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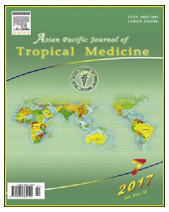

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Production, characterization and biological activities of acidic exopolysaccharide from marine *Bacillus amyloliquefaciens* 3MS 2017

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ABSTRACT

Objective: To evaluate *in-vitro* antioxidant, anti-inflammatory and antitumor abilities against human breast adenocarcinoma (MCF7) and human prostate cancer (PC3) as well as the suppressor effect of bacterial exopolysaccharide (BAEPS) on Ehrlich ascites carcinoma (EAC).

Methods: *In-vitro* antioxidants characters of BAEPS were determined using various methods, while anti-inflammatory activity was estimated against cyclooxygenase (COX-1 and COX-2). *In-vitro* study, anticancer against MCF7 and PC3 were assessed by the mitochondrial dependent reduction of yellow MTT. In *in-vivo* study against EAC progression, mice were inoculated with EAC cells and then were orally administered BAEPS at 200 mg/kg after 24 h (equals to 0.10 of determined LD_{50})/10 d.

Results: BAEPS was acidic exopolysaccharide contained uronic acid (12.3%) and sulfate (22.8%) with constitution of glucose, galactose and glucuronic acid in a molar ratio 1.6:1.0:0.9, respectively, with a molecular mass of 3.76×10^4 g/mol. BAEPS appeared potent antioxidant characters as free radical scavenging, oxygen reactive species scavenging and metal chelation, while its reducing power was low. BAEPS showed selective anti-inflammatory activity against COX-2 than COX-1, COX-2 selective. BAEPS exhibited potent and selective effect to breast cell cancer MCF7, the death percentage was 65.20% with IC_{50} = 70 μg/mL and IC_{90} = 127.40 μg/mL. BAEPS decreased counted viable EAC cells and induced non-viable cells. BAEPS improved all assessed hematological parameters. These improvements were reflected in the increasing median survival time and significant increment ($P < 0.05$) in life span.

Conclusions: BAEPS has anti-tumor activity with a good margin of safety. The anti-tumor activity of BAEPS may be due to its content from sulfated groups and uronic acids and they have antioxidant and anti-inflammatory properties.

1. Introduction

Reactive oxygen species (ROS) or free radicals are a hazard agent lead to many diseases, including chronic inflammation. Inflammation and oxidative stress together are responsible for several chronic diseases like cancer, and aging. Inflammatory cells release reactive species in the inflammation place leading to extreme oxidative stress. Contrary, some of reactive oxygen/nitrogen species able to start intracellular was signaling cascade that induces pro-inflammatory gene expression. Thus, inflammation and oxidative stress are closely related pathophysiological events that are tightly linked with one another [1].

Either endogenous antioxidant or exogenous antioxidant can be protecting the cells and body organ from ROS. The function of these antioxidants is neutralized of these danger radicals. These antioxidants divided into nutrient deprived, enzymes and metal binding proteins. Ascorbic acid, tocopherols, carotenoids, glutathione and lipoic acid are nutrient-derived antioxidants. Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase are antioxidant enzymes which catalyze free radical. Ferritin, lactoferrin, albumin, and ceruloplasmin are metal binding proteins antioxidants, which isolate free iron and copper ions as well as other phytonutrients antioxidant found in vegetarian foods like phenols [2].

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Cancer is the main common cause of death all over the world. It affects millions annually. Carcinogenesis is an out of control growth and multiplicity of abnormal cells which invade either surround or distant tissues. It includes main biological process concerning disordered cell replication death, disorganization of organ structure and the ability to invade various tissues to form malignant tumors [3]. Epidemiology investigations demonstrated that the older the age, the more the increase in the incidence of most cancers exponentially. Cancer injury is elevated yearly; the increment was determined as fourteen million cases a year in 2012. Simultaneously, the number of dead cases of cancer was increased to reach thirteen million a year [4]. There are many factors considered as cancer risk factors like obesity, hormones, diet style, especially high fat diet [4]. Many chemical materials are used in cancer management by minimizing the effect of risk factors to decrease mortality. One of these cancer-treating chemicals is a non-steroidal anti-inflammatory drug, which affect cyclooxygenases. Pro-inflammatory molecules, like prostaglandin and interleukins are biosynthesized through the consumption of arachidonic acid by COX. This conversion stimulates tumor growth and enhances carcino-materials to affect genetic substances that enhance tumor growth. In addition, genetic substances oppositely affect the immune system as well as activation of carcinogens to do damage in a genetic matter [5]. Application of synthetic materials in chemotherapy caused a multifarious malfunction in many organs. Hence, searching for natural therapeutic sources became a target for pharmaceutical researchers so that they could avoid these malfunctions specially, those used in traditional medicine with acceptable effects [6]. On the other hand, many studies set up to find new anti-cancer agents from algae and plants.

More attention targeted natural substances in the scientific research for bioactive compounds in developing new drugs and supplementary foods. Natural exo-polysaccharides (EPSs) play important roles in many biological activities, and they can work as growth determinant in pathogens. Microbial EPSs attract more attention in medicine [7]. Several of these EPSs have been established to play as a scavenger of different types of free radicals in the laboratory (*in-vitro*) and as antioxidants to prevent oxidative stress damage in living organism's cells [8]. Marine bacteria often produce EPS with new structures and various activities according to their specific marine environment [9]. In addition, EPSs are considered as important bioactive natural materials used in medical applications as they have antioxidant properties, immune stimulating effects and antitumor effects. EPS isolated from different sources, like plants and algae, were documented in their suppressive effect on proliferation of varied tumor types in an *in-vivo* and *in-vitro* models [10]. Current research carried out to clarify the chemical characterization of bacterial exopolysaccharide (BAEPS) produced from marine *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) 3MS 2017 and evaluate its biological activities as antioxidant, anti-inflammatory and anticancer.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), peroxidase, H_2O_2, ABTS (2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt, sodium nitroprusside (SNP), sulfanilamide, ortho-H_3PO_4, naphthylethylene diamine dihydrochloride, nicotinamide adenine dinucleotide (NADH), butylated hydroxytoluene (BHT), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), ferrous chloride, trichloroacetic acid (TCA), potassium ferricyanide, polyoxyethylenesorbitan monolaurate (Tween-20), ascorbic acid (vitamin C), BHT, and, Leuco-2,7-dichlorofluorescien diacetate, hematin, arachidonic acid and cyclooxygenases enzymes (COX-1 from sheep, EC. 1.14.99.1 or COX-2) were purchased from Sigma-Aldrich, USA. Ammonium thiocyanate was bought from E. Merck. All chemicals and solvents are analytical grade.

2.2. Production of exopolysaccharide

2.2.1. Sampling

Two samples were collected for study from marine sediment at different locations for isolation of bacteria, Marsa-Alam area (Rhizosphere around the mangrove tree) and El-Ain El-Sokhna beach. Sediments collected in sterile tubes by divers and were reserved in a refrigerator until processed in laboratory.

2.2.2. Isolation of bacteria

The samples were positioned in a new 100 mL of sterile seawater and shaking at 150 rpm for 15 min and then a serial dilution was performed [11]. The isolated bacteria showing mucous growth on the same medium therefore they were further screened for EPS production by inoculation into shake flasks.

2.2.3. Screening for production of EPS

Isolates were screened for creation of EPS using media composed of the following ingredients (g/L), glucose 20, $CaCO_3$ 0.1, NH_4NO_3 0.8, K_2HPO_4 0.6, KH_2PO_4 0.05, $MnSO_4 \cdot 4H_2O$ 0.1 and yeast extract 1.0 [12].

2.2.4. Production and isolation of EPS from liquid medium

The isolate, producing EPSs, was grown in a liquid medium ingredient (g/L). This medium consisted of glucose 20, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.5, $MnSO_4.4H_2O$ 0.05, $FeSO_4 \cdot 7H_2O$ 0.01, and $CaCl_2 \cdot 2H_2O$ 0.01. The mentioned ingredients mentioned was dissolved in 75% seawater [13]. Culture was centrifuged at 5 000 rpm for 40 min at 4 °C to remove bacterial cells; the supernatant was subjected to deproteinization by TCA (5%). The pH of the supernatant was adjusted to 7.0 and dialyzed 3 times against flowing distilled water using dialysis tube (MWCO 2000). The supernatant was completed to three volumes with absolute ethanol and left at 4 °C to 24 h. The precipitated EPSs were separated by centrifugation at 5 000 rpm, for 25 min, re-dissolved in distilled water, dialyzed with distilled water and fractionation by precipitation using 1, 2, 3 and 4 volumes of chilled absolute ethanol collected, washed by acetone and dried at 50 °C, the main fraction was named as BAEPS.

