

## RESEARCH ARTICLE

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# Exopolysaccharide from Marine *Bacillus velezensis* MHM3 Induces Apoptosis of Human Breast Cancer MCF-7 Cells through a Mitochondrial Pathway

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### Abstract

**Objective:** The production of new natural pharmaceutical agents that increase the efficiency of chemotherapy without affecting the normal cells is the goal of all researchers. Therefore, the present study expects to evaluate the antioxidant and anticancer studies against MCF-7 cell lines of EPS produced by novel Egyptian marine bacterial strain. **Methods:** Marine bacterium was isolated, purified and identified by 16S rRNA gene amplification and sequence analyses. MHMEPS (the produced EPS) was analyzed by Fourier Transform Infra-red (FTIR), monosugars identification by HPLC, molecular weight estimation and sulfur content were determined. While, in-vitro antioxidants characters was determined using various methods and anticancer studies against MCF-7 cell lines. **Results:** *Bacillus velezensis* MHM3 produced 5.8 g/L of MHMEPS. The chemical analysis of MHMEPS showed 24% uronic acid and 18.19% sulfate and monosugars glucuronic acid, glucose, fructose and rhamnose with molar ratio of 4.00: 2.00: 1.00: 0.13, correspondingly, with an overall weight average molecular weight  $M_w$  of  $1.145 \times 10^4$  g/mol and the number average of molecular weights  $M_n$  of  $5.155 \times 10^3$  g/mol. The FTIR analysis and periodate oxidation indicate the existence of  $\beta$ -(1-4) linkage acidic polysaccharide. MHMEPS showed antioxidant scavenging activity against DPPH•, H<sub>2</sub>O<sub>2</sub> and Metal chelating activity, respectively. So, reducing power method give high activity at 500  $\mu$ g/ml. MHMEPS hinder the proliferation of MCF-7 cells at 5-80  $\mu$ g/ml compared to the control group. Moreover, induced apoptosis was associated with activation of caspase-3. Also increased cytochrome C levels significantly in a dose-dependent manner compared with the control. The Caspase-3 activity was raised in MHMEPS treated MCF-7 cells compared with the control ( $p < 0.05$ ) in a dose-dependent manner. Therefore, the result of DNA fragmentation was confirmed by DNA ladder assay. We presume that MHMEPS has high potential at its low concentration, as a novel restorative agent for the treatment of MCF-7 cells, with no cytotoxicity against normal cells.

**Keywords:** Exopolysaccharide- marine *Bacillus velezensis* MHM3- apoptosis human breast cancer MCF-7 cells

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### Introduction

Breast cancer is the widely recognized malignancy in the female populace and was the driving reason for disease related death among ladies between the ages 35– 54 everywhere throughout the world (Ruocco et al., 2016). Microbial exopolysaccharides (EPSs) are emitted into the outer condition. Moreover, an eatable polysaccharide (PGL) from marine algae (*Gracilariopsis lemaneiformis*) have valuable antitumor properties to hinder cell proliferation by actuating apoptosis which largely interceded by fas/fasI in cancer cell (Yani et al., 2017). The properties of EPSs made changes in the industrial and medical divisions because of their of practical applications and prospects. These applications have been broad in regions, for example, pharmacological, nutraceutical, useful food,

cosmeceutical, herbicides and bug sprays among others (Raposo et al., 2013; Abid et al., 2018), while prospects incorporates utilizes as anticoagulant, antithrombotic, immunomodulation, anticancer, an aversion and treatment of cardiovascular sicknesses, antiviral and antimicrobial impacts (Nwodo et al., 2012). EPSs from microorganisms are reporting to have free radical scavenging activity, superoxide radical scavenging, reducing properties, lipid peroxidation hindrance, concealment of expansion and oxidative anxiety etc. (Shengjie et al., 2014; Selim et al., 2018). Polysaccharides segregated from various sources genera are fit for giving antitumor action; these incorporate the enactment of macrophages, T-lymphocytes and natural killer cells, which can emit to secrete inflammatory mediators of cytoki as the tumor necrosis factor, interferon, and

