

# Production Of A Bioactive Exopolysaccharide In Nanocrystalline Form *Pseudovibrio* Sp.

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**Abstract :** A strain of *Pseudovibrio* sp. strain 4MS 2020 with high exopolysaccharide production was isolated from sawa lake in Iraq and identified based on morphological, physiological, biochemical characteristics and phylogenetic examination of 16S rDNA sequences. *Pseudovibrio* sp. strain 4MS 2020 was optimized for production of maximum yeild of EPS which gave 10.8 g/l after 3 days of incubation with pH 7, 40°C, RPM 150, 750 µl inoculum sizes, yeast extract as nitrogen source. While, 20 g/l sucrose as a carbon source. EPSS2 was a major fraction of EPS contained sulfate (53.9%) and has no uronic acid. The monosaccharide composition of these fraction composed of glucose, rhaminose and arabinose with molar ratio 6.0 : 3.0 : 1.0, respectively. Then EPSS2 was Prepared as Nanocrystalline Exopolysaccharide (NCEPSS2). Therefore, EPSS2 and NCEPSS2 were subjected to antioxidant and anticancer activities, scince it showed that EPSS2 give maximum activity against DPPH radical found 88.8 % at 1500 µg/ml after 120 min, while against H<sub>2</sub>O<sub>2</sub> radical found that the activity was 64.8 at 1500 µg/ml after 60 min. while, NCEPSS2 give antioxidant activity was 99.3 % at 1500 µg/ml after 120 min. and the maximum antioxidant activity was 81.6 % at 1500 µg/ml after 60 min. for H<sub>2</sub>O<sub>2</sub> radical. By using MTT assay, EPSS2 and NCEPSS2 was effect on the proliferation of HCT-116, A-549, HepG-2 and MCF-7 cells. the calculated IC<sub>50</sub> for EPSS2 at cell lines HCT-116 was 230 µg/ml and HepG-2 showed a IC<sub>50</sub> values 203 µg/ml. While, the calculated IC<sub>50</sub> for cell line MCF-7 and A-549 showed no cytotoxic level for these cell lines. While, NCEPSS2 calculated IC<sub>50</sub> for cell line HCT-116 was 119 µg/ml. While, the cell line HepG-2 showed a IC<sub>50</sub> values 107 µg/ml. IC<sub>50</sub> values of cell line A-549 showed 97.7 µg/ml and the calculated IC<sub>50</sub> for MCF-7 was 200 µg/ml

**Keywords:** exopolysaccharides, Nanocrytalline exopolysaccharides, *Pseudovibrio* sp., antioxidant, anticancer.

## INTRODUCTION

An exopolysaccharide (EPS) is a sugar polymer that produced mainly by microorganisms as bacteria, either in a form bound to the cell wall, called capsular polysaccharide (CPS), or in a free form liberated into the culture medium, known as a slime EPS (Sutherland, 1972). However, it is believed that the physiological function of EPS is the first line of biological defense against phagocytosis, phage attack. The exopolysaccharides have strong biological activity and play roles in the regulation of cell division and differentiation, immune regulation, as well as have antitumor, antioxidant, and antiviral activities

(Yahya *et al.*, 2019 and Abdelhamid *et al.*, 2020). Analysis of exopolysaccharides has shown, they have high antioxidant activity, and this may explain the pharmacological basis of polysaccharide prevention of anile, inflammation and atherosclerosis (Zhang *et al.*, 2003). Li *et al.* (1992) Dysregulated activation of inflammation and oxidative stress has been recognized as one principal causes of inflammatory diseases like arthritis, diabetes, Alzheimer's disease and even cancers (Balkwill and Mantovani, 2012; Schacter and Weitzman, 2002). Exopolysaccharides (EPSs) contribute to various physiological activations in human beings as antitumor, antiviral, anti-inflammatory agents, anticardiovascular disease and anti-neurodegenerative disease specific Alzheimer's disease (Calazans *et al.*, 1997; Mahmoud *et al.*, 2014). Recently, major attention has been focused on marine polysaccharides due their structural and functional diversity beside it's natural source compounds. The novel active EPSs from marine bacteria hold an excellent potential. Application in biology and pharmacology (Luo and Fang, 2008). The EPSs have many advantages, including non-toxic and safe, unique physical and chemical properties, simply separated from bacteria and may be produced at large scale (Czaczyk and Myszka, 2007).

## MATERIALS AND METHODS

### 1. *Sampling and isolation of bacteria*

Samples were collected from marine sediment sources from sawa lake at Iraq. Bacterial isolation using serial dilution method Hayakawa and Nonomura (1987) on marine media contain (g/l): glucose 20, CaCO<sub>3</sub> 1.0, NH<sub>4</sub>NO<sub>3</sub> 0.8, KH<sub>2</sub>PO<sub>4</sub> 0.05, K<sub>2</sub>HPO<sub>4</sub> 0.6, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, MnSO<sub>4</sub>. 4H<sub>2</sub>O 0.1, yeast extract 0.1, dissolved in 750 ml sea water completed to 1 L (Kim *et al.*, 1998)

### 2. *Screening for EPSs production from liquid culture*

Bacterial isolates were screened for production of EPSs in a liquid production medium contain peptone 5.0, meat extract 3.0, FeSO<sub>4</sub> 0.01, agar 15, g/l dissolved in 750 ml sea water completed to 1 liter for distal water and adjusted pH 7 (Harrigan and McCance 1976) and cultivated at 37°C for 3 days at 120 rpm. After incubation, the culture medium was centrifuged at 5000 rpm for 10 min, the supernatant was mixed with Trichloroacetic acid (10%) and left overnight at 4°C and centrifuged at 5000 rpm again to remove protein. The pH of the clear solution was adjusted to 7. The supernatant was completed to four volumes with ethanol 95% and left overnight at 4°C. The precipitated EPSs were separated by centrifugation at 5000 rpm for 20 min washed twice with acetone and dehydrated by ether (Shene *et al.*, 2008).

### 3. *Assessment of antioxidant activity*

The antioxidant activity of different isolated EPSs was detected at 30, 60, 90 and 120 min. The free radical scavenging activity (RSA) was assessed by the decolouration of an the solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical evaluated spectrophotometrically at 517 nm (Brand-Williams *et al.*, 1995).

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

### 4. *Identification of bacterial isolates*

Isolate produced the highest amount of EPSs with high antioxidant activity was identified on the basis of its morphological and biochemical characteristics (Bergey *et al.*, 1994). While, the identification was confirmed by phylogenic analysis (Tamura *et al.*, 2011), genomic DNA from the bacterial isolate was isolated and quality was evaluated on 1.2% agarose gel, a single band of high Mw DNA has been observed. A Polymerase Chain Reaction was performed using 5'-TCCGTAGGTGAACCTTTGCGG-3' and the reverse primer was 5'-TCCTCCGCTTATTGATATGC-3' (Gardes and Bruns, 1993). Data were submitted to GenBank database and DNA sequence was compared to the GenBank database in the national Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) using the BLAST program Selected sequences of the greatest similarity to the 16S rRNA sequences of the bacterial isolate were aligned and generating the phylogenetic tree. The 16S rRNA gene sequences of the bacteria were

deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession numbers was MW000888.