2.2.5. Identification of promising isolate

The promising isolate which produced high amounts of EPSs and antioxidant by DPPH was identified based on morphological, biochemical and physiological characteristics of the possible producer determined by adopting standard methods [14].

The strain was confirmed with 16S rRNA sequence and compared with other bacterial sequences by using NCBI BLAST. Taxonomic association of the sequences was retrieved from classifier plan of ribosomal database scheme (RDP-II) [15]. RDP-II hierarchy was based on the novel phylogenetically reliable senior order bacterial taxonomy.

2.2.6. Fourier-transform infrared spectroscopy (FTIR)

The BAEPS was mixed with KBr powder, was ground and was pressed into a 1 mm pellets for FTIR measurements in the range of 400–4000 cm^{-1} on a Bucker scientific 500-IR Spectrophotometer [16].

2.2.7. Chemical analysis

Uronic acids were determined at 525 nm by the *m*-hydroxybiphenyl colorimetric method [17]. Sulfate was determined using the turbidly method [18]. 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 [17].

2.2.8. Molecular weight determination

The molecular weight of BAEPS was determined to an Agilent 1100 HPLC with a refractive index detector (RID), Water Company Ireland according to Jun *et al.* [19].

2.3. Antioxidant characters determination

2.3.1. DPPH radical scavenging activity

The free radical scavenging activity of BAEPS was estimated by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) [20].

2.3.2. ABTS radical cation scavenging activity

Free radical activity against ABTS cation was measured using the method of Miller and Rice-Evans [21] and Arnao *et al.* [22].

2.3.3. Scavenging of hydrogen peroxide

Using the method of Ruch *et al.* [23], H_2O_2 scavenging ability of BAEPS was determined and was compared to reference materials; vitamin C and BHT and the percentage of scavenging of hydrogen peroxide was calculated [24].

2.3.4. Superoxide anion scavenging activity

Superoxide anion scavenging activity of BAEPS was determined and was predestined by comparison with these of vitamin C and BHT [24,25].

2.3.5. Nitric oxide radical scavenging activity

NO radical scavenging activity of BAEPS was assayed by using a SNP generating NO system. In aqueous physiological pH solution NO generates from SNP and reacts with oxygen to produce nitrite ions. Nitrite ions were measured by the Greiss reagent [26].

2.3.6. Lipid peroxidation-ammonium thiocyanate assay

The lipid peroxidation inhibition property of BAEPS and standards was determined according to the method of Gülçin *et al.* [27].

2.3.7. Ferrous ions chelating capacity

The ferrous ions chelating capacity of BAEPS was estimated by the method of Dinis *et al.* [28] and was compared with reference compounds and the percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula of Gülçin *et al.*, 2003 [24].

2.3.8. Reduction of ferric ions (Fe^{3+}) power

The ferric ions (Fe^{3+}) reducing power of BAEPS and reference materials were assayed spectrophotometrically [29].

2.4. Anti-inflammatory activity

According to the method of Larsen *et al.* [30] anti-inflammatory activity of BAEPS as cyclooxygenase inhibition assay were performed and estimated in comparison with celecoxib as standard compound.

2.5. Cytotoxic effect on breast cancer MCF7 and prostate cancer PC3 cell lines

Cell viability of human Caucasian breast adenocarcinoma (MCF7) and human prostate cancer (PC3) were assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [31]. MCF7 and PC3 cells were generously provided by Dr. Stig Linder, Professor in Oncology and Pathology department in Karlinska Institute, Sweden. Cells were suspended in RPMI 1640 medium (MCF7 and PC3) [32,33]. The absorbance was measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. DMSO is the vehicle used for dissolution of BAEPS and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

$$(\text{Reading of extract}/\text{Reading of negative control}) - 1) \times 100$$

A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

2.6. Anti-tumor properties of BAEPS in Ehrlich ascites carcinoma (EAC) model in mice

2.6.1. Ethical issues

This research protocol was approved by the Medical Research Ethics Committee, National Research Centre, Egypt as a part of the project protocol under registration No 6/014 entitled 'New drug discovery for breast and prostate cancers from Egyptian medicinal plants and polysaccharides derived from natural sources'.

2.6.2. Toxicity study

BAEPS was tested with gradient doses to determine its toxicity in female albino mice ($n = 8$) in an oral administration route [34] started with 200 mg/kg up to 3000 mg/kg with increasing the dose by 200 mg/kg body weight. Animals (80 mice) were observed for any changes and mortality through forty-eight hours.

2.6.3. Tumor cell

EAC cells were obtained from the National Cancer Institute, Cairo, Egypt. Animals were injected with 2×10^6 cell/mouse.

2.6.4. Experimental scheme

Seventy albino female mice (20–25 g) from National Research Centre animal house were fed on standard diet and water *ad libitum* and were acclimatized to normal laboratory conditions, 25–27 °C, 60%–65% humidity, in a polypropylene cage for one week before starting experiment. After adaptation for one week, Mice were classified to five groups with fourteen female albino mice each. Group I: mice were administrated normal saline in an oral administration, served as negative control group for ten sequential days. Group II: mice were injected with EAC (2×10^6 cell/mouse), served as positive control group (tumor bearing mice). Group III: mice were injected with EAC with incubation for 24 h, they were administered reference drug; 5-fluorouracil (FU) as 20 mg/kg body weight (B.W.)/d for 10 d [35]. Group IV: mice were administrated with 0.10 of BAEPS LD_{50} orally at 200 mg/kg B.W./d for ten sequential days and were served as BAEPS-control group. Group V: mice were injected with EAC with 24 h incubation time, they were administered 0.10 of BAEPS LD_{50} orally at 200 mg/kg B.W./d for ten sequential days and were served as treated group.

After 10 d, 6 mice/group for all groups were fasted, overnight and then blood samples were collected from cardiac puncture for hematological analysis. Whereas, eight mice were kept alive to evaluate changes in animal's life span [36]. Activity of BAEPS against tumor development and the survive of animals was evaluated by assessment of tumor volume, packed cell volume, viable and non-viable cell count, median survival time (MST) and raise in life span percentage besides tumor volume and weight. Mice were anesthetized and dissected then the ascitic fluid was collected from peritoneal cavity. Tumor volume was determined by a graduated centrifuge tube and was weighed [37]. Evaluation of viable and non-viable tumor cell count was carried out using Trypan blue (0.4% in normal saline) dye technique [37]. Cell count was estimated as follows:

$$\text{Cell count} = (\text{No. of cell} \times \text{dilution})/\text{area}) \times \text{thickness film}$$

Average of surviving time and increment in life span were monitored and were measured according to the following formula:

$$\text{Increases of life span}(\%) = (T - C/C) \times 100$$

where, T was number of days in which animals still alive while C was number of days for bearing mice [37].

Effect of BAEPS on tumor volume and weight was performed by collection of ascitic fluid from peritoneal cavity and then was measured by graduated centrifuge tube and weighed immediately [38]. Contrary, animal blood samples were introduced for determination of hematological parameters according to Dacie and Lewis [39]. Liver function enzymes were spectrophotometrically assessed according to Reitman and Frankel [40].

2.7. Statistical analysis

All data were mentioned as mean ± SE. Data were analyzed by ANOVA one way ($n = 6$). $P < 0.05$ was considered as significant difference.

3. Results

3.1. Identification of *B. amyloliquefaciens* 3MS 2017

The most bacterial isolate producing the highest amount of EPS was identified according to a large diversity of morphological, physiological and biochemical features. This isolate was found to be gram-positive cells, short rod shaped, positive of citrate and catalase tests and negative in both of indole and Voges–Proskauer tests. 16S rRNA sequencing was carried out for selected *B. amyloliquefaciens* 3MS 2017 whose identity was determined by performing a sequence similarity search using NCBI (Figure 1). The sequences were submitted to GenBank with the accession number MF321890.