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interleukin. Polysaccharides can discourage the E-selectin protein and gene expression, which restrain tumor cell-to-cell adhesion. Other mechanism incorporates antiproliferative impacts, apoptosis enlistment and tumor cells differentiation (Ahmed and Elmenoufy, 2016). Apoptosis is an imperative administrative mechanism in the improvement of tissues, including biological events, for example, chromosome build up, DNA laddering, membrane blebbing, and cytochrome C discharge, which prompts the evacuation of pointless cells (Yan and Shi, 2005). Two noteworthy pathways intervening the procedure of apoptosis, the death receptor pathway (extrinsic), furthermore the mitochondrial pathway (intrinsic) (Sayers, 2011). Mitochondria assume a noteworthy part in the apoptotic procedure. Besides, both the extrinsic and the intrinsic pathways get unite at the mitochondrial level and trigger mitochondrial layer permeabilization (Wong, 2011). The goal of the present study was isolation and recognition of bacterial strain that produced EPS. In addition, to analyze the in-vitro cytotoxic activities of EPS in breast adenocarcinoma (MCF-7) cells utilizing MTT cytotoxicity assay. In addition to the current examination tried the conceivable mechanism of action involves induction of apoptosis.

## Materials and Methods

### *Bacterial strains*

Thirteen isolates were isolated from the sediment of the seashore of El-Ein El Sokhina (Red Sea Coast, Suez governorate, Egypt). The methods of sampling and isolating strain have been described (Asker et al., 2015).

### *Screening and identification of bacterial strain*

The isolates of marine bacteria were screened for production of EPSs. The pure isolates were inoculated into a 250 mL flask containing 50 ml of screening marine nutrient medium and cultivated at 37°C for 72 h at 100 rpm. After centrifugation at 5,000 rpm for 30 min, EPS production at supernatant was determined according to Dubois et al., (1956). Strain MHM3, which give high production of EPS, was identified based on morphological, biochemical and physiological characteristics (Cappucino and Sherman, 2004). Also, phylogenetic analysis based on the 16S rRNA gene sequence was made as described by Tamura et al., (2011).

### *Culture conditions production of EPS*

MHM3 isolate was grown in liquid medium containing (g/L) sucrose 50; peptone 4 and yeast extract 2; and dissolved in 750 ml seawater at pH 7 (Jing et al., 2013). The fermentation cultures were then incubated at 37°C with shaking at 100 rpm for 3 days. The fermented broth was collected and centrifuged at 4,000 rpm at 4°C for 30 min to remove bacteria cells. Trichloroacetic acid TCA 5% was added and left over night at 4°C and centrifuged at 5,000 rpm to remove protein. The pH of the clear solution was adjusted to 7.0 with NaOH solution (Liu et al., 2011). Four volumes of cold absolute ethanol was added to the supernatant and the precipitate was collected by centrifuged. The precipitate obtained was

re-dissolved in deionized water followed by dialysis against deionized water for 48 h. The dialyzed solution was subjected to fractional precipitated by 1, 2, 3, and 4 volumes of cold absolute ethanol. The yield major fraction obtained by two volumes of absolute ethanol was lyophilized and coded MHMEPS. 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 and Luo, 2007). The yield of MHMEPS was determined by Dubois method.

### *Analysis of monosaccharide composition*

The monosaccharide composition was determined by HPLC on shim pack SCR-101N column (Shimadzu) with water deionized as the mobile phase at 0.5 mL/min (El-Sayed et al., 2005). Sulfate was measured using the turbidimetric method (Dodgson and Price, 1962) with Na<sub>2</sub>SO<sub>4</sub> as standard. Uronic acid contents were determined using the *m-hydroxybiphenyl* colorimetric procedure (Filisetti-Cozzi and Carpita, 1991).

### *Fourier-transform infrared spectrometric analysis (FT-IR)*

The MHMEPS was analyzed with a FT-IR spectrophotometer Bucker scientific 500-IR using disk technique with KBr powder as matrix over a wave number range 4,000-500 cm<sup>-1</sup> (Ray, 2006).

### *Molecular weight determination*

The molecular weight of MHMEPS was determined to a high-performance chromatography (HPLC, Agilent 1100 Series System, Hewlett-Packard, Germany) with refractive index (RI) detection, Water Company Ireland according to Jun et al., (2009).