#### 5. *Medium optimization for production of EPSs*

Optimization of media components required for maximum EPSs production by bacterial strain was evaluated at broth production media. Subsequently the medium component studied included the effect of different incubation time (2, 3, 4, 5 and 6 days), different pH (5, 6, 7, 8 and 9 adjusted with 1 N HCl or 1 N NaOH), different temperature (25, 30, 35, 40, 45 and 50 °C), different RPM (static, 50, 100, 150 and 200), different inoculum size (250, 500, 750 and 1000 µl.) different additional carbon sources (glucose, xylose, arabinose, mannose, galactose, fructose, sucrose, maltose and starch at 1% w/v), different concentration of sucrose (10, 15, 20, 25 and 30 g/l) and different organic nitrogen sources (yeast extract, malt extract, peptone, beef extract, potassium nitrate, ammonium oxalate, ammonium molybdate and ammonium sulfate).

#### 6. *Production and Fractionation of EPSs*

The promising isolates which give highest antioxidant activity was selected to production EPSs. The fermented broth was collected and centrifuged at 4000 rpm at 4°C for 30 min to remove bacteria cells. TCA (5%) was added and left over night at 4°C and centrifuged at 5000 rpm for 20 min 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 centrifuged. The precipitate was re-dissolved in deionized water followed by dialysis against deionized water for 72 h. The dialyzed solution was subjected to fractional precipitated by 1, 2, 3, and 4 volumes of cold absolute ethanol. The yield of major fraction obtained by three volumes of absolute ethanol was washed by acetone, diethyl ether, dried at 40°C and coded as EPSS2. The UV absorption spectrum was recorded using a UV-Vis Spectrophotometer 2401PC (Shimadzu, Japan) between 200 and 800 nm, in order to examine the existence of proteins and nucleic acids (Wang, 2007).

#### 7. Analysis of EPSS2

The FTIR spectra of EPSS2 was performed with KBr pellets, (2.0 mg sample and 200 mg KBr using the FTIR-UNIT Bruker Vector 22 Spectrophotometer), according to Brock-Neely (1957). While, Uronic acids were determined at 525 nm by the *m*-hydroxybiphenyl colorimetric method (Filisetti-Cozzi and Carpita, 1991). Sulfate was determined using the turbidly method (Dodgson and Price, 1962). The monosaccharide composition was determined (Agilate Pack, serics1, 200), equipped with Aminex carbohydrate HP-87C column (300 ×7.8 mm) with water deionized as the mobile phase at 0.5 mL/min (Randall *et al.*, 1989).

#### 8. Assessment of Antioxidant activity

##### 8.1. *DPPH assay*

EPSS2 with different concentrations 100, 300, 500, 1000 and 1500 µg/ml were used for assessment of antioxidant activity with DPPH assay which the mixture of 2 ml of DPPH solution and of EPSS2 were used. The mixture was shaken vigorously and left to stand for 30, 60, 90 and 120 min in the dark, and the absorbance was measured at 517nm and the scavenging activity was calculated as follows:

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

##### 8.2. *Hydrogen peroxide scavenging (H<sub>2</sub>O<sub>2</sub>) assay*

The ability of EPSS2 to scavenge hydrogen peroxide can be estimated according to Ruch *et al.*, (1989). Different concentrations of EPSS2 (200, 400, 600, 800, 1000 and 1500 µg/ml) in distilled water is added to H<sub>2</sub>O<sub>2</sub> and absorbance determined at 230 nm. The percentage of H<sub>2</sub>O<sub>2</sub> scavenging is calculated as follows:

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

#### 9. Preparation of Nanocrystalline Exopolysaccharide (NCEPSs)

Exopolysaccharide (EPS) (0.11 gm) was hydrolyzed by diluted sulfuric acid (64 %, 250 ml) and incubated at room temperature for 24 hours under static conditions. At the end of the incubation period, the color of the suspension becomes dark brown. Then the EPS suspension was diluted with de-ionized water to stop the hydrolysis reaction, allowed to settle overnight until the suspensions were layered and the clear top layer was decanted off. After that, the supernatant washed with de-ionized water until no layered was found by centrifuge at 5000 rpm for 10 min. The supernatant was taken and the previously step was repeated (Several times). The final washed was conducted using dialysis page with de-ionized water for several days until the water pH remained constant. Afterwards, ultrasonication was conducted for 20 min at an output power of 1200W. Finally, the NCEPSs suspension sample was subjected to freeze-drying. All processings were done under dark conditions. The NCEPSs was evaluated for their surface and shape characteristics by transmission electron microscopy. The TEM image was carried out using: Electron microscope JEOL-JEM-1230 (Beck-Candanedo *et al.*, 2005).

#### 10. Evaluation of Cytotoxic Effects using different cell line

For cytotoxicity assay, the cells of MCF-7 cells (human breast cancer cell line, HepG-2 cells (human Hepatocellular carcinoma), A549 (human Lung carcinoma) and HCT-116 (colon carcinoma) were seeded in 96-well plate at a cell concentration of  $1 \times 10^4$  cells per well in 100 $\mu$ l of growth medium. Fresh medium containing different concentrations of the EPS BU was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method (Mossman, 1983). The number of viable cells and the percentage of viability were calculated as

$$[1 - (\text{OD}_t / \text{OD}_c)] \times 100$$

Where, OD<sub>t</sub> is the mean optical density of wells treated with the tested sample and OD<sub>c</sub> is the mean optical density of untreated cells.

## RESULTS AND DISCUSSION

### 1. Isolation, Screening, and Identification of the EPS producing bacteria

A total 25 bacterial isolates were isolated from marine source according to their special morphological characteristics colony for screening program to production of EPSs. Among them 10 strains were found to be producer EPSs. The antioxidant activity for these isolates was quantitatively assessed by DPPH free radical scavenging activity technique Table (1). The highest yield of EPS (8.74 g L<sup>-1</sup>) was obtained by a marine bacterium isolated from Sawa lake (S2) has high antioxidant activity 95 % after 90 min. EPSs from marine microorganisms are important to new medication revelation (Han *et al.*, 2005; Miranda *et al.*, 2008 and Xu *et al.*, 2009). The results are in a good agreement with Hu *et al.*, (2001) and Pilar *et al.*, (2005) who reported that the polysaccharides assume an imperative part as free radical scavengers and antioxidant for the counteractive action of oxidative harm in living organisms. To characterize strain S2 further, standard morphological, physiological and biochemical plates showed that strain S2 was a Gram-negative short rod, which Dull Colony surface, rough Colony texture, undulate Edge, Irregular Large colony and has No pigments. While, it gave positive results with Catalase test, Voges-Proskauer test, Simon citrate test, Nitrate reduction. Furthermore, The most valuable strategy for setting up the relatedness of higher taxa is the comparative investigation of the ribonuclease resistant oligonucleotides of the 16S ribosomal RNA (rRNA) (Huddleston *et al.*, 1997). Phylogenetic analysis based on 16S rDNA sequence revealed its close relationship to *Pseudovibrio* sp. The nucleotide sequence

was deposited in the GenBank sequence database. So, it was identified as *Pseudovibrio* sp. strain 4MS 2020 with accession number MW000888. Figure (1) showed Phylogenetic tree of the partial sequence of 16S rRNA of the local isolate *Pseudovibrio* sp. strain 4MS 2020 respects to closely related sequences available in Gen Bank databases.