3.2. Composition and characterization of BAEPS

The purified BAEPS from *B. amyloliquefaciens* 3MS 2017, a creamy powder, was used for following analysis. It had a positive reaction to absorption at 280 nm in the UV spectrum and the Bradford test, referring to no protein and/or nucleic acid founded. The BAEPS contain uronic acid (12.3%) and sulfate (22.8%). These results indicated that the BAEPS was acidic EPSs. The monosaccharide composition of BAEPS is composed of glucose, galactose and glucouronic acid with molar ratio 1.6: 1.0: 0.9, respectively. The weight average molecular weight (*Mw*), number average of molecular weights (*Mn*) and polydispersity (*Mw*/*Mn*) of the BAEPS were determined by GPC as shown in Figure 2A. The EPSs in the GPC chromatogram were widely dispersed molecules polydispersity index (*PI* = 1.1) and had an overall *Mw* of 3.76×10^4 g/mol and *Mn* of 3.38×10^4 g/mol (Figure 2A). In Figure 2B, the 3933.11 and 343.03 cm^{-1} peaks showed deformation of O—H, related to the intermolecular and intramolecular hydrogen bond. The peaks at 3077.51 and 2881.16 cm^{-1} were attributed to deformations of the C—H for the secondary ($-CH_2-$) and primary ($-CH_3$) bonds, respectively. The major absorption observed at 1646.91 and 1651.73 cm^{-1} was referred to the stretching vibration of COO^-. BAEPS showed an intense absorption peak at 1120.44 cm^{-1}

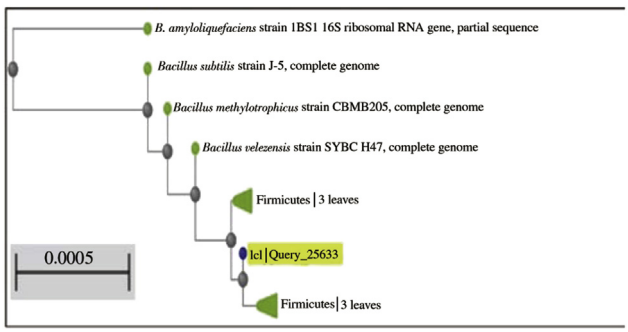

Figure 1. Phylogenetic tree of the partial sequence of 16S rRNA of the local isolate *B. amyloliquefaciens* 3MS 2017 with respect to closely related sequences available in GenBank databases.

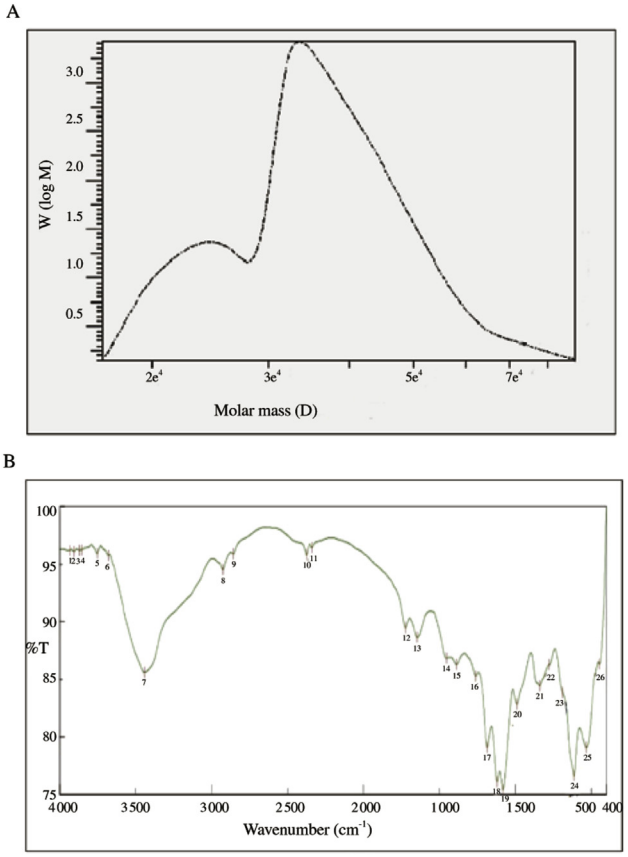

Figure 2. Molecular weight (part A) and Fourier-transform infrared spectrum (part B) of BAEPS.

common to the sulfate groups. Moreover, the band at 922.77 and 838.88 cm^{-1} indicated the forms of the glucosyl residue.

3.3. Evaluation of BAEPS antioxidants characters

3.3.1. DPPH free radical scavenging activity

The free radical scavenging ability of BAEPS was estimated using DPPH methods and evaluated by comparison with two standard components, vitamin C and BHT as represented in Table 1. BAEPS appeared high DPPH radical scavenging activity and significantly magnified when BAEPS concentration was elevated from 100 to 1000 μg/mL [(85.55 ± 2.83)% and (99.39 ± 0.63)% respectively], comparing to vitamin C [(77.17 ± 3.00)% and (100.00 ± 0.04)% respectively] and BHT [(77.46 ± 2.46)% and (98.32 ± 1.68)% respectively] ($P < 0.05$). Depending on IC_{50} values, the scavenging activity of BAEPS and standard materials on the reduction against DPPH radical in order of vitamin C [(2.87 ± 0.16) μg/mL] > BHT [(2.33 ± 0.32) μg/mL] > BAEPS [(0.21 ± 0.01) μg/mL] with significant differences ($P < 0.05$).

3.3.2. ABTS radical cation scavenging activity

The ABTS/H_2O_2 discoloration method is performed to evaluate the ABTS radical cation scavenging activity of BAEPS and reference materials at sequenced concentrations (Table 1). Compared to two reference materials, BAEPS showed moderate scavenging $ABTS^+$ cation radical evident by discolored a bluish green complex of ABTS/H_2O_2 and was increased concentration dependently. At the lowest concentration, 100 μg/mL BAEPS appeared moderate activity, (37.75 ± 2.20)% increased gradually to (67.44 ± 1.56)% by increasing concentration to 1000 μg/mL;

Table 1
Free radicals scavenging, ROS scavenging, NO radical scavenging, lipid peroxidation inhibition, and Fe^{2+} chelation activities (inhibition %) and total ferric reducing power (absorbance) of different concentrations (100–1000 μg/mL) of BAEPS and reference materials, vitamin C and BHT.

Material	Concentration	DPPH radical scavenging	ATBS radical scavenging	H_2O_2 scavenging	O^{-2} scavenging	NO radical scavenging	Lipid peroxidation inhibition	Metal chelation	Reducing power
BAEPS	100	85.55 ± 2.83^{ab}	37.75 ± 2.20^{ab}	61.72 ± 3.20^{ab}	60.55 ± 1.62^{ab}	63.54 ± 2.60^{ab}	59.73 ± 1.77	63.77 ± 3.68^{ab}	0.003 ± 0.001^{ab}
	250	98.40 ± 1.69^{ab}	43.11 ± 2.89^{ab}	73.92 ± 2.10^{ab}	72.06 ± 1.00^{ab}	76.42 ± 2.58^{a}	68.94 ± 1.06^{ab}	69.77 ± 1.84^{ab}	0.005 ± 0.001^{ab}
	500	98.75 ± 1.71	47.22 ± 1.78^{ab}	85.45 ± 0.55^{a}	83.42 ± 2.68^{ab}	85.09 ± 2.60	74.76 ± 4.22^{ab}	70.25 ± 1.75^{ab}	0.005 ± 0.001^{ab}
	1000	99.39 ± 0.63	67.44 ± 1.56^{ab}	92.17 ± 2.83^{a}	91.44 ± 2.51^{ab}	90.18 ± 1.82^{b}	80.67 ± 3.30^{ab}	71.14 ± 2.12^{ab}	0.005 ± 0.001^{ab}
Vitamin C	100	77.17 ± 3.00	78.32 ± 1.68	73.39 ± 2.30	66.21 ± 2.52^{b}	66.32 ± 1.68^{b}	77.55 ± 2.37^{b}	75.55 ± 2.45	0.190 ± 0.020
	250	90.31 ± 1.60	80.94 ± 1.06^{b}	82.77 ± 2.25	80.37 ± 2.63	73.68 ± 3.29^{b}	90.11 ± 3.00	82.33 ± 1.67^{b}	0.580 ± 0.030^{b}
	500	98.28 ± 2.88	99.89 ± 0.10	90.41 ± 2.40^{b}	95.34 ± 2.40	86.15 ± 2.09	98.01 ± 3.00^{b}	91.52 ± 0.45^{b}	0.640 ± 0.030^{b}
	1000	100.00 ± 0.04	100.00 ± 0.0	95.81 ± 1.60	97.75 ± 2.20	93.27 ± 3.20^{b}	100.00 ± 0.00	98.19 ± 1.86	0.780 ± 0.060^{b}
BHT	100	77.46 ± 2.46	77.85 ± 2.57	72.21 ± 3.20	73.22 ± 2.78^{a}	69.83 ± 2.52^{a}	74.63 ± 2.89^{a}	77.41 ± 1.59	0.190 ± 0.010
	250	91.10 ± 1.90	84.88 ± 0.05^{a}	80.00 ± 3.00	83.61 ± 2.00^{a}	77.98 ± 2.39^{a}	88.58 ± 1.79	87.93 ± 1.07^{a}	0.490 ± 0.020^{a}
	500	97.88 ± 2.13	99.91 ± 0.10	87.61 ± 2.70^{a}	97.21 ± 2.20^{a}	88.72 ± 3.50	93.74 ± 2.00^{a}	94.16 ± 3.76^{a}	0.540 ± 0.040^{a}
	1000	98.32 ± 1.68	100.00 ± 0.00	93.53 ± 2.45	98.11 ± 1.89^{a}	96.44 ± 3.56^{a}	100.00 ± 0.00	99.09 ± 0.77	0.610 ± 0.060^{a}