### *Periodate oxidation*

Desulphated MHMEPS (30 mg) was dissolved in 12.5 ml of distilled water was mixed with 12.5 ml of 30 mM NaIO<sub>4</sub>. The solution was kept in the dark at room temperature; 0.1 ml aliquots were withdrawn at 24h intervals, diluted to 25 ml with distilled water and read in a spectrophotometer at 223 nm (Linker et al., 2001). Periodate consumption was calculated based on the change of the absorbance at 223 nm. The solution of periodate product (2 ml) was used to assess the amount of formic acid by titration with 0.005 M NaOH.

### *Radical-scavenging activity (RSA) of MHMEPS*

#### *DPPH scavenging activity*

The free radical scavenging activity of MHMEPS was estimated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according Brand-Williams et al., (1995).

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

The ability of MHMEPS to scavenge hydrogen peroxide can be estimated according to the method of Ruch et al., (1989). The percentage of scavenging of hydrogen peroxide was calculated according to Gülcin et al., (2003).

#### *Reducing power method (RP)*

This method was based on the principle of increase

in the absorbance of the reaction mixture. Increased in the absorbance indicates an increase in the antioxidant activity (Jayaprakash et al., 2001). In the method described by Oyaizu (1986) different concentration of MHMEPS (200, 300, 400, 500 µg/ml) were assayed spectrophotometrically at 700 nm against blank sample.

#### Metal chelating activity

The chelation of ferrous ions was estimated using the method of Dinis et al., (1994). 0.1 mL of solution of MHMEPS with different concentrations (200, 300, 400, 500 µg/ml) dissolved in distilled water was added to solution of 0.5 mL ferrous chloride (0.2 mM). The reaction is started by the addition of 0.2 mL of ferrozine (5mM) and incubated at room temperature for 10 min and then the absorbance is measured at 562 nm. EDTA used as a positive control.

#### Cell culture

Breast Aden carcinoma cells (MCF-7) were purchased from ATCC (American Type Culture Collection). The cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) (Lonza, Belgium) at 37°C in humidified air containing 5% CO<sub>2</sub>. All media were supplemented with 10% FBS (Fetal Bovine Serum), 100 u/ml penicillin, 2 mM L-glutamine, 100 u/ml streptomycin sulfate.

#### Cytotoxicity assay

MTT was used as a colorimetric assay to assess cell viability (Mosmann, 1983). It was utilized in examined to evaluate MHMEPS activity on cell proliferation. The cells (5×10<sup>4</sup>) were allowed to attach overnight and were then treated with different concentrations of MHMEPS (5, 10, 20, 40 and 80, µg/ml in a FBS-free medium) for 48 h. MTT was then mixed with MCF-7 cells at 37°C for 2 h in a humidified CO<sub>2</sub> incubator at 5% CO<sub>2</sub>. MTT formazan product was dissolved in dimethyl sulphoxide (DMSO) and absorbance was then measured at 570 nm using ELISA plate reader (Bio Tech Instruments, USA). Cell viability was expressed as a percentage of the control (untreated) culture value. Experiments for each extract were carried out in triplicate. The results were compared with the cytotoxic activity of paclitaxel, a known anticancer drug.

#### Protein expression analysis Bax, Bcl-2, cytochrome C, P53 and caspase-3

Levels of protein expression of Bax Sun

Red, Biotechnology Company, China), caspase 3 (Wkea Med Supplies Corp., China), cytochrome C and P53 (SunLong Biotech Co., LTD, China) were measured by ELISA kits to evaluate the apoptotic pathway in MCF-7 cells exposed to MHMEPS.

#### Caspase-3 activity analysis

The activity of caspase-3 assay was carried out according to Caspase-3 Activity Assay Kit (Beyotime, Haimen, China). The caspase-3 activity assay was based on spectrophotometric detection of the chromophore p-nitronanilide (p-NA), after its cleavage from the labeled substrate, acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA).

#### Statistical analysis

All experiments were performed in triplicates. Data were expressed as means ± SE. Differences between values of EPSs-treated versus untreated cells were compared by the One Way ANOVA and LSD test using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). Values with P < 0.05 were considered significant.