## 2. Effect of some factors on the production of EPSs

*Pseudovibrio* sp. strain 4MS 2020 can produce maximum level of EPS (8.8 g/l) cell dry weight (3.7 g/l) was obtained after three days of incubation then the EPS decreased. These results reveal that the relationship between EPS productivity and period of incubation is variable depending on the organism (Figure 2. A). pH affected on the level of EPSs production by *Pseudovibrio* sp. strain 4MS 2020. Thus maximal yield was noticed at pH 7 was 7.9 g/l and cell dry weight was 3.8 g/l either excess or lowering in the pH of the medium produced in reduced EPSs production (Figure 2.B). While, Incubation temperature of the fermentation medium is informed to effect the growth of this strain thus the maximal EPSs production (9.1 g/l) at cell dry weight (3.9 g/l) was noticed at 40°C. Diversity in temperature in any way leads to reduce of EPS production (Figure 2.C). EPS production by *Pseudovibrio* sp. strain 4MS 2020 affected by RPM degree (Figure 2.D). Thus maximal yield at RPM 150 was 9.8 g/l and cell dry weight was 3.7 g/l. Then, bacterial inoculum sizes in the fermentation medium have strong effect on the production of EPSs. Figure (2.E) Show the maximal EPS production (10.2 g/l) at cell dry weight (3.8 g/l) was noticed at 750 µl. Change in inoculum size at any way leads to reduce of EPSs production. Different carbon sources were added into medium at 1% level resulted in a increase in the EPSs production (Figure 3.A). Sucrose was supported maximal yield (10.2 g/l) at cell dry weight (3.8 g/l) and All the other carbon sources also showed considerable amount of EPS production. While Figure 2.F showed that different sucrose concentration can effect on the production of EPS. Sucrose with (20 g/l) can give the maximum yield of EPS productivity (10.5 g/l) and cell dry was 3.8 g/l. While, effect of additional organic and inorganic nitrogen sources into medium resulted in a respectable increase in the EPS production. From all the diverse nitrogen sources tested, yeast extract was supported maximal EPS output (10.8 g/l) with cell dry weight (3.8 g/l). All the other nitrogen sources also showed various amount of EPSs production (Figure 3.B). Potassium nitrate was the least effective as a nitrogen source for production of EPSs (3.4 g/l) with cell dry weight (1.6 g/l). Maheswari and Karthiga, (2016) studied the carbon and nitrogen sources were used in the optimization process. Among the carbon sources used Adenitol showed maximum amount of exopolysaccharides production by *Pseudomonas putida*. Among the nitrogen sources used (NH<sub>4</sub>)<sub>2</sub> CO<sub>3</sub> showed maximum amount of exopolysaccharides production by *Pseudomonas putida*. Where, the maximum amount of EPS was observed in jaggery and in nitrogen source tryptone was the best source for *Frateria aurentia* (Sivakumar *et al.*, 2012). Whereas, Wang *et al.* (2004) stated that among the carbon sources tested, maximum EPSs (10.45 g/l) be there found in the maltose medium. Among the nitrogen source tested, the maximum EPSs production (10.53 g/l) be there found in the peptone.

## 3. Isolation, partial purification and composition of EPSS2

Exopolysaccharide production reached a maximum of 10.8 gL<sup>-1</sup> after scaling up fermentation for 3- days. crude extract of EPS which subjected to partial purification and fractionation by re-dissolved in deionized water followed by dialysis in contradiction of deionized water for 72 h. The dialyzed solution was precipitated by 1, 2, 3, and 4 volumes of cold absolute ethanol. The main fraction of EPS (75%) was obtained after fractionated with three volume of ethanol precipitation from the crude EPS then coded EPS2. It was gray in color, in a form of odorless powder and soluble in water but insoluble in ethanol and other organic solvents.

The EPSS2 fraction was contained sulfate (53.9%) and has no uronic acid. The monosaccharide composition of these fraction composed of glucose, rhaminose and arabinose with molar ratio 6.0 : 3.0 : 1.0, respectively. These indicate that the this fraction is acidic heteropolysaccharide. The FTIR spectrum fraction exhibited a significant as showed at Figure (4), broad characteristic peak at around at 3298.57 cm<sup>-1</sup> region was attributed to the expansion vibration of O–H in the ingredient sugar residues (Kanmani

*et al.*, 2011). The EPS fraction also appears to have a particular band at 1660.90  $\text{cm}^{-1}$ , which is dominated by circle vibrations (Sun *et al.*, 1998), The band at 1069.71  $\text{cm}^{-1}$  indicated the SO= 3 . interfered with stretching vibration of C-O glycosidic bond vibration and the strap at 834.00  $\text{cm}^{-1}$  suggested the  $\beta$ -pyranose (Cheng *et al.*, 2008).

#### 4. Characterization of Nanocrystalline Exopolysaccharide (NCEPSS2)

Crystallization is a very significant property that affects all mechanical and physical properties of all kind of polymers especially EPSs. Nanocrystalline Exopolysaccharide (NCEPSs) was separated from EPS from *Lactobacillus plantarum* NRRL B- 4496 produced by sulfuric acid hydrolysis sonication treatment (El-Waseif and El-Ghwas, 2015). Therefore, EPSS2 was Prepared as Nanocrystalline Exopolysaccharide (NCEPSS2). Transmission electron microscope (TEM) showed that, the NCEPSS2 in the reaction mixture has a uniform shape and showing varying sizes under magnification of 50 nm and 100 nm as observed in Figure (5).

#### 5. Antioxidant activity of EPSS2 and NCEPSS2

the antioxidant activity was decide quantitatively at different times (30, 60, 90 and 120 min). Figure (6.A) Showed that the whole antioxidant activity was increased by increasing the concentrations of the compound from 100, 300, 500, 1000 and 1500  $\mu\text{g/ml}$ . Maximum antioxidant activity was 88.8 % at 1500  $\mu\text{g/ml}$  after 120 min. So, the  $\text{IC}_{50}$  value against DPPH radical found about 500  $\mu\text{g/ml}$  after 60 min. While, with using  $\text{H}_2\text{O}_2$  scavenging activity the ability of EPSS2 to scavenge hydrogen peroxide can be estimated by different concentrations (200, 400, 600, 800 and 1000  $\mu\text{g/mL}$ ) demonstrated that the most extreme activity was 64.8 at 1500  $\mu\text{g/ml}$  after 60 min. The  $\text{IC}_{50}$  value against  $\text{H}_2\text{O}_2$  radical found about 1500  $\mu\text{g/ml}$  after 15 min (Figure 6.B). Therefore, By using nanofom of different concentration of EPS fraction (NCEPSS2) to determine of the antioxidant activity against DPPH and  $\text{H}_2\text{O}_2$  radical. Maximum antioxidant activity was 99.3 % at 1500  $\mu\text{g/ml}$  after 120 min. and  $\text{IC}_{50}$  value found about 500  $\mu\text{g/ml}$  after 30 min. While it showed that Maximum antioxidant activity was 81.6 % at 1500  $\mu\text{g/ml}$  after 60 min. The  $\text{IC}_{50}$  value against  $\text{H}_2\text{O}_2$  radical found about 1000  $\mu\text{g/ml}$  after 30 min. Xiao *et al.*, (2016) investigated that the antioxidant activities of two EPS isolated from *Brasenia schreberi* and examines the antioxidant activity by DPPH,. Demonstrated that the  $\text{IC}_{50}$  estimations was (31.189 mg/ml and 1.863 mg/ml).