BHT; butylated hydroxytoluene; DPPH: 1,1diphenyl-2-picryl-hydrazyl free radical; $ABTS^+$: 2,20-azinobis (3-ethylbenzthiazoline-6-sulfonic acid). Data presented as mean ± SE. ANOVA one-way was used for data analysis (n = 3, $P < 0.05$). Data are followed with small letter; a means significant difference with vitamin C, b means significant difference with BHT.

with respect to vitamin C, (78.32 ± 1.68)% to (100.00 ± 0.01)% respectively and BHT, (77.85 ± 2.57)% to (100.00 ± 0.0)% respectively ($P < 0.05$). To scavenge 50% of $ABTS^+$ cation radical BAEPS [(408.64 ± 20.00) μg/mL] was significantly higher than vitamin C [(7.59 ± 0.30) μg/mL] and BHT [(4.22 ± 0.30) μg/mL] ($P < 0.05$).

3.3.3. Hydrogen peroxide scavenging ability

The ability of BAEPS to scavenge H_2O_2 was assayed as represented in Table 1 and compared with that of vitamin C and BHT as reference materials. H_2O_2 scavenging ability of BAEPS was found to be (61.72 ± 3.20)% at the lowest concentration 100 μg/mL and was elevated progressively to (92.17 ± 2.83)% when concentration reached 1 000 μg/mL. On the other hand, the same concentration exhibited H_2O_2 scavenging activity started from (73.39 ± 2.30)% to (95.81 ± 1.60)% with vitamin C and (72.21 ± 3.20)% to (93.53 ± 2.45)% with BHT ($P < 0.05$). BAEPS had a low IC_{50} by about [(30.04 ± 2.50) μg/mL] but it remained significantly greater than these of vitamin C [(4.38 ± 0.12) μg/mL] and BHT [(4.17 ± 0.20) μg/mL] ($P < 0.05$).

3.3.4. Superoxide anion radical scavenging activity

Superoxide anion (SOR) produced in the PMS–NADH–NBT system from dissolved oxygen by PMS–NADH coupling reaction reduces NBT (yellow dye NBT^{2+}). Data figure out in Table 1 that the inhibition percentage of generated SOR by BAEPS at gradual concentrations compared with reference materials at the same concentrations. BAEPS exhibited SOR scavenging percentage ranged from (60.55 ± 1.62)% with the lowest concentration (100 μg/mL) to (91.44 ± 2.51)% at the highest one (1 000 μg/mL), with respect to that of vitamin C [(66.21 ± 2.52)% and (97.75 ± 2.20)% respectively] and BHT [(73.22 ± 2.78)% and (98.11 ± 1.89)% respectively] ($P < 0.05$). BAEPS exhibited low IC_{50} value [(35.28 ± 3.20) μg/mL] while vitamin C exhibited [(20.09 ± 1.91) μg/mL] and BHT exhibited [(8.03 ± 0.50) μg/mL] with significant differences among them ($P < 0.05$).

3.3.5. Nitric oxide scavenging activity

NO radical scavenging ability of BAEPS was estimated by a SNP generating NO system. NO released from SNP in aqueous solution at physiological pH reacts with oxygen to generate nitrite ions which were measured. As data pointed out in Table 1, the BAEPS significantly decreased nitrite which was liberated in SNP assay medium represented as strong $NO^{\cdot}$ scavenging effect, compared to references materials. The $NO^{\cdot}$ scavenging capacity was concentration dependent. Therefore, the NO scavenging activity of BAEPS significantly rose from (63.54 ± 2.60)% at the lowest concentration (100 μg/mL) to (90.18 ± 1.82)% at the highest concentration (1 000 μg/mL). BAEPS showed NO scavenging percentage close to these of vitamin C [(66.32 ± 1.68)% and (93.27 ± 3.20)% respectively] and BHT [(69.83 ± 2.52)% and (96.44 ± 3.56)% respectively] at each concentration ($P < 0.05$). The amount of BAEPS to capture 50% of generated NO was [(18.78 ± 1.02) μg/mL], which was nearly equal with that of BHT [(19.84 ± 1.22) μg/mL] and significantly lower than that of vitamin C [(10.31 ± 1.70) μg/mL] ($P < 0.05$).

3.3.6. Inhibition of lipid peroxidation

Lipid peroxidation inhibition activity of BAEPS was determined by the thiocyanate method. In thiocyanate system, linoleate radicals oxidized ferrous ion to hydroperoxides to form ferric ion and then ferric ion is monitored spectrophotometrically as a thiocyanate complex at 500 nm. BAEPS showed linoleic acid preventive effect against peroxidation in the emulsion (Table 1). Linoleic acid peroxidation stopped by BAEPS with concentration dependently manner, therefore, the lowest linoleic peroxidation inhibition activity [(59.73 ± 1.77)%] recorded with the lowest concentration (100 μg/mL). Meanwhile the highest percentage [(80.67 ± 3.30)%] recorded with the highest concentration (1 000 μg/mL) in comparison to the percentage recorded by the same concentration of vitamin C [(77.55 ± 2.37)% and (100.00 ± 0.01)% respectively] and BHT [(74.63 ± 2.89)% and (100.00 ± 0.00)% respectively]. BAEPS [(26.69 ± 2.30) μg/mL] was required to prevent 50% of linoleic acid oxidation into peroxide and significantly higher than vitamin C [(4.28 ± 0.20) μg/mL] and BHT [(2.68 ± 0.30) μg/mL] ($P < 0.05$), which were respectively required to get the same effect.

3.3.7. Ferrous ions chelating capacity

Ferrous ion (Fe^+) chelating ability of BAEPS and standard materials; vitamin C and BHT, are represented in Table 1. The chelating effect of Fe^{2+} of BAEPS and standard materials was estimated among the transition metals by forming complexes between ferrozine and Fe^{2+}. BAEPS appeared moderate ferrous ion chelating ability compared with vitamin C and BHT. BAEPS inhibited the formation of the Fe^{2+}-ferrozine complex that meant they are able to capture ferrous ion before ferrozine complex formed. BAEPS recorded ferrous ion chelating percentage (63.77 ± 3.68)% with the lowest concentration and elevated with low rate to (71.14 ± 2.12)% by the highest one, with respect to vitamin C [(75.55 ± 2.45)% and (98.19 ± 1.86)%, respectively] and BHT [(77.41 ± 1.59)% and (99.09 ± 0.77)%, respectively] at the same concentrations, respectively ($P < 0.05$). Although, IC_{50} of BAEPS was nearly to that of vitamin C and BHT was [(1.10 ± 0.06) μg/mL, (1.62 ± 0.15) μg/mL and (1.79 ± 0.20) μg/mL, respectively].

3.3.8. Fe^{3+} reducing antioxidant power

The Fe^{3+} reductive capability of BAEPS was measured by the Fe^{3+}–Fe^{2+} transformation and compared to references materials; vitamin C or BHT. In the total reductive capability using the potassium ferricyanide reduction method Fe^{3+} are reduced to the ferrous form or when more electrons are donated by antioxidant components. Table 1 demonstrated that, BAEPS promotes weak effect as reducing power agent using the potassium ferricyanide reduction method, with insignificant changes as concentration was changed.