## Results

#### Characterization of the bacterial isolate

Entirely 13 different stable bacterial isolates were isolated from El-Ein El Sokhina, they successfully produced EPSs. Depending on the growth rate and EPSs yield, MHM3 was found to be a highly producer of EPS (5.8 g/L). The promising bacterium was recognized via morphological, physiological, and biochemical characteristics and 16S rRNA according to phylogenetic analysis. The microbial properties of MHM3 bacterium was aerobic, motile, gram-positive bacillus that gave a positive outcome at iodine, citrate, starch nitrate and Voges-Proskauer tests. The partially sequenced 16S rRNA genes exhibited high comparability (99%), with that of *Bacillus* and the closet strain was *Bacillus velezensis*. In this way, it was distinguished as *Bacillus velezensis* MHM3 with accession number MF186594 Figure 1, the phylogenetic analysis result. Subsequently, the chemical composition of the MHMEPS from *Bacillus velezensis* MHM3 was studied especially the sugar composition. Monosaccharide composition analysis showed that the MHMEPS isolated from fermentation *Bacillus velezensis* MHM3 were glucuronic acid, glucose, fructose and rhamnose with molar ratio of

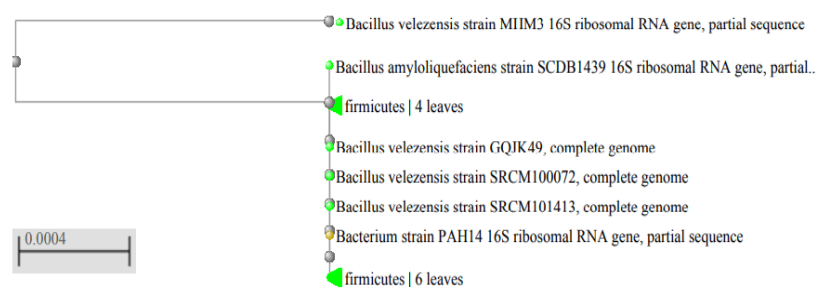


Figure 1. Phylogenetic Tree of the Partial Sequence of 16S rRNA of the Local Isolate *Bacillus velezensis* MHM3 Respects to Closely Related Sequences Available in Gen Bank Databases

Table 1. Metal Chelating Activity and Reducing Power Method Activity of MHMEPS

Concentration $\mu\text{g/ml}$	200	300	400	500
Metal chelating activity (%)	0.00	34.98	58.48	73.71
Reducing power method (abs) absorbance	0.1000	0.1346	0.1366	0.1499

4.00:2.00:1.00:0.13, respectively.

*Periodate oxidation*

For periodate oxidation the purified desulphated MHMEPS was oxidized with  $\text{NaIO}_4$  via usual manner. It consumed 0.6 moles of periodate/1 mole of anhydrosugar and liberated moles of formic acid equivalent for each anhydrosugar unit of MHMEPS after 21 days.  $\text{HCOOH}$  is originating from the reducing as well as non-reducing terminal unit of D-unit. The presence of (1-4)  $\beta$ -type linkage are also confirmed by the OH groups resulting in the consumption of periodate ions in the periodate reaction.

*FT-IR spectra of the MHMEPS*

The infrared spectra of MHMEPS showed a trademark wide exceptional extending peak at around  $2,934.16\text{ cm}^{-1}$  for the C-H stretching band and  $3410.49\text{ cm}^{-1}$  for the OH. The peak towards  $1424.17\text{ cm}^{-1}$  was due to uronic acids resulted from the presence of the  $\text{COO}^-$ . The band in the region pyranose ring of  $1341.25\text{ cm}^{-1}$ . Absorption at  $836.955\text{ cm}^{-1}$  for  $\alpha$  configuration.

