stretching vibration of C–H in the sugar ring. The EPS fraction also appears to have a particular band at  $1675.84$ cm<sup>-1</sup>, which is dominated by circle vibrations of C=O and COO group (Sun et al., 1998), the absorptions around  $1438.64$ cm<sup>-1</sup> represented CH<sub>2</sub> and OH bonding. While, stretching vibration of C–O glycosidic bond vibration and the band at  $838.883$ cm<sup>-1</sup> suggested the β-pyranose (Cheng et al., 2008). Therefore, FTIR spectroscopy and HPLC analysis were highly likely that the EPSSH3 belonged to a β-type hetero-polysaccharide.

#### *Antioxidant activity of EPSSH3*

DPPH free radical scavenging activity technique was used to decide quantitatively the antioxidant activity at different times (30, 60, 90 and 120min). When DPPH free radical encounters a proton-donating or electron accepting substrate such as an antioxidants, the radicals will be scavenged, the blue-purple color will be turned to yellow and the absorbance will be reduced (Shimada et al., 1992). The decrease in absorbance is taken as a measure for radical-scavenging activity. In Fig. 5, it is clear that there is a direct proportionality the increment of the compound concentrations from 100, 300, 500, 1000 and 1500μg/ml and the antioxidant activity. Maximum antioxidant activity was

92.9±1.3% at 1500µg/ml after 120min. The IC<sub>50</sub> value against DPPH radical was around 500µg/ml after 60min.

The hydroxyl radical is considered to be a the most potent and reactive oxidant, which can react with all biomacromolecules leading to impairment of their functions (Qi et al., 2005). Thus, the removing of H<sub>2</sub>O<sub>2</sub> is very important for antioxidant defense in cells or food systems (Aruoma, 1998). The scavenging ability of the purified polysaccharide on hydrogen peroxide is shown in Fig. 6. In this figure, the ability of EPSSH3 to scavenge hydrogen peroxide was estimated using different concentrations (200, 400, 600, 800 and 1000µg/ml). The highest activity was 68.5±1.48% at 1500µg/ml after 60min. The IC<sub>50</sub> value against H<sub>2</sub>O<sub>2</sub> radical was approximately 1000µg/ml after 60min. Therefore, by after reducing power method (RP), the antioxidant activity of EPSSH3 with concentrations (400, 600, 800 and 1000µg/ml) was measured and showed that the maximum activity was detected at 1000µg/ml and the metal chelating activity for EPSSH3 the red color of the ferrozine-Fe<sup>2+</sup> complexes was decreased by different concentration of EPSSH3 (400, 600, 800 and 1000µg/ml) demonstrated that the most extreme activity was 90.2% at 1000µg/ml (Table 4).

The bioactivity of EPSSH3 might be affected by their structural features, such as chemical composition, molecular mass, types of glycosidic

linkage, and conformation. Differences in origin materials, extraction and drying procedures that influence the physiochemical properties will lead to differences in antioxidant activity (Cheung et al., 2012; Wang et al., 2012; Gou et al., 2014 and Shen et al., 2014) and a similar observation for uronic acid content was reported (Mateos-Aparicio et al., 2010). Additionally, it is suggested that the number of hydroxyl or charged groups in polysaccharides are responsible for the overall radical scavenging ability (Guo et al., 2005). Our results of chemical analysis showed that EPSSH3 had high content of negatively charged COO<sup>-</sup> groups as well as hydroxyl groups which gave that EPS the antioxidant activities.

Manivasagan et al. (2013) isolated an EPS from marine *Streptomyces violaceus* MM72. This EPS showed strong DPPH radical-scavenging, superoxide scavenging, metal chelating and reducing power activities. So that EPS produced by *S. violaceus* MM72 could be used in industry replacing synthetic antioxidant compounds. While, He et al. (2007) extracted a polysaccharide from the broth of cultured *Streptomyces virginia* H03 and assessed the antioxidant activity of the polysaccharide *in vitro*. It was reported that the purified polysaccharide showed significant antioxidant activity against, hydrogen peroxide which was found to be 76.96% at 350µg/ml While, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity reached to 79.2% at the dose of 350µg/ml.