3.4. Anti-inflammatory activity of BAEPS

Celecoxib showed COX-1 inhibition percentage from (52.50 ± 2.50)% at 100 μg/mL to (67.82 ± 2.20)% with 1 000 μg/mL. Celecoxib was effective on COX-2 and recorded inhibition percentage from (41.23 ± 1.77)% at 100 μg/mL to (72.49 ± 2.20)% at 1 000 μg/mL. Data in Figure 3A figured out BAEPS selective inhibitory effect against COX-2. BAEPS inhibited COX-2 by about (14.40 ± 0.60)% at 100 μg/mL with dependent increment with increasing concentration to reach 100% at 500 and 1 000 μg/mL. COX-2 50% inhibition required [(340.75 ± 7.70) μg/mL] of BAEPS which was more than celecoxib [(312.48 ± 9.69) μg/mL], data in Figure 3C. BAEPS

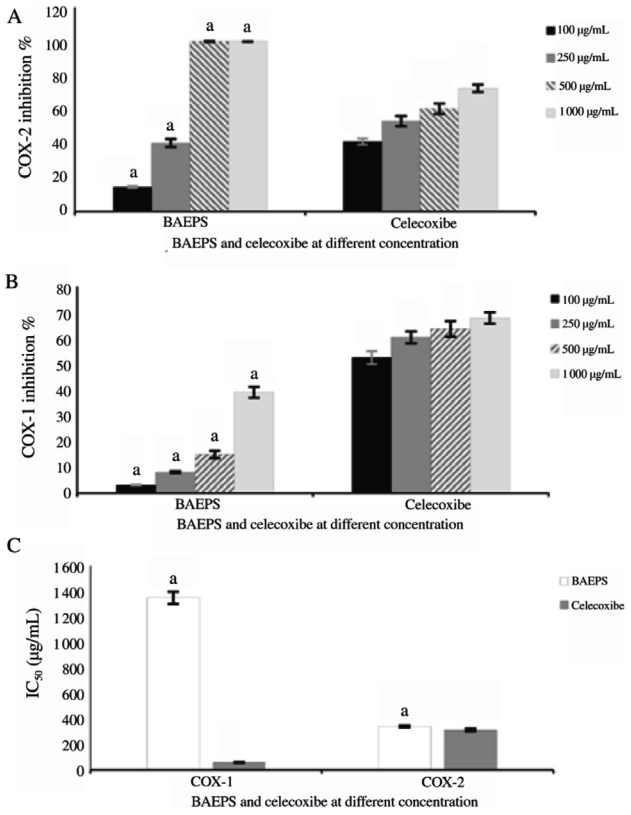

Figure 3. Cyclooxygenases inhibition activity of different concentrations (100–1 000 μg/mL) of BAEPS and reference drug; celecoxib, COX-2 (A) and COX-1 (B), IC_{50} (C).

presented inhibitory effect against COX-1 less than COX-2 at all concentration comparable to the same concentration of celecoxib. The COX-1 inhibition percentage recorded for BAEPS ranged from (3.08 ± 0.17)% to (38.98 ± 2.10)% (at 100 and 1 000 μg/mL, respectively) (Figure 3B). BAEPS exhibited COX-1 inhibition percentage less than that exhibited by celecoxib. Therefore, IC_{50} of BAEPS was very big [(1348.43 ± 48.00) μg/mL] compared to IC_{50} of celecoxib [(57.92 ± 3.40) μg/mL] as Figure 3C. It is evident from these mentioned data that, BAEPS could correspond selective anti-inflammatory effect evident by low potency against COX-1 and high potency against COX-2, therefore, it showed low COX-2/COX-1 ratio (0.25) which indicated potent anti-inflammatory activity with fewer side effects on the stomach and kidney.

3.5. Cytotoxic effect of BAEPS

The cell viability assay was used to investigate the cytotoxic effect of BAEPS on PC3 and MCF7. BAEPS showed low anti-cancer effect against PC3 and recorded 5.90% death percentage at 100 μg/mL while, BAEPS exhibited potent and selective effect to breast cancer cell MCF7 and recorded 65.20% death percentage with IC_{50} 70 μg/mL and IC_{90} 127.40 μg/mL.

3.6. Acute toxicity studies

BAEPS produced from *B. amyloliquefaciens* 3MS 2017 was administered up to 3 000 mg/kg B.W. The lethal dose of half number of animals was recorded at 2 000 mg/kg B.W. for BAEPS.

3.7. Role of BAEPS in suppression of EAC

The total cell count of EAC was significantly reduced by administration of BAEPS which may attribute to inhibition of cell cycle. The total cell counts of bearing mice treated with BAEPS was [$(4.50 \times 10^7 \pm 0.73)$ cell/mL], while cell count of FU group was [$(4.86 \times 10^7 \pm 0.87)$ cell/mL] compared to untreated animals [$(10.0 \times 10^7 \pm 1.13)$ cell/mL] (Table 2). The viable cell count [$(9.18 \times 10^7 \pm 1.10)$ cell/mL] was decreased to a minimal number when animals administered BAEPS [$(0.82 \times 10^7 \pm 0.33)$ cell/mL]. The same effect was presented by administration of FU but it remained less effective than BAEPS [$(1.41 \times 10^7 \pm 0.69)$ cell/mL]. In an opposite manner, the non-viable cell count was increased; non-viable cell percentage as compared to total cell count was (81.77 ± 0.75)% for BAEPS whereas it was (2.80 ± 0.95)% for untreated animals. Therefore, the percentage of viable cell count was decreased to be [(18.00 ± 0.71)% and (18.22 ± 0.91)%] for FU and BAEPS which point to the powerful effect in inhibition of EAC progression.

Injection of carcinoma cells showed an adverse effect on animal MST [(20.00 ± 1.81) d] with amplification of tumor volume and weight [(3.00 ± 0.61) cm^3 and (3.59 ± 0.49) g] while FU decreased tumor volume and weight with increasing MST as well as significant increments in life span. The same trend was recorded with BAEPS with higher increments than FU as mentioned in Table 2.

Results of carcinoma cells viability are in accordance with those collected from the hematological parameters. The BAEPS affected the carcinoma viability. It determined hematological parameters. Hemoglobin concentration of infected mice was significantly decreased to [(6.53 ± 0.96) mg/dL] whereas it was improved with FU treatment [(7.11 ± 1.06) mg/dL]. In parallel, treating animals with BAEPS significantly upgrade hemoglobin concentration to reach [(10.55 ± 0.97) mg/dL]. Administration of BAEPS made sufficient improvements in all determined

Table 2
Effect of BAEPS Exo-polysaccharide on viable and non-viable tumor cell count and survival parameters of bearing mice.

Group	Tumor cell count					Survival parameters			
	Total	Viable	Non-viable	Viable %	Non-viable %	Tumor volume	Tumor weight	MST	Increase in lifespan %
Tumor bearing mice	10.00 ± 1.13	9.18 ± 1.10	0.82 ± 0.45	97.23 ± 1.64	2.77 ± 0.95	3.00 ± 0.61	3.59 ± 0.49	20.00 ± 1.81	0.00 ± 0.00
FU-treated	4.86 ± 0.87[a]	1.41 ± 0.69[a]	3.45 ± 0.61[a]	18.00 ± 0.71[a]	82.00 ± 0.68[a]	1.52 ± 0.23[a]	0.73 ± 0.34[a]	41.00 ± 0.47[a]	105.00 ± 1.56
BAEPS treated	4.50 ± 0.73[a]	0.82 ± 0.33[ab]	3.68 ± 0.27[a]	18.22 ± 0.91[a]	81.77 ± 0.75[a]	0.81 ± 0.22[ab]	0.52 ± 0.18[ab]	70.00 ± 1.24[ab]	250.00 ± 1.51[b]

Data are presented as mean ± SD. ANOVA one-way was used for data analysis ($n = 6$, $P < 0.05$). FU: 5-flouro uracil treated group, MST: median survival time. a, significant as compared to bearing mice, b, significant as compared to FU group.

Table 3
Influence of BAEPS exo-polysaccharide on mice hematological parameters.