*Molecular weight determination*

The weight average molecular weight ( $M_w$ ) was

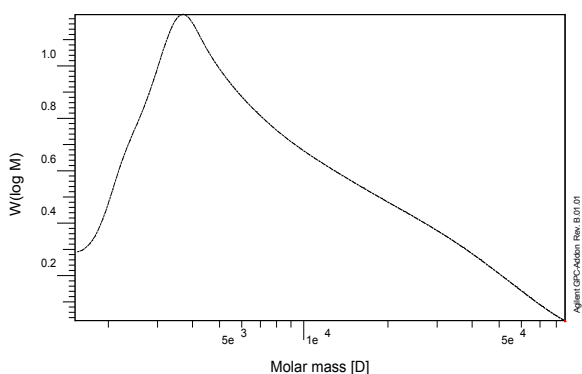


Figure 2. Molecular Weight of MHMEPS

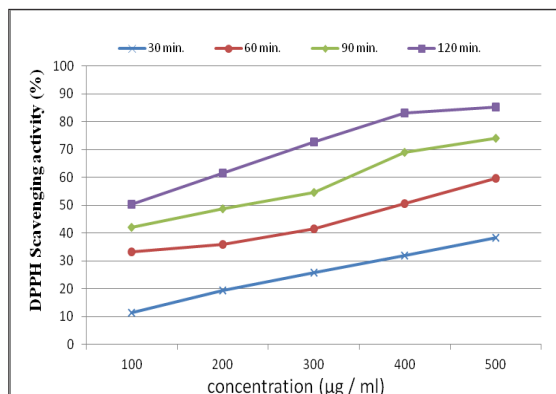


Figure 3. DDPH Radical Scavenging Activity of MHMEPS

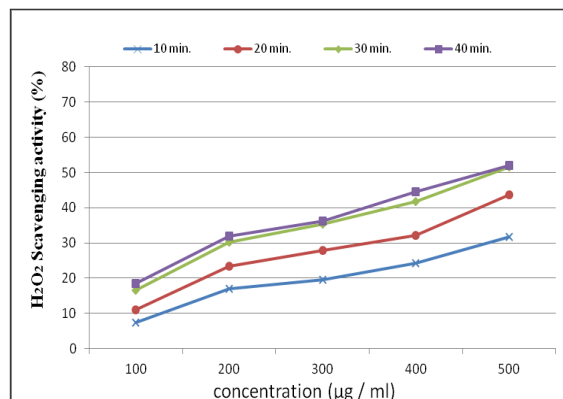


Figure 4.  $\text{H}_2\text{O}_2$  Radical Scavenging Activity of MHMEPS

$1.145 \times 10^4\text{ g/mol}$ , the number average of molecular weights ( $M_n$ ) was  $5.155 \times 10^3\text{ g/mol}$  and polydispersity ( $M_w/M_n$ ) of the MHMEPS ( $\text{PI} = 2.2.$ ) Figure 2.

*Antioxidant activities of MHMEPS*

Inside the DPPH test, the antioxidants were able to reduce the 1,1-diphenyl-2-picrylhydrazyl radical to the yellow shaded diphenyl picrylhydrazine. MHMEPS was significantly scavenged the DPPH and increased with increasing concentration and the time. The maximum antioxidant activity at  $500\text{ }\mu\text{g/ml}$  was 85.4% after 120 min. with  $\text{IC}_{50}$  at about  $400\text{ }\mu\text{g/ml}$  after 60 min. Figure 3. While, the ability of MHMEPS to scavenge  $\text{H}_2\text{O}_2$  was noticed as seen in Figure 4. Whereas the concentration and time increased the  $\text{H}_2\text{O}_2$  scavenging ability increased until  $500\text{ }\mu\text{g/ml}$ , which give 52.1 % after 40 min. and showed that  $\text{IC}_{50}$  at about  $500\text{ }\mu\text{g/ml}$  after 30 min. Metal chelating activity of MHMEPS showed high activity 73.71% with  $500\text{ }\mu\text{g/ml}$ . Consequently, reducing power method was give high absorbance (0.1499) at  $500\text{ }\mu\text{g/ml}$  Table 1.

