processes

MDPI

Article

Extraction, Purification and Characterization of Exopolysaccharide from *Lactiplantibacillus plantarum* B7 with Potential Antioxidant, Antitumor and Anti-Inflammatory Activities

Abeer A. Ageeli and Sahera F. Mohamed *

Department of Physical Sciences, Chemistry Division, Collage of Science, Jazan University, P.O. Box 114, Jazan 45142, Saudi Arabia; abeerali@jazanu.edu.sa
* Correspondence: smohamed@jazanu.edu.sa; Tel.: +966-557879150

Abstract: In recent years, exopolysaccharides (EPSs) have emerged as significant substances due to their impressive biological properties. This research intends to analyze the EPS extracted from probiotic bacteria and assess its various biological activities. The promising probiotic bacteria isolated from human breast milk was isolated and identified as *Lactiplantibacillus plantarum* B7 by 16S rRNA sequencing. The EPS yield of this strain was quantified as 5.2 g/L. The crude extract (EPSc) was subjected to purification by chromatography on DEAE-cellulose and Sephadex G-100 columns, giving two main fractions named EPSF1 and EPSF2. Structural features were investigated by HPLC, FTIR, GPC and ^{1}HNMR. Chromatographic analysis indicated that EPSF1 and EPSF2 were composed of mannuronic acid, mannose and glucose in a molar ratio of 2.6:2.15:1.00 and 3.92:2.65:1.00 with a molecular weight of 4.36×10^4 and 5.27×10^5, respectively. Multiple in vitro assays of EPSc, EPSF1 and EPSF2 showed potent radical scavenging activity on DPPH, ABTS, hydroxyl radical scavenging activity (HRS) and superoxide scavenging activity. Also, they showed reducing power of 0.69, 0.61 and 0.58, respectively, at 1000 µg/mL. EPSc, EPSF1 and EPSF2 displayed negligible toxicity against WI-38 human normal lung cells but had cytotoxic effects against human colon cancer (Caco-2), (IC_{50} = 122.13 ± 0.01, 72.5 ± 0.12 and 81.6 ± 0.1 µg/mL), HepG2 liver cancer (IC_{50} = 112.5 ± 0.01, 60.3 ± 0.1 and 62.0 ± 0.03 µg/mL) and human prostate cancer (PC3) (IC_{50} = 109.6 ± 0.03, 65.7 ± 0.01 and 70.3 ± 0.04 µg/mL). While anti-inflammatory as hemolysis inhibition was 79.3 ± 0.05, 93.5 ± 0.05 and 87.9 0.03% at 500 µg/mL, respectively. The results indicate that EPSF1 showed promising antioxidant, antitumor and anti-inflammatory activities.

Keywords: probiotics; *L. plantarum* B7; exopolysaccharides; antioxidant; antitumor; anti-inflammatory

check for updates

Academic Editor: Elwira Sieniawska

Received: 16 February 2025
Revised: 15 March 2025
Accepted: 19 March 2025
Published: 21 March 2025

Citation: Ageeli, A.A.; Mohamed, S.F. Extraction, Purification and Characterization of Exopolysaccharide from *Lactiplantibacillus plantarum* B7 with Potential Antioxidant, Antitumor and Anti-Inflammatory Activities. *Processes* **2025**, *13*, 935. https://doi.org/10.3390/pr13040935

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Probiotics are bacteria that, when properly consumed, confer health advantages to the host and, when introduced into food, enhance the microbial balance in the human gastrointestinal system, hence promoting general health [1]. Several studies have indicated that probiotics have medicinal advantages, including anticancer activity, cholesterol lowering and immunological enhancement [2]. The probiotic microorganisms employed are frequently extracted from the gastrointestinal system or other sites, including feces, milk, fermented foods or pickles. The FDA has deemed lactic acid bacteria, a normal part

of the gastrointestinal microflora, to be safe [3]. Probiotic bacteria play an effective role in stimulating immune responses and antimicrobial effects through various mechanisms. They produce bacteriocins, which are protein substances that have an inhibitory effect on the growth of pathogenic bacteria and have unique systems for the decomposition of various digestive materials, in addition to being some natural food additives, such as organic acid taste and texture enhancers [4–6].