#### 6. Antitumor activity against different cell lines

By using MTT assay, EPSS2 fraction was effect on the proliferation of HCT-116, A-549, HepG-2 and MCF-7 cells was studied as presented in Figure (7). the calculated  $\text{IC}_{50}$  for cell line HCT-116 was 230  $\mu\text{g/ml}$  and the cell line HepG-2 showed a  $\text{IC}_{50}$  values 203  $\mu\text{g/ml}$ . While,  $\text{IC}_{50}$  for cell line MCF-7 and A-549 showed no cytotoxic level for these cell lines. while, there are increase in the activity of EPSS2 in Nano form (NCEPSS2) aganist different cell lines as the calculated  $\text{IC}_{50}$  for cell line HCT-116 was 119  $\mu\text{g/ml}$ . While, the cell line HepG-2 showed a  $\text{IC}_{50}$  values 107  $\mu\text{g/ml}$ . the cell line A-549 showed a cytotoxic level as concluded from the high calculated  $\text{IC}_{50}$  values 97.7  $\mu\text{g/ml}$ . While, the calculated  $\text{IC}_{50}$  for cell line MCF-7 was 200  $\mu\text{g/ml}$  Figure (8). Umezawa *et al.* (1983) screened EPSs of marine bacteria for their anticancer activity in contradiction of sarcoma-180 solid tumor in mice. Also, Zhang *et al.* (2002) reported that glycoproteins from *E. cloacae* showed antitumor effect on mice and Wu and Chen (2006) suggested that Sulfated EPSs can inhibited the metastasis and propagation of tumor cells by binding to growth factors and cell linkage molecules.

In general EPS has monosaccharide composition diversity with high molecular weight and exhibit more interactions with many different receptors at cell surface especially on dendritic cells such as toll like receptors (TLRs) and C-type lectins which have carbohydrate recognition domain (CRD). so, it enhancing complement system and innate immune system against tumor (Brown and Gordon, 2005; Willment *et al.*, 2005). Polysaccharides with high molecular weight cannot enter the cells but, instead they bind with receptors of cancer cell so, it controlling cell signaling and cellular transduction. Zhou *et al.*, (2017) reported that *Lactobacillus plantarum* NCU116 can produce EPS116 and it inhibites the proliferation and survival of mouse colorectal carcinoma CT26 cells through induction of apoptosis via c-

Jun, Fas/ FasL pathways. Furthermore, the anticancer activity of EPS116 may be TLR-2 dependent. Asker *et al.*, (2018) isolated eleven isolates from marine sediment of the Mediterranean and Red Seas based on their production of EPS and study its cytotoxic activity against HepG2 cells found that the EPSs produced from marine bacteria are very promising for treating the HepG2 cells.

## CONCLUSION

From these results we conclude that, marine bacteria *Pseudovibrio* sp. strain 4MS 2020 can produce an Exopolysaccharide under optimized condition and it has antioxidant and anticancer activity. While these activities could be increased by preparing of this Exopolysaccharide in Nanocrystalline form

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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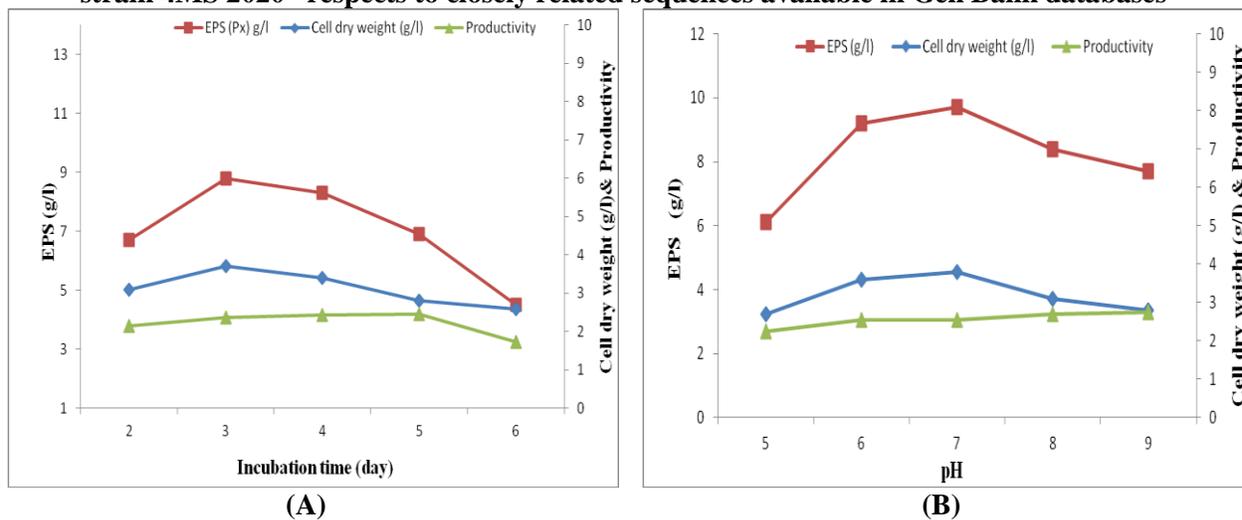
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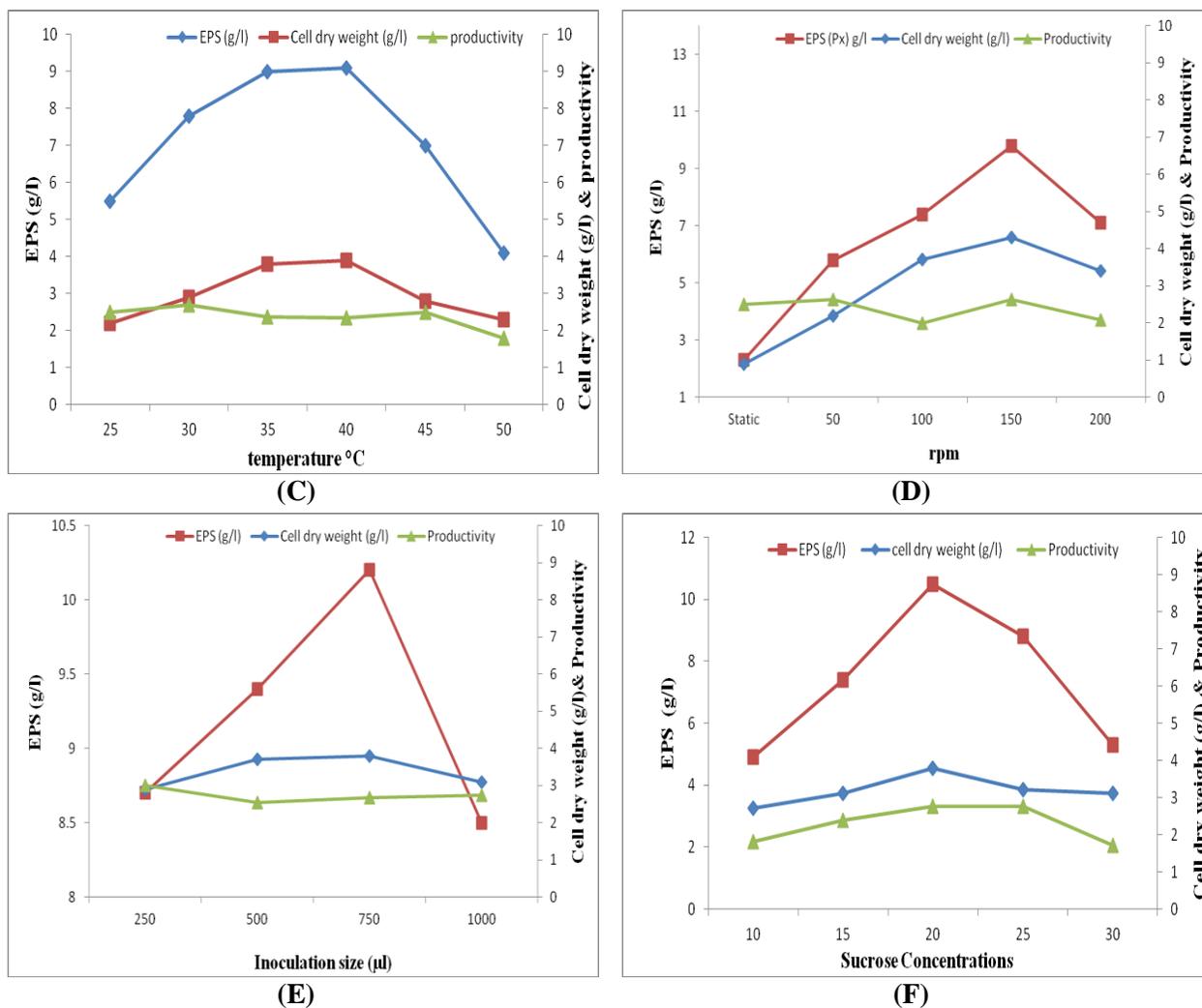
**Table (1) DPPH free radical scavenging activity (%) for marine bacterial EPS at different concentrations with different periods.**