Group	Hb concentration	RBC's count	PCV	Platelets count	WBC	Neutrophil	Lymphocytes	Monocytes	Eosinophil	Basophiles
Negative control	11.50 ± 1.10^{ab}	11.12 ± 1.00^{ab}	41.12 ± 1.25^{a}	300 ± 1.41^{ab}	7.00 ± 0.98^{ab}	25.63 ± 0.70^{ab}	65.00 ± 1.02^{ab}	3.41 ± 1.24^{ab}	4.00 ± 0.78^{ab}	2.00 ± 0.30^{ab}
Tumor bearing mice	6.53 ± 0.96	7.24 ± 1.11	31.32 ± 1.52^{b}	100 ± 1.61^{b}	11.41 ± 1.00^{b}	60.00 ± 0.84^{b}	29.00 ± 0.67^{b}	5.50 ± 0.13^{b}	3.00 ± 0.09	2.50 ± 0.14^{b}
FU-treated	7.11 ± 1.06	7.53 ± 0.97	38.54 ± 2.16^{a}	185 ± 1.86^{a}	9.24 ± 1.08^{a}	36.33 ± 2.11^{a}	56.24 ± 1.46^{a}	4.16 ± 0.74^{a}	3.27 ± 0.85	0.00 ± 0.04^{a}
BAEPS control	12.95 ± 0.86^{ab}	8.55 ± 0.84^{ab}	44.10 ± 1.35^{ab}	295 ± 0.79^{ab}	5.50 ± 0.79^{ab}	24.00 ± 1.00^{ab}	68.00 ± 1.70^{ab}	1.00 ± 0.53^{ab}	2.00 ± 0.67^{ab}	5.00 ± 0.28^{ab}
BAEPS treated	10.55 ± 0.97^{ab}	6.45 ± 1.06^{ab}	35.86 ± 0.98^{ab}	285 ± 1.00^{ab}	4.60 ± 0.95^{ab}	40.00 ± 0.97^{ab}	55.00 ± 1.16^{a}	1.00 ± 0.37^{ab}	2.00 ± 0.19^{ab}	2.00 ± 0.41^{ab}

Data are presented as mean ± SD. ANOVA one-way was used for data analysis ($n = 6$, $P < 0.05$). a, significant difference with tumor bearing mice, while b, significant difference with FU group. Hb: hemoglobin (mg/dL); RBC: red blood cell ($\times 10^3/mm^3$); PCV: packed cell volume (mm); WBC: white blood cell ($\times 1000/mm$).

Table 4
Efficacy of BAEPS exo-polysaccharides on liver function of tumor bearing mice in Ehrlich model.

Group	ALT	AST	AST/ALT ratio
Negative control	29.33 ± 0.98^{ab}	25.83 ± 0.79^{ab}	0.88 ± 0.25^{ab}
Tumor bearing mice	110.00 ± 1.56	450.00 ± 1.75^{b}	4.09 ± 1.00^{b}
FU-treated	90.00 ± 1.31^{a}	192.00 ± 1.42^{a}	1.92 ± 0.92^{a}
BAEPS-control	28.26 ± 0.06^{ab}	24.95 ± 0.92^{ab}	0.88 ± 0.21^{ab}
BAEPS-treated	80.23 ± 1.13^{ab}	168.00 ± 1.36^{ab}	2.09 ± 1.00^{ab}

Data are presented as mean of six animals ± SD. ANOVA one-way was used for data analysis ($n = 6$, $P < 0.05$). a, significant difference with tumor bearing mice, while b, significant difference with FU group.

hematological parameters. Packed cell volume, platelets count and lymphocytes percentage were suppressed by Ehrlich ascites carcinoma injection while administration of BAEPS induced packed cell volume, platelets count and lymphocytes percentage. Concerning the neutrophil percentage, it was magnified by carcinoma cell injection while it was depressed by application of BAEPS (Table 3). There is no deleterious effect on animals hematological parameters with administration of BAEPS as BAEPS-control group enhanced hemoglobin concentration and the balance between neutrophils and lymphocytes percentages remained in normal control level, the ameliorative effect of BAEPS was close to FU drug.

Aspartate amino transaminase (AST, U/L) and alanine amino transaminase (ALT, U/L) were elevated with inoculation of carcinoma cells; AST reached [(450.00 ± 1.75) U/L] and ALT reached [(110.00 ± 1.56) U/L] with respect of the negative control. In an opposite manner, administration of BAEPS produced ameliorative effect on liver function parameters. It decreased ALT activity from [(110.00 ± 1.56) U/L to (80.23 ± 1.13) U/L]. AST activity was significantly reduced from [(450.00 ± 1.75) U/L] for bearing mice to (168.00 ± 1.36) U/L in treated BAEPS animals (Table 4) which reflect the improvement of BAEPS on liver function.

4. Discussion

Most microbes in the marine are encompassed by EPSs, which may help microbial communities to tolerate extremes of salinity, temperature, and nutrient availability. Because of the charming chemical and rheological properties of the EPSs generated by microorganisms, the studies performed to test their potential applications in biotechnology [41]. Recently most bacteria have been found to have produced the highest quantity of EPSs in the stationary phase of growth; this result might be related to the competition occurring during the growth phase between EPS and cell-wall polymer biosynthesis. While, there are microorganisms releasing the maximum amount of EPS through the exponential phase. The product and quality of microbial EPSs are highly influenced by the environmental and nutritional status [42]. Mainly EPSs produced by marine bacteria are heteropolysaccharides containing different units of monosaccharides coordinated in a range of about ten to compose repeating units. Most EPSs are linear, with Mw ranging from (1×10^5) to (3×10^5) Da [43]. Several EPSs are neutral molecules; however, the majorities of them are polyanionic for the existence of sulfate, pyruvate, phosphate and uronic acids. Furthermore, the linkages between

monosaccharides that have been most generally found are β-(1–4)- or β-(1–3)-linkages in the backbone characterized by strong hardness and α-(1–2)- or α-(1–6)-linkages in the more malleable ones. The physical properties of EPSs are mightily affected with the way of the monosaccharides and are arranged jointly and the aggregation of the one polymer chains [44]. The IR of BAEPS displayed peaks at 3933.11 and 3423.03 cm^{-1} due to vibration of O−H in the ingredient sugar residues [45]. Particular peaks 1646.91 and 1651.73 cm^{-1} which is dominated by circle vibrations and is interfered with stretching vibration of C−O glycosidic bond vibration [46]. The strong absorption at 1079.94 and 1081.87 cm^{-1} was controlled by glycosidic linkage υ-(C−O−C)-stretching vibration [47]. The band at 1120.44 cm^{-1} could be imputed to the existence of sulfate groups as S═O and C−O−S [48]. While, the band at 922.77 cm^{-1} indicated the α-pyranose form of the glucosyl residue, and the strap at 838.88 cm^{-1} suggested the β-pyranose form [48].

The antioxidant characters of strong antioxidants agents may be refer to different mechanisms as free radical scavenging, prohibition of uninterrupted hydrogen abstraction, forbidding of chain initiation of lipid peroxidation, binding of transition metal ion catalysts, decomposition of peroxides, inhibition of free radical generating enzymes, activation of internal antioxidant enzymes and reductive capacity [49].

$DPPH^{\bullet}$ and $ABTS^{+}$ radicals need to scavenge to donate an electron or an active hydrogen atom from the antioxidant compounds. Components have active hydroxyl groups, like ascorbic acid, tocopherols, polyphenols, are strong free radical scavengers antioxidants [49]. Addition, H_2O_2 leads to transition metal ion-dependent OH radicals-mediated oxidative DNA damage. The H_2O_2 scavenging ability may be due to donate electrons to H_2O_2, neutralizes it to water [50].

Also, SOR helps in other ROS formation like; hydrogen peroxide, hydroxyl radical, and singlet oxygen, which stimulate oxidative damage status in lipids, proteins and DNA [51]. The toxic effect of SOR is through its ability to inhibit iron–sulfur bloc containing enzymes, which are ticklish in a broad variety of metabolic pathways. SOR able to decrease certain iron complex like cytochrome C [27].

Fe^{2+} chelation activity may give protection against oxidative damage by removing Fe^{2+} that may otherwise participate in OH generating Fenton type reactions causing a reduction on ROS generation and lipid peroxidation [24]. The unsaturated fatty acids, which contain multiple double bonds and the methylene CH_2-groups were attacked by ROS especially reactive hydrogen atoms, and initiate the radical peroxidation chain reactions. While, antioxidants scavenging peroxide and •OH radicals resulting inhibition the formation of linoleic acid hydroperoxides [49]. Therefore, capture Fe^{2+} and scavenging H_2O_2 may prevent linoleic acid oxidation.