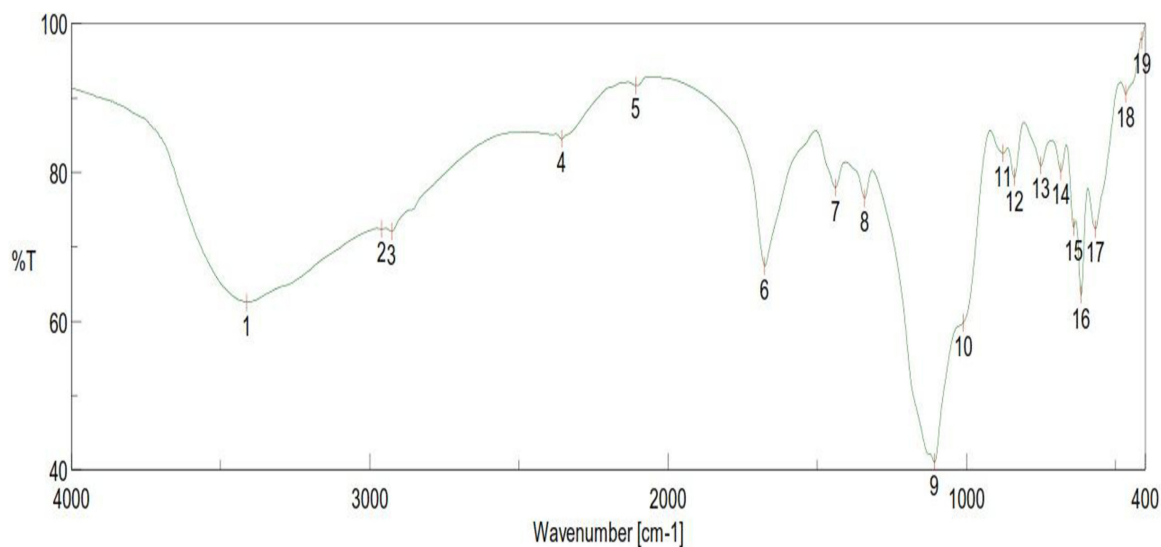


Fig. 4. FTIR spectrum of EPSSH3.

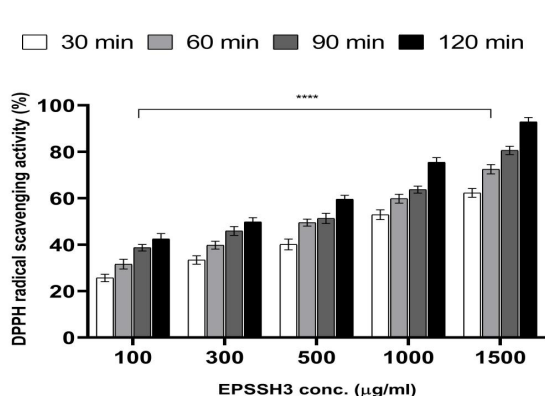


Fig. 5. Scavenging activity of EPSSH3 against DPPH at different time [Significance: P value < 0.0001\*\*\*\*].

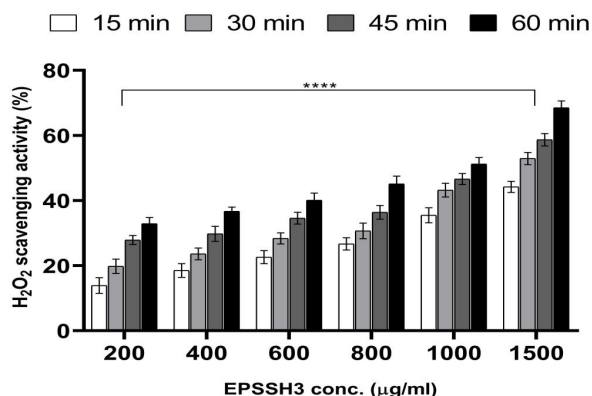


Fig. 6. Scavenging activity of EPSSH3 against H<sub>2</sub>O<sub>2</sub> at different time [Significance: P value < 0.0001\*\*\*\*].

TABLE 4. Reducing power (RP) activity and metal chelating scavenging activity of EPSSH3.