Isolate no.	EPS conc.	DPPH free radical scavenging activity (%) with time			
		10 min	30 min	60 min	90 min
S 2	5 mg	31.4	42.3	50.4	65.6
	10 mg	54.5	63.1	75.8	89.4
	20 mg	72.7	79.4	84.5	95.5
S 3	5 mg	10.5	15.2	21.6	32.1
	10 mg	19.7	24.4	30.1	38.7
	20 mg	24.3	32.9	38.8	45.9
S 5	5 mg	14.3	23.9	35.6	42.2
	10 mg	25.8	33.2	40.4	49.5
	20 mg	36.1	45.5	52.7	58.8
S 10	5 mg	8.7	9.1	10.8	12.7
	10 mg	13.2	14.4	18.7	20.0
	20 mg	15.1	17.8	21.3	25.7
S 12	5 mg	35.0	48.4	54.4	59.5
	10 mg	45.3	59.7	68.7	77.1
	20 mg	61.1	72.8	79.9	89.2
S 14	5 mg	11.2	19.1	27.5	35.5
	10 mg	18.6	26.0	38.9	46.8
	20 mg	22.7	30.9	39.3	48.6
S 15	5 mg	17.5	29.3	41.8	52.4
	10 mg	25.3	38.7	52.5	69.3
	20 mg	48.9	64.1	81.4	88.8
S 17	5 mg	15.7	24.4	32.8	33.7
	10 mg	19.3	28.3	34.5	37.6
	20 mg	23.5	35.1	38.9	40.0
S 19	5 mg	28.7	40.5	51.1	64.5
	10 mg	42.2	61.6	70.7	83.3
	20 mg	63.4	70.5	79.8	87.9
S 20	5 mg	13.1	14.9	16.7	19.5
	10 mg	15.4	17.2	19.4	20.6
	20 mg	15.9	17.8	20.1	22.2

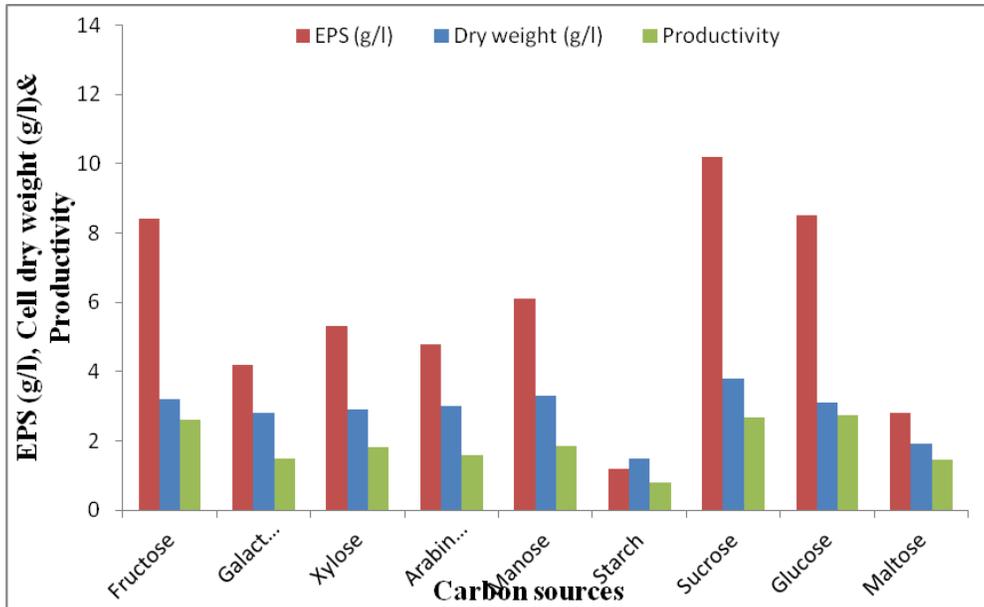


**Figure (1) Phylogenetic tree of the partial sequence of 16S rRNA of the local isolate *Pseudovibrio* sp. strain 4MS 2020 respects to closely related sequences available in Gen Bank databases**