In this research, BAEPS showed potent free radical scavenging activity, capture H_2O_2 radical and Fe^{+2}, reduce Fe^{+3} and inhibited SOR generation. Many scientific reports illustrated that polysaccharides have many biological activities as antioxidant activity dependent on their structure [16,51–53].

Gülçin [54] reported that the compounds with structures containing two or more of the following functional groups: −OH, −SH, −COOH, $-PO_3H_2$, −C═O, $-NR_2$, −S− and −O− in a favorable structure–function configuration can show metal chelation activity. The mentioned active groups can donate an electron or hydrogen atom to eliminate free radicals or reactive species and exhibited antioxidant properties [54]. As well as, active groups like OH, −SH, −COOH, −C═O, $-NR_2$, −S− and −O− can contend with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite [45]. In this study, tested BAEPS contains many important antioxidants active groups can donate a hydrogen atom or electron and compete with oxygen to react with NO as OH, C−H, C−O, C═O, C−O−C, S═O and C−O−S. From the above presentation together can conclude that, the antioxidant activities of BAEPS may be attributed to the presence of its active groups OH, C−H, C−O, C═O, C−O−C, S═O and C−O−S. In addition, BAEPS appeared ability to capture Fe^{2+} ion, reduction Fe^{+3}, hydrogen peroxide abstraction and scavenging SOR, which could be attributed to its lipid peroxidation inhibition ability.

Nitric oxide (NO) the signaling molecule works an important function in the pathogenesis of inflammation. It appears an anti-inflammatory ability in normal state. Contrary, NO works as a pro-inflammatory moderator, it promotes inflammation through overproduction in abnormal status. NO is implicated in the pathogenesis of inflammatory troubles of the joint, gut and lungs. Hence, NO inhibitors appear a key role in the handling of inflammatory diseases [55]. Our study demonstrated that BAEPS had high NO inhibition percentage with low IC_{50} 18.78 μg/mL.

COX-2 selective inhibitor is a non-steroidal anti-inflammatory drug (NSAID) which essentially goals to inhibit COX-2, like of celecoxib, rofecoxib. These drugs decreased the risk of peptic ulceration. The selective inhibition of COX-2 has been suggested to be an approach for treating inflammatory disorders and related inflammatory diseases. COX-2 enzyme binds to arachidonic acid resulting in the release of metabolites that induce pain and inflammatory responses. Therefore, the development of strong COX-2 inhibitors to remove pain and treat inflammation related diseases. Activation of inflammation and oxidative stress is defined as one principal cause of inflammatory related diseases like rheumatoid arthritis, diabetes, Alzheimer's disease and even cancers [56].

Many EPSs were reported for their anti-inflammatory activities through their cyclooxygenases inhibitory activity [16]. The selective anti-inflammatory activity of BAEPS may be because of its structure. The inhibitory action of BAEPS against nitric oxide formation with those of its effect as selective cyclooxygenase inhibitory agent supports the possibility of using it as an anti-inflammatory agent.

Tumor cells depend on the ascitic fluid to get their nutritional requirements needed for their growth and tumor progression that decrease life span. Through this point of view, many agents are considered as anti-cancer agents when they decrease this nutritional source to increase life span with disappearing blood leukemia [57]. As mentioned from this study, tested polysaccharide not only decreased tumor volume, but also decreased viable tumor cell count that reflected on the significant increment in life span. The anti-tumor effect of BAEPS is proven through many investigations that were previously mentioned in all evaluated characteristic tumor parameters.

ROS able to make mutation by damaging genetic molecules and cell division. These reactive molecules may interrupt cell signaling and growth or attach DNA to induce DNA damage, the first risk factor for cancer [58]. Moreover, ROS induce inflammatory response and aggregate inflammatory cascade to increase the evidence of cancer inside inflamed tissues. Based on this idea, antioxidants can play main role in depression of

tumor evidence or its progression through suppression of premalignant lesions and inhibition of cancer development. Many polysaccharides isolated from organisms showed *in-vitro* and *in-vivo* antioxidant properties including water-soluble polysaccharide of EPS from *Paenibacillus lactes* NRC1 [8]. In this study, the tested BAEPS; BAEPS showed powerful antioxidant characters compared to two standard materials; vitamin C as a natural antioxidant and BHT as a synthetic one. Thus, the anti-tumor activity of BAEPS may be attributed to the antioxidant capacity of BAEPS.

Cyclooxygenases are important enzymes in lipid metabolism that catalyze the oxygenation of polyunsaturated fatty acids, especially arachidonic, to produce the prostaglandin, which are potent cell-signaling molecules associated with the initiation, maintenance and resolution of inflammatory processes [59]. Prostaglandin can stimulate growth of tumor cells and suppress immune surveillance. Additionally, COX activates carcinogens to take up forms that damage the genetic material [5]. PSs play a double role; activates the immune system and inhibit the inflammation process [60]. This study demonstrated that the tested BAEPS; BAEPS appeared anti-inflammatory effect against cyclooxygenases; COX-1 and COX-2 and showed selectivity against COX-2 than COX-1 as well as nitric oxide inhibition activity.

Finally, the present research isolated and partially purified an acidic exo-polysaccharide from *B. amyloliquefaciens* 3MS 2017. BAEPS showed potent antioxidant activities as a radical scavenger and hydrogen peroxide also as a metal ion chelator. The inhibition of NO formation with selective inhibitory property of COX-2 activity is considered to be a promising approach to the treatment of various inflammations related diseases like cancer. BAEPS showed an antitumor activity against EAC, that it reduced counted viable EAC cell and increased non-viable cells also it improved all assessed hematological parameters. The antitumor ability of BAEPS may be attributed to its molecular weight, sulfate and COO^- groups and β-glycosidic linkages. All these data point to the potential of improving BAEPS as a prospect agent as a tumor chemotherapy concerning on its effect against EAC model.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid Med Cell Longev* 2016; **2016**. 5698931; http://dx.doi.org/10.1155/2016/5698931.

[2] Mathew BB, Tiwari A, Jatawa SK. Free radicals and antioxidants: a review. *J Pharm Res* 2011; **4**(12): 4340-4343.

[3] Leblanc GA. Acute toxicity. In: Hodgson E, editor. *A textbook of modern toxicology*. Hoboken, New Jersey: John Wiley & Sons, Inc; 2004, p. 255.

[4] World Health Organization. *WHO library cataloguing-in-publication data World health statistics* [Online]. Geneva: World Health Organization; 2015. Available from: http://www.who.int [Accessed on 7th June, 2017]

[5] Daba AS, Ezeronye OU. Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms. *Afr J Biotech* 2003; **2**(12): 672-678.

[6] Fauziah O, Hanachi P, Yogespiriya S, Asmah R. Reducing effects of *Strobilanthes crispus* leaf extract in hepatocarcinogenesis in rats. *Int J Cancer Res* 2005; **1**(2–3): 109-112.

[7] Singh S, Khar A. Biological effects of curcumin and its role in cancer chemoprevention and therapy. *Anti-Cancer Agents Med Chem* 2006; **6**(3): 259-270.

[8] Mahmoud MG, Mohamed SS, Ibrahim AY, El Awady ME, Youness ER. Exopolysaccharide produced by *Paenibacillus lactes* NRC1: its characterization and anti-inflammatory activity via cyclooxygenases inhibitory activity and modulation of inflammation related cytokines. *Der Pharma Chem* 2016; **8**(5): 16-26.

[9] Delbarre-Ladrat C, Sinquin C, Lebellenger L, Zykwinska A, Colliec-Jouault S. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. *Front Chem* 2014; **2**(1): 1-15.

[10] Ahmed OM, Ahmed MR. Anti-proliferative and apoptotic efficacies of ulvan polysaccharides against different types of carcinoma cells *in-vitro* and *in-vivo*. *Cancer Sci Ther* 2014; **6**(6): 202-208.

[11] Harrigan WF, McCance ME. *Laboratory methods in food dairy and microbiology*. New York: Academic Press; 1976, p. 204,267,209,308.

[12] Kim S, Ahu S, Seo W, Kwan G, Park Y. Rheological properties of a novel high viscosity polysaccharide A49 pol., produced by *Bacillus polymyxa*. *J Microb Biotech* 1998; **8**(2): 178-181.