EPSSH3 concentration (µg/ml)	RP activity (absorbance at OD <sub>700</sub> nm)	Metal chelating activity (%)
400	0.1553	85.2
600	0.185	86.1
800	0.2225	86.4
1000	0.278	90.2

## Conclusion

Because of the wide range of pharmaceutical activities of the microbial exopolysaccharides, marine *Streptomyces globisporus* BU2018 was subjected for producing a bioactive exopolysaccharide (EPSSH3) which showed high antioxidant activity using different methods. EPSSH3 had an ability to scavenge DPPH free radical (92.9±1.3%) and hydrogen peroxide (68.5±1.48%). Also, EPSSH3 showed high metal chelating activity (90.2%) and reducing power. EPSSH3 was β-type heteropolysaccharide containing (65.13%) uronic acid content. The monosaccharide composition was glucuronic acid: galacturonic acid: rhamnose: fructose with molar ratio 4.0: 3.0: 1.0: 1.0, respectively. This indicated that the importance of marine microorganisms in production of bioactive metabolites.

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إنتاج وتقييم للنشاط المضاد للأكسدة للسكر العديد الخارجى المعزول من *Streptomyces globisporus* BU2018 البحرية

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تعد السكريات العديدة الخارجية واحدة من أهم نواتج الأيض الأولية والتي لها العديد من الإستخدامات خاصة فى مجال الصناعات الدوائية وعلوم الطب الحيوى. فقد وجد أن لها الكثير من الكفاءات الحيوية كمضادات للأكسدة ومضادات للسرطان ومضادات للفيروسات كما أنه تم اختبارها كمضادات للإلتهابات ومنشطات للجهاز المناعى وموانع لتخثر الدم وتكوين الجلطات. وتم التركيز فى هذه الدراسة على الأنشطة كمضادات للأكسدة للسكر العديد الذى سمي ب-EPSSH3 المنتج بواسطة سلالة بكتيرية تنتمى لطائفة الأكتينوبكتريا المعزولة من البحر الأحمر تحديدا من شواطئ مدينة شرم الشيخ و التى تم تعريفها بواسطة 16 SrRNA على انها *استريبتومييس جلوبيسپورس* وتم التأكد بإجراء الإختبارات الفسيولوجية والكيمياء حيوية وفحص الشكل الظاهرى. من خلال الفحص الكيمائى لهذا السكر العديد تبين أنه بوليمر غير متجانس يتكون من سكريات احادية مختلفة هى حامض الجلوكويورونيك: حامض جلاكتويورونيك: رامنوز: فركتوز بنسبة 1.0 : 1.0 : 3.0 : 4.0 على التوالى و له وزن جزيئى  $10 \times 8.15$  جرام/مول. تم تقييم دور السكر العديد كمضادات للأكسدة على النحو التالى أولا: على قدرته على كسح الشوارد الحرة باستخدام مركب DPPH ثانيا: على مركب فوق أكسيد الهيدروجين ثالثا: تعيين القدرة الإختزالية رابعا: تعيين قدرته على الإرتباط بالمعادن. تم تعيين قيمة  $IC_{50}$  للسكر العديد على مركب DPPH ووجدت أنها 500 ميكروجرام/مل بعد 60 دقيقة وعلى فوق أكسيد الهيدروجين 1000 ميكروجرام/مل بعد 60 دقيقة كما وجد أن له قدرة إختزالية وقدرة على الإرتباط بالمعادن بلغت 90.2 % عند تركيز 1000 ميكروجرام/مل.

من هذه النتائج نستخلص أن عديد السكر له كفاءة كمضاد للأكسدة تعتمد إعتادا كبيرا على تركيبه الكيمائى والمجموعات الوظيفية الموجودة به والتي لها القدرة على اكتساح الإلكترونات الحرة. فهذه البوليمرات تحتوى على مجموعات الهيدروكسيل والكاربوكسيل التى لها تستطيع إختزال هذه المركبات عن طريق إعطاء أيون الهيدروجين أو إكتساب الإلكترونات نظرا لوجود ذرة الأوكسجين ذات السالبية الكهربية العالية.