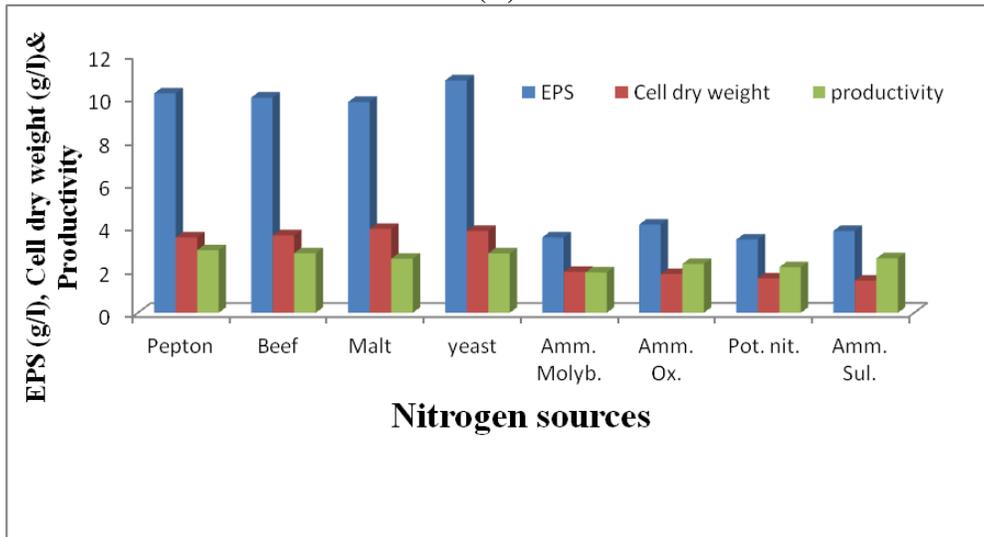




**Figure (2).** Effect of some factors on the production of EPSs from *Pseudovibrio* sp. strain 4MS 2020 (A) incubation time; (B) pH; (C) temperature; (D) aeration; (E) inoculum size and (F) Sucrose concentration



(A)



(B)

**Figure (3).** Effect of carbon and nitrogen sources on the production of EPSs from *Pseudovibrio* sp. strain 4MS 2020i (A) carbon sources and (B) nitrogen sources.

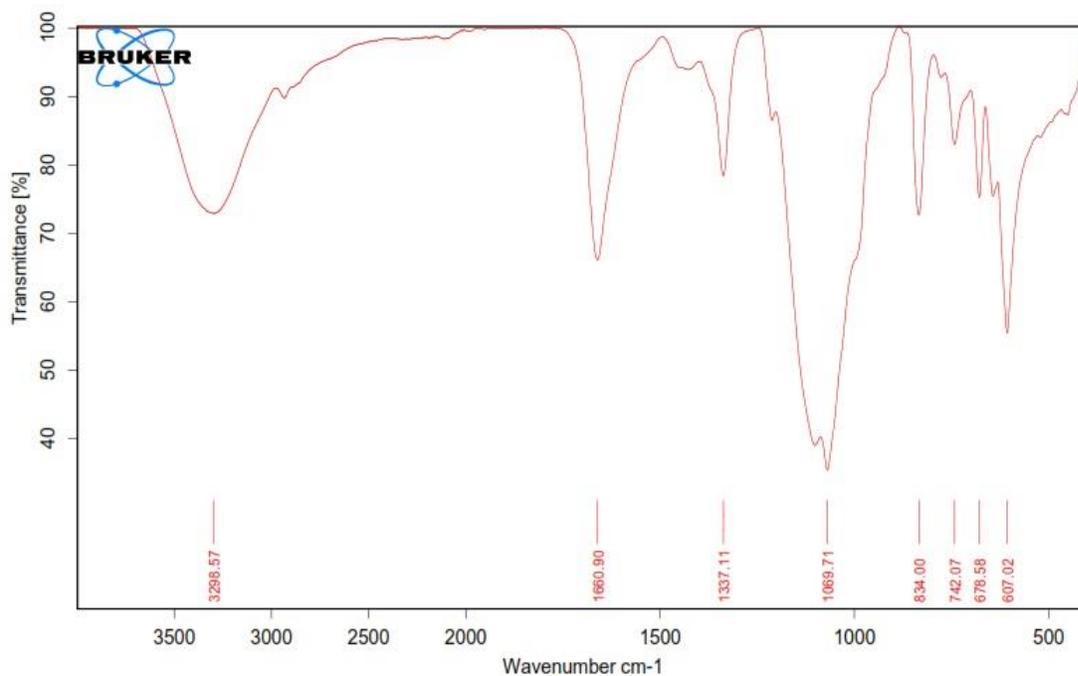
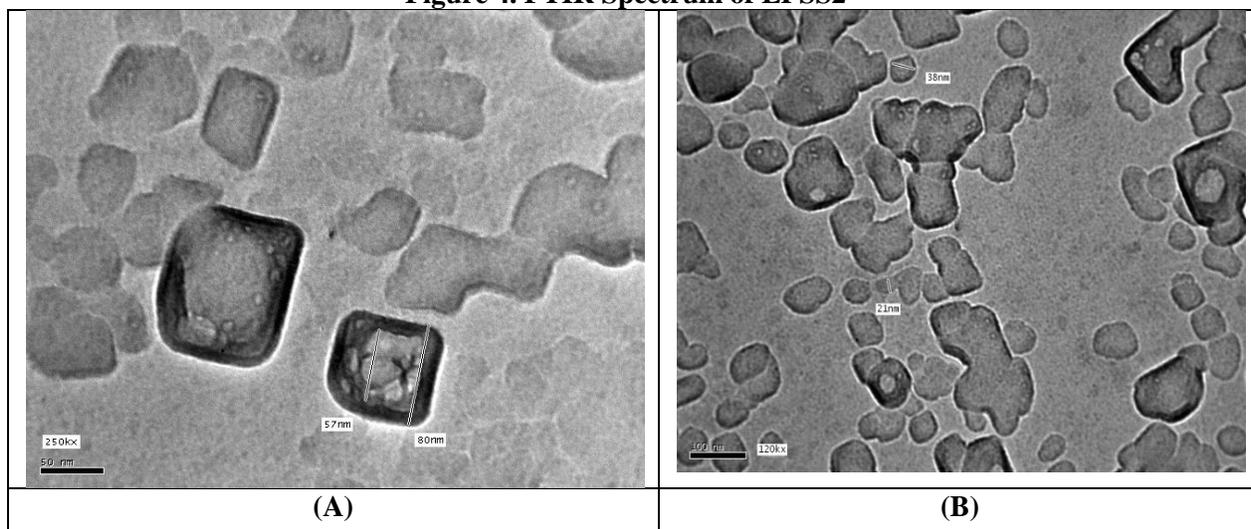
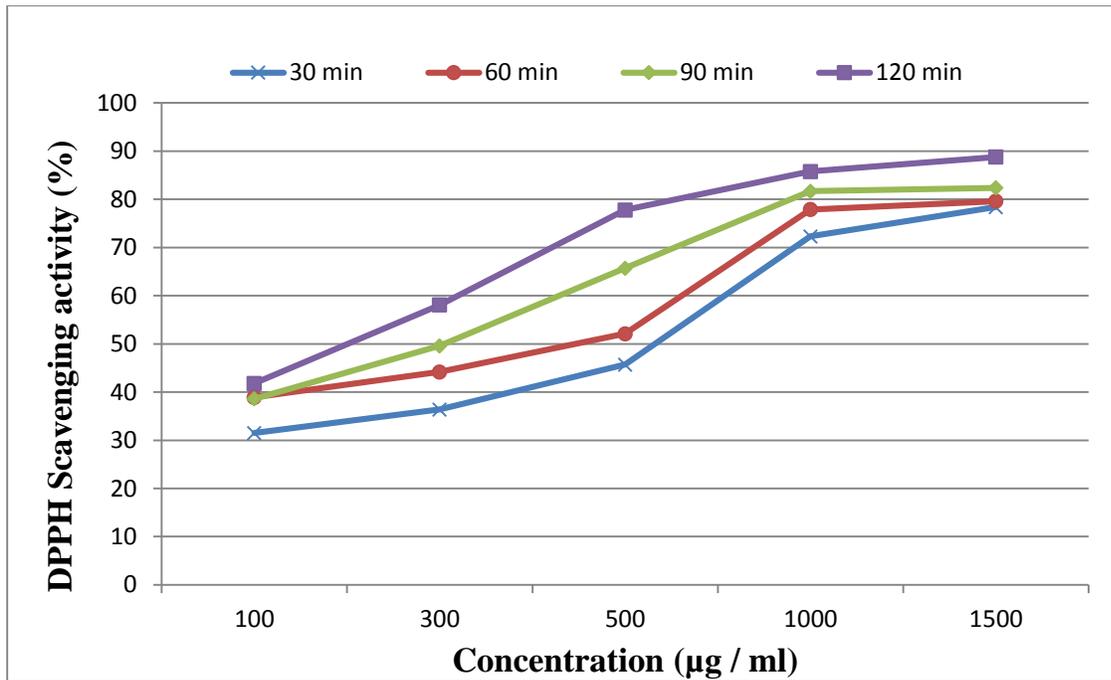