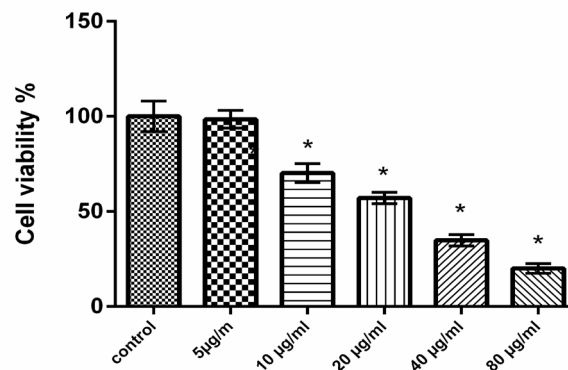


Figure 5. Effect of MHMEPS on Viability of MCF-7 Cells. Cells were exposed to serial dilutions of MHMEPS, for 72h. Cell viability was after 72 h was measured using MTT assay. The data are expressed as percentages of control cells. Each point represents the mean  $\pm$  SD of three independent experiments. \*  $P < 0.05$  vs. control.



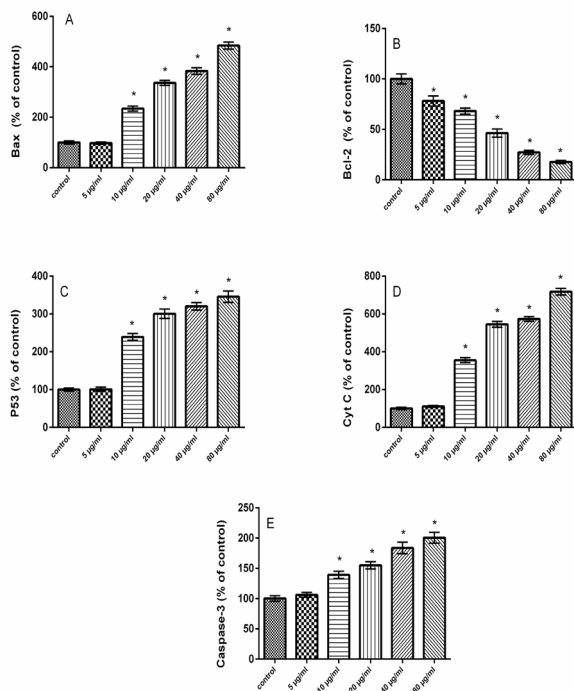


Figure 6. Effects of MHMEPS on Pro-apoptotic Proteins in MCF-7 Cells. The protein expression of (A) Bax, (B) Bcl-2, (C) cytochrome c, (D) p53 and (E) caspase 3 levels were examined by ELISA in supernatant from MCF7 cells treated with serial dilutions of MHMEPS for 48 h. The data are expressed as percentages of control cells. Each point represents the mean  $\pm$  SD of three independent experiments. \*P<0.05 vs. control.

#### Inhibitory effect of MHMEPS on MCF-7 cells

The cytotoxicity of MHMEPS on MCF-7 cells proliferation was estimated by the MTT assay, Paclitaxel, a generally utilized hostile to tumor medication, was used as a source of reference drug Figure 5. MHMEPS fundamentally reduced the proliferation of MCF-7 cells at 5-80  $\mu\text{g/ml}$  comparing with the control group, indicating a dose-dependent effect of MHMEPS on cell viability. The  $\text{IC}_{50}$  value for MHMEPS was 26.316  $\mu\text{g/ml}$ , whereas paclitaxil  $\text{IC}_{50}$  was 1.659  $\mu\text{g/ml}$ .

#### Effect of MHMEPS on Apoptosis Related Proteins

The levels of Bax, Bcl-2, P53, cytochrome c

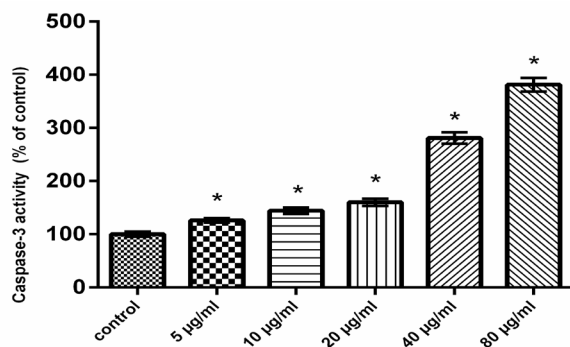


Figure 7. Effects of MHMEPS on Caspase-3 Activity in MCF-7 Cells. The data are expressed as percentages of control cells. Each point represents the mean  $\pm$  SD of three independent experiments. \*P<0.05 vs. control.