[13] Boyle CD, Reade AE. Characterization of two extracellular polysaccharides from marine bacteria. *Appl Environ Microbiol* 1983; **46**(2): 392-399.

[14] Shene C, Canquil S, Rubilar M. Production of the exopolysaccharides by *Streptococcus thermophilus*: effect of growth conditions on fermentation kinetics and intrinsic viscosity. *Int J Food Microb* 2008; **124**(3): 279-284.

[15] Maidak BL, Olsen GJ, Larson N, Overbeek MJ, Woese CR. The ribosomal database project (RDP). *Nucleic Acid Res* 1997; **25**(1): 109-110.

[16] Ibrahim AY, Mahmoud MG, Asker MMS. Anti-inflammatory and antioxidant activities of polysaccharide from *Adansonia digitata*: an *in vitro* study. *Int J Pharm Sci Rev Res* 2014; **25**(33): 174-182.

[17] El-Sayed OH, Ismail SA, Ahmed YM, Abd El-Samei M, Asker MMS. Studies on the production of sulfated polysaccharide by locally isolated bacteria. *J Egypt Pharm* 2007; **4**(1): 439-452.

[18] Dodgson KS, Price RG. A note on the determination of the ester sulfate content of sulfated polysaccharides. *Biochem J* 1962; **84**(1): 106-110.

[19] Jun HI, Lee H, Song GS, Kim YS. Characterization of the pectic polysaccharide from pumpkin peel LWT. *Food Sci Technol* 2006; **39**(5): 554-556.

[20] Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. *Biosci Biotech Biochem* 1998; **62**(6): 1201-1204.

[21] Miller NJ, Rice-Evans CA. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem* 1997; **60**(3): 331-337.

[22] Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem* 2001; **73**(2): 239-244.

[23] Ruch RJ, Cheng SJ, Klaunig JF. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989; **10**(6): 1003-1008.

[24] Gülçin I, Büyükokuroğlu ME, Oktay M, Beydemir S, Küfrevioğlu ÖI. Antioxidant and antimicrobial activities of *Teucrium polium* L. *J Food Tech* 2003; **1**(1): 9-17.

[25] Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci* 1997; **60**(10): 763-771.

[26] Marcocci I, marguire JJ, Droy–lefaiz MT, Packer L. The nitric oxide scavenging properties *Ginkgo biloba* extract. *Biochem Bioph Res Comm* 1994; **201**(2): 748-755.
[27] Gülçin I, Gungor Sat I, Beydemir S, Elmastas M, Kufrevioglu Irfan O. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender *(Lavandula stoechas* L.). *Food Chem* 2004; **87**(3): 393-400.
[28] Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys* 1994; **315**(1): 161-169.
[29] Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr Diet* 1986; **44**(6): 307-315.
[30] Larsen LN, Dahl E, Bremer J. Peroxidative oxidation of leucodichlorofluorescein by prostaglandin H synthase in prostaglandin biosynthesis from polyunsaturated fatty acids. *Biochim Biophys Acta* 1996; **1299**(1): 47-53.
[31] Mosmann T. Rapid colorimetric assays for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**(1–2): 55-63.
[32] Thabrew MI, Hughes RD, McFarlane IG. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J Pharm Pharmacol* 1997; **49**(11): 1132-1135.
[33] El-Menshawi BS, Fayad W, Mahmoud K, El-Hallouty SM, El-Manawaty M, Olofsson MH, et al. Screening of natural products for therapeutic activity against solid tumors. *Indian J Exp Biol* 2010; **48**(3): 258-264.
[34] Bruce RD. An up-and-down procedure for acute toxicity testing. *Fundam Appl Toxicol* 1985; **5**(1): 151-157.
[35] Raju A, Arulanandham A, Pradeep R, Lakshmi N. Anticancer activity of *Jasminum angustifolium* Linn against Ehrlich ascites carcinoma cells bearing mice. *J Exp Integr Med* 2012; **2**(3): 271-275.
[36] Lippman ME, Dickson RB. Mechanisms of normal and malignant breast epithelial growth regulation. *J Steroid Biochem* 1989; **34**(1–6): 107-121.
[37] Karmakar I, Dolai N, Kumar RBS, Kar B, Roy SN, Haldar PK. Antitumor activity and antioxidant property of *Curcuma caesia* against Ehrlich's ascites carcinoma bearing mice. *Pharm Biol* 2013; **51**(6): 753-759.
[38] Kuttan G, Vasudeven DM, Kuttan R. Effect of a preparation from *Viscum album* on tumor development *in vitro* and in mice. *J Ethnopharmacol* 1990; **29**(1): 35-41.
[39] Dacie JV, Lewis SM. *Laboratory investigation in hemolytic anemia in practical haematology*. 5th ed. New York: Churchil Livingstone; 1975, p. 40.
[40] Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; **28**(1): 56-63.
[41] Guezennec J. Deep-sea hydrothermal vents: a new source of innovative bacterial exopolysaccharides of biotechnological interest? *J Ind Microbiol Biotechnol* 2002; **29**(4): 204-208.
[42] Decho AW. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanogr Mar Biol Rev* 1990; **28**(7): 73-153.
[43] Vanho oren PT, Vandamme EJ. Biosynthesis, physiological role, use and fermentation process characteristics of bacterial exopolysaccharides. *Rec Res Dev Ferment Bioeng* 1998; **1**(2): 253-299.
[44] Cipriani TR, Mellinger CG, Souza LM, Baggio CH, Freitas CS, Marques MCA, et al. Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydr Polym* 2008; **74**(2): 274-278.
[45] Umezawa H, Okami Y, Kurasawa S, Ohnuki V, Ishizuka M, Takeuchi T, et al. Marinactan, antitumor polysaccharide produced by marine bacteria. *J Antibiot* 1983; **36**(5): 471-477.
[46] Wang H, Ooi EV, Ang PO. Antiviral polysaccharides isolated from Hong Kong brown seaweed *Hydroclathrus clathratus*. *Sci China* 2007; **50**(5): 611-618.
[47] Zhang L, Wei L, Takashi N, Hiroshi F. Double-stranded helix of xanthan: rigidity in 0.01M aqueous sodium chloride containing 0.01N hydrochloric acid. *Biopolymers* 1987; **26**(3): 333-341.
[48] Zhang HN, Lin ZB. Hypoglycemic effect of *Ganoderma lucidum* polysaccharides. *Acta Pharmacol Sin* 2004; **25**(2): 191-195.
[49] Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J Cell Mol Med* 2014; **14**(4): 840-860.
[50] Wettasinghe M, Shahidi F. Scavenging of reactive species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chem* 2000; **70**(1): 17-26.
[51] Liang TW, Tseng SC, Wang SL. Production and characterization of antioxidant properties of exopolysaccharide(s) from *Peanibacillus mucilaginosus* TKU032. *Mar Drugs* 2016; **14**(1): 2-12.
[52] He F, Yang Y, Yang G, Yu L. Components and antioxidant activity of the polysaccharide from *Streptomyces virginia* H03. *Z Naturforsch C* 2008; **63**(3–4): 181-188.
[53] Pietta PG. Flavonoids as antioxidants. *J Nat Prod* 2000; **63**(7): 1035-1042.
[54] Gülçin I. Antioxidants and antiradical activities of L-carnitine. *Life Sci* 2006; **78**(8): 803-811.
[55] Bogdan C. Nitric oxide and the immune response. *Nat Imm* 2001; **2**(10): 907-916.
[56] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**(9255): 539-545.
[57] Mazumdur UK, Gupta M, Maiti S, Mukherjee D. Anti-tumor activity of *Hydrophilla spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian J Exp Biol* 1997; **35**(3): 473-477.
[58] De Groot H. Reactive oxygen species in tissue injury. *Hepatogastroenterology* 1994; **41**(4): 328-332.
[59] Charlier C, Michaux C. Dual inhibition of cyclooxygenase-2 (COX-2) and 5 lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *Eur J Med Chem* 2003; **38**(7–8): 645-659.
[60] Besednova NN, Zaporozhets ST, Makaren DL, Kova AT, Kuznetsova PS, Kryzhanoskii NT, et al. Anti-inflammatory effects of sulphated exopolysaccharides extracted from brown marine algae. *Biol Bull Rev* 2012; **2**(6): 525-532.