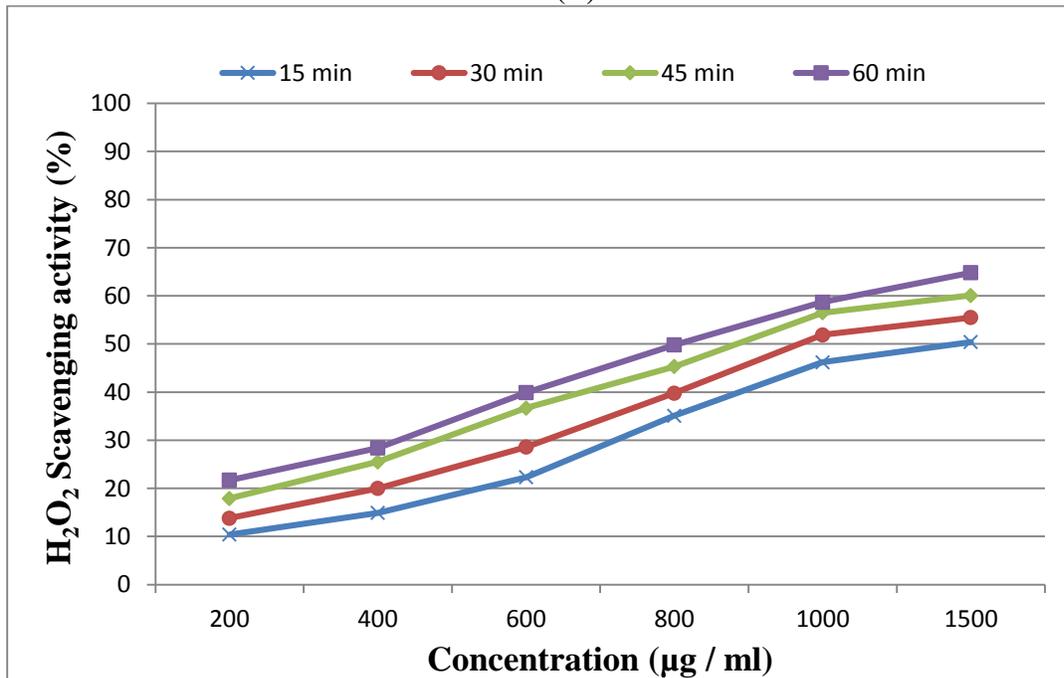
Figure 4. FTIR Spectrum of EPSS2



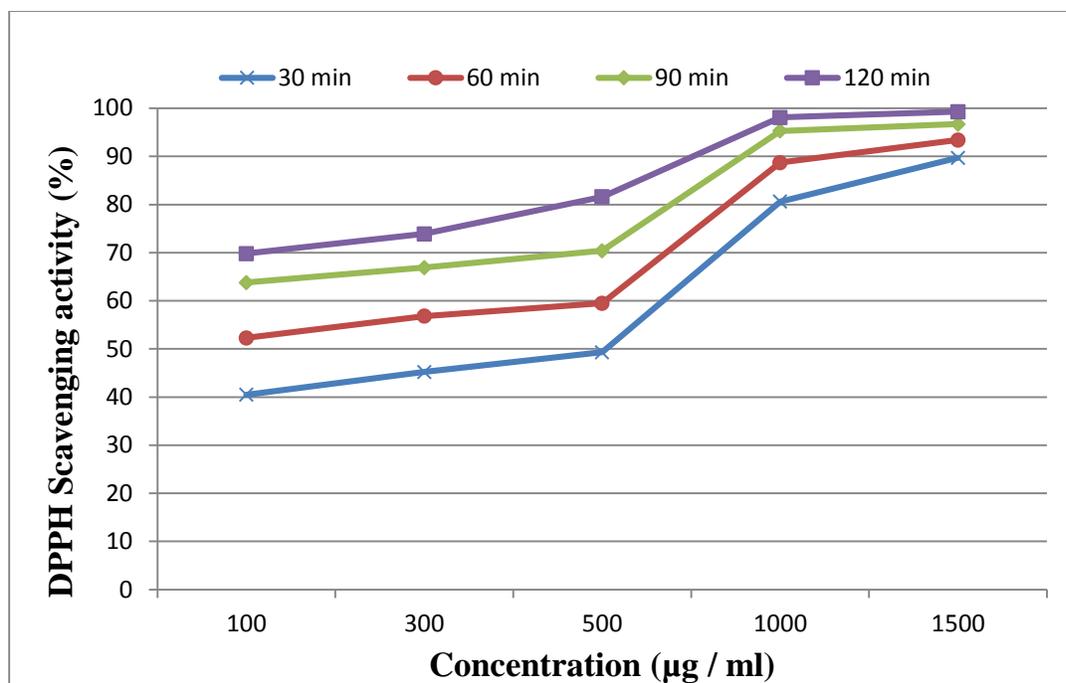
**Figure. 5: Transmission electron microscopy (TEM) of NCEPSS2 under different magnifications (A) : 50nm & (B): 100nm.**