and caspase-3 in cells treated with MHMEPS were determined using ELISA kits to explore the possible mechanism of MHMEPS on MCF-7 cells Figure 6. The results demonstrated that P53 and Bax levels were elevated, but Bcl-2 was decreased in a dose-dependent manner compared with control Figure 6, a,b,c,. Previous results mean that due to the decreased the ratio of Bcl-2/Bax, the mitochondrial membrane permeability transition pore was opened in these cells. Therefore, cytochrome c level was increased in cytosol Figure 6, d. Moreover, caspase-3 level was enhanced in MCF-7 treated with mhmEPS compared with control. It was noticed that MHMEPS induced apoptosis was related with activation of caspase-3 and increased cytochrome c levels significantly (P<0.05) in a dose-dependent manner comparing with control Figure 6, e.

#### Effect of MHMEPS on caspase-3 activity

Caspase-3 activity was estimated in MCF-7 cells to investigate the effect of MHMEPS on the proteolytic phase of apoptosis. Caspase-3, a key protease that is activated during the early stages of apoptosis. Treatment of MHMEPS raised caspase-3 activity in MCF-7 cells compared with the control (p<0.05) in a dose-dependent manner Figure 7.

## Discussion

Most microscopic organisms in the marine natural surroundings are incorporated by EPSs, which might help bacterial groups to endure limits. For the reason that of the rheological and chemical properties of the EPSs created by microscopic organisms, the examinations were done to test their approaching biotechnological applications (Guezennec, 2002). Lately marine bacteria have been created the most astounding amount of EPSs; this outcome may be related with the opposition happening through the development stage amongst EPS and polymer biosynthesis. The quantity and quality of bacterial EPS are exceedingly affected by the ecological and nutritional condition (Decho, 1990). Generally EPSs created by marine microorganisms are contain diverse of sugars matched in a range of ~ 10 repeating sugars, with Mw extending from 1 -3 $\times 10^5$  Da (Vanho oren and Vandamme, 1998). More than a few EPSs are neutral molecules; but the greater parts of them are anionic due to the presence  $\text{PO}_4^-$  or  $\text{SO}_3^-$ , pyruvate and  $\text{COO}^-$ . Furthermore, the linkages between units that have been most by and large found are (1-3)- or/and (1- 4)- linkages in the backbone characterized by sturdy rigidity and (1-2)- and/or (1-6)-linkages in the more malleable ones. EPSs physical characters are forcefully influenced with the method for the units, agreed as one and the polymerization of the polymer chains (Cipriani et al., 2008). MHMEPS IR showed peaks at 2934.16  $\text{cm}^{-1}$  for the C-H stretching band and 3410.49  $\text{cm}^{-1}$  for the OH (Santhiya et al., 2002). The peak towards 1,424.17  $\text{cm}^{-1}$  was due to uronic acids resulted from the existence of the  $\text{COO}^-$  (Manrique and Lajolo, 2002). The band in the region pyranose ring of 1,341.25  $\text{cm}^{-1}$ . Absorption at 836.955  $\text{cm}^{-1}$  for  $\alpha$ -configuration.

The antioxidant typeset of study antioxidants agents might be due to the different mechanisms as free radical scavenging, lipid peroxidation, exclusion of continuous H concept, metal ion catalysts, generating enzymes with free radical, decomposing of peroxides, internal antioxidant enzymes activation and reductive power (Lü et al., 2010). ABTS<sup>+</sup> and DPPH<sup>·</sup> radical desire to hold an electron or/ and an H<sup>·</sup> from the anti-oxidant substances.

Anti-oxidant compounds have active OH<sup>·</sup>, akin to tocopherols, polyphenols, ascorbic acid are physically powerful free radical scavengers anti-oxidants. Moreover, H<sub>2</sub>O<sub>2</sub> causing conversion metal ion reliant OH<sup>·</sup> mediated oxidative DNA break. H<sub>2</sub>O<sub>2</sub> holding capacity might be a result of donation of electrons to H<sub>2</sub>O<sub>2</sub>, neutralizes it to H<sub>2</sub>O (Wettasinghe and Shahidi, 2000). SOR help in other ROS arrangement like; H<sub>2</sub>O<sub>2</sub>, OH<sup>·</sup>, and O<sup>·</sup> that stimulate oxidative break type in proteins, DNA and lipids (Liang et al., 2016). Lethal SOR affect during its capacity to capture Fe-sulfur coalition having enzymes. The activity of Fe<sup>2+</sup> chelation might give protection against oxidative damage via removing Fe<sup>2+</sup> that might partake in OH generating Fenton type reactions leads to a decrease on lipid peroxidation and ROS generation (Gülçin et al., 2003). ROS particularly reactive H, besides the initiation of the radical peroxidation chain reactions, assaulted the unsaturated fatty acids that have different double bonds furthermore the CH<sub>2</sub>-groups. At the same time, antioxidants scavenging peroxide and OH<sup>·</sup> hindrance the arrangement of hydroperoxides in linoleic acid (Lü et al., 2010). In this investigation, MHMEPS indicated powerful free radical scavenging activity, inhibit H<sub>2</sub>O<sub>2</sub> radical and Fe<sup>2+</sup>, decline Fe<sup>3+</sup> and capture SOR generation. A few logical studies represented that EPSs have numerous activities like antioxidant activity according to their molecular weight, compositions, repeating units, and configuration structure (Liang et al., 2016).

Because of genuine side effects of chemotherapy in cancer patients, the look for new natural items to restrain malignant development is one the objectives of cancer chemoprevention. Polysaccharides got from microbial and plant sources have a great effect as an antitumour agent (Ahmed and Elmenoufy, 2016). Short fermentation process besides easily formed and stable emulsions are the major biotechnological focal points of microbial polysaccharides (Yu et al., 2001). MCF-7 cells were chosen as a cancer cell model because of the wide spread of breast cancer comparing with other types of cancer.

Apoptosis is a prospective target when developing novel anticancer medications as it spoke to the real pathway of tumor cells death. Anticancer medications induce apoptosis through down controlling of anti-apoptotic proteins (like Bcl-2), besides the up control of pro-apoptotic proteins (like Bax), also activation of caspases (Lee et al., 2011). The present results demonstrate the MHMEPS dose dependent inhibitory effect on MCF-7 cells, indicating that MHMEPS possess antitumor activity.

To know the mechanism of MHMEPS that initiate apoptosis of MCF-7 cells, various proteins associated with apoptosis were determined. Bax (a pro-apoptotic factor) moves from the cytosol to mitochondrial outer membrane in order to form heterodimers with Bcl-2

protein and the complex makes pores and mediates cytochrome c release. It was revealed that high Bax/Bcl-2 ratio was provoking of apoptotic activity (Lee et al., 2011). The principal mediators of the characteristic pathway of apoptosis are pro- and anti-apoptotic proteins (Williams and Smith, 1993). During the process of apoptosis, mitochondrial dysfunction is an early occasion happening. The integrity of the mitochondrial outer membrane was disrupted because of any imbalance of the expression levels of anti- and pro-apoptotic Bcl-2 family proteins (Breckenridge and Xue, 2004). Current results showed that, MHMEPS decreased Bcl-2 level whereas Bax level was increased in MCF-7 cells. It indicated that MHMEPS might activate mitochondria-mediated apoptosis by increasing the permeability of the mitochondrial membrane. Moreover, MHMEPS incites the release of cytochrome c from the mitochondria to the cytosol. Therefore, this demonstrate that MHMEPS participating in inducing the damage of mitochondria and controlling apoptotic proteins in MCF-7 cells. Apoptosis is a type of physiological cell demise portrayed at the biochemical level by the initiation of cysteine proteases family known as caspases that results in DNA fragmentation (Hengartner, 2000). Caspase-3, the main executioner of apoptosis, is one of the deeply studied proteases. caspase-3 activation incites cells into an irreversible apoptosis pathway (Jiang et al., 2012). In the current results, MHMEPS increased the caspase-3 activity in MCF-7 cells.

#### Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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