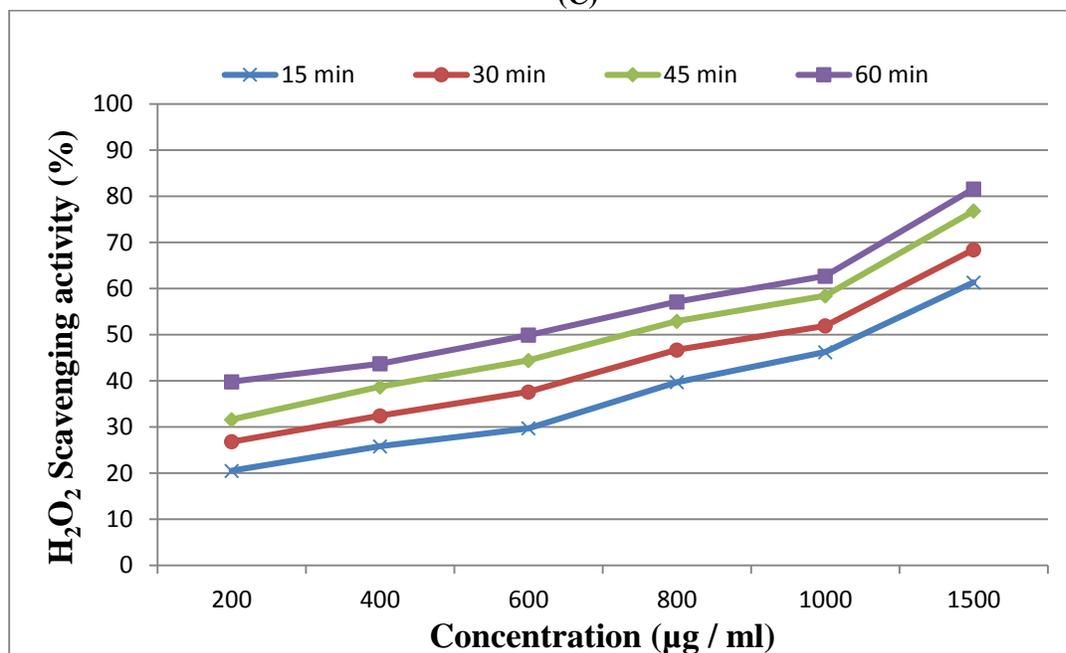
(A)



(B)



(C)



(D)

Figure 6. Scavenging activity of EPSS2 at different time (A) DPPH and (B): H<sub>2</sub>O<sub>2</sub> and NCEPSS2 (C) DPPH and (D): H<sub>2</sub>O<sub>2</sub>

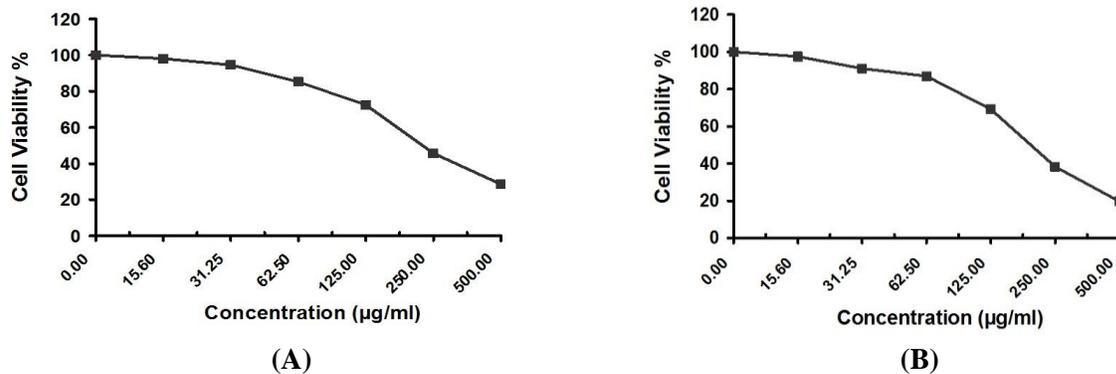


Figure 7. Evaluation of cytotoxicity against different cell line (A): HCT & (B): HepG2

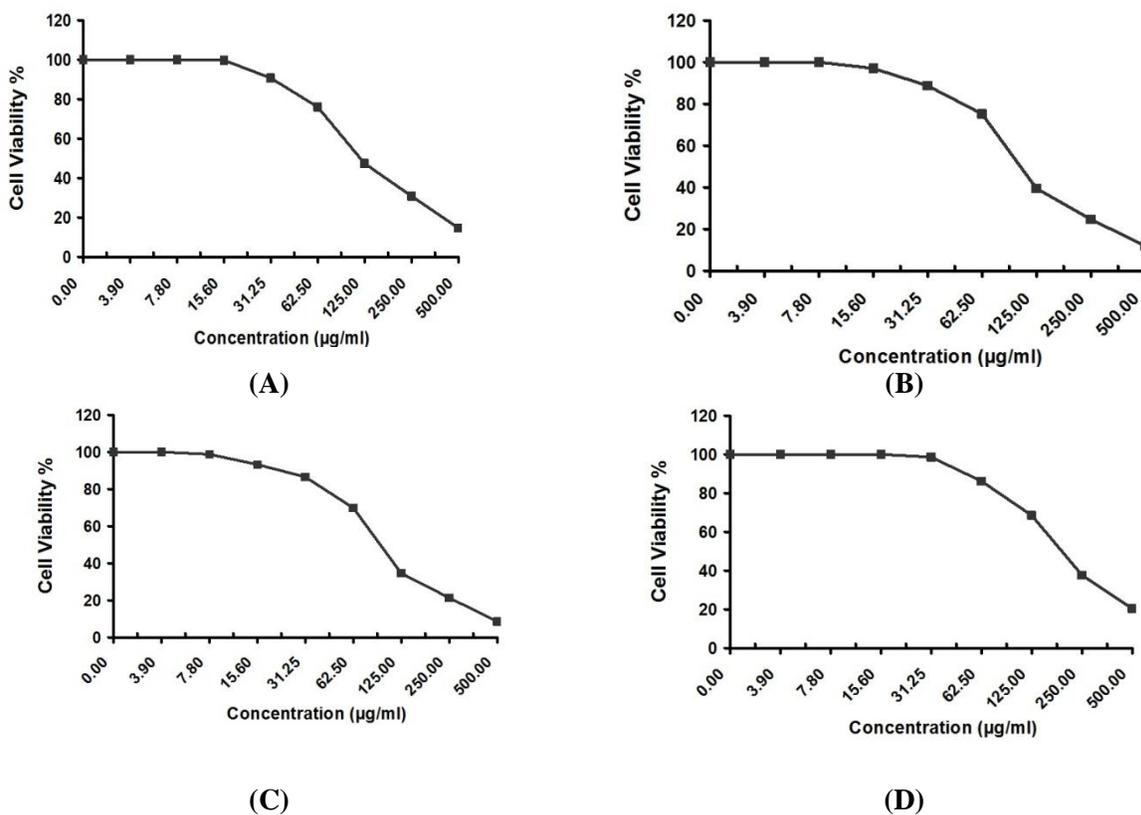


Figure 8. Evaluation of cytotoxicity against different cell line (A): HCT & (B): HepG2 & (C) A-549 & (D) MCF7