Countless types of exopolysaccharides (EPSs) are produced from probiotic bacteria [7]. These molecules are recovered from the microorganisms in the form of tightly bound capsules (CPs) or are released into their surrounding EPSs [8]. The most prominent function of EPSs has been protection against desiccation, phagocytosis, cell recognition, attack by bacilli, antibiotics or toxic compounds and osmotic stress. During the past few decades, EPSs have gained great interest among scientific communities since they are generally regarded as safe (GRAS). Therefore, probiotic EPSs are being used in the treatment of human disorders [9]. They play a role in enhancing the texture and sensory properties of fermented dairy products such as cheese and yogurt. A wide variety of research has been reported emphasizing the health-promoting potential of EPSs. For example, in cancer cell lines, EPSs can trigger both apoptosis and autophagy [10], have antioxidant activity [11], modulate the immune system response [12], have anti-inflammatory, antidiabetic, antiulcer and low-density lipid (LDL)-lowering activity [13–16] and act as anti-biofilm agents to stop the adhesion of pathogenic bacteria. Many research groups have indicated that EPSs are able to reduce the growth of tumors through the subsequent shared processes: (i) inhibition of tumor initiation by depletion of active molecules; (ii) direct anticancer activity, such as induction of cancer cell death; (iii) immunomodulatory activity with chemotherapy; and (iv) tumor metastasis inhibition. Recently, many EPSs have been isolated from different natural sources [17–19], and the focus has been on the biological activities of EPSs due to their high ability to induce cancer cell death and their low toxicity to normal cells [20]. Also, EPSs from lactic acid bacteria (LAB) exhibit high levels of biodegradability, are non-toxic and possess excellent biocompatibility [21]. Consequently, their potential in cancer prevention has garnered growing interest recently.

Lactiplantibacillus plantarum (previously known as *Lactobacillus plantarum*) strains are classified as lactic acid bacteria (LAB) and are facultative heterotrophs that flourish in versatile and nutrient-dense settings, including the urogenital tracts, as well as vegetables and fermented foods. *L. plantarum* is characterized by the production of various fermented foods and vitamins. It is non-pathogenic and comprises 218 subsystems and 32,918 genes, along with five classes of sugars that fulfill essential functions [22–24]. EPSs from *L. plantarum* YW32 have very good antioxidants, inhibit pathogenic bacteria and suppress tumor cell growth in vitro [6], while EPSs from *L. plantarum* MM89 display immunomodulatory properties in immuno-suppressed mice by enhancing lymphocyte proliferation, the spleen index and IgA content and enhancing the immune cells' immunostimulatory function [12]. These promising features have highlighted EPSs as a potentially beneficial element for improving human health. Some previous studies have been conducted on extracting, characterizing and evaluating exopolysaccharides from different *L. plantarum* strains, such as WLPLO4, YW32 [6] and MM89 [12].

The objective of this study is to extract and refine the EPS from the bacterial strain *L. plantarum* B7. The isolation of *L. plantarum* B7 from human breasts was performed, and its identification was confirmed by 16S rRNA sequencing. The extracted exopolysaccharide was characterized by Fourier-transform infrared (FT-IR), the monosaccharide composition was determined using HPLC and the molecular weights of the separated fractions were determined using gel permeation chromatography (GPC). Furthermore, the biological activities of the crude extract EPSc, as well as its fractions EPSF1 and EPSF2, were evalu-

ated in vitro, including antioxidant, anticancer and anti-inflammatory activity. This work provides a pre-fundamental basis for the usage of EPSs for human health and biotechnological applications.

2. Methodology

2.1. Materials

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid (vitamin C. Vit. C), 2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), ferrous chloride and trichloroacetic acid (TCA)—all chemicals of analytical grade—were purchased from Sigma-Aldrich, USA. Normal lung cells WI-38 (ATCC: CCL-25), the human cancer cell hepatocellular carcinoma (HepG2), prostate carcinoma (PC3) and colon carcinoma (Caco-2) (ATCC: CCL-185) were purchased from the National Cancer Institute (NCI), Cairo University, Egypt. All microorganisms—*Bacillus subtitles* ATCC6633, *Escherichia coli* ATCC 225922, *Staphylococcus aureus* NRRL B-767, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231—were obtained from the culture collection unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2.2. Screening, Isolation, Characterization and Identification of EPS-Producing Bacterial Strain from Human Breast Milk

Milk specimens were gathered in sterile containers from ten wholesome maternal volunteers. The collected samples were stored at 4 °C until being transported to the laboratory. A ten-fold dilution of each sample was prepared in 0.9% sterile saline solution and homogenized for 10 min by vortex mixing. Each dilution (100 µL) was plated on de Man, Rogosa and Sharpe (MRS) agar pH 5.8 to isolate Lactiplantibacillus species [6]. The plates that were inoculated were kept at 37 °C for 48 h. The chosen colonies were then gathered and purified utilizing the streak plate method on an MRS agar medium [25]. The isolates were primarily identified through the assessment of staining characteristics (Gram-positive), a catalase-negative test and an examination of cell morphology, following the guidelines in Bergey's manual of systematic bacteriology [26–28]. Bacterial isolates that are Gram-positive, catalase-negative and oxidase-negative were preserved in the same medium with glycerol at a temperature of −20 °C.

2.2.1. Screening of EPS Producing Isolates

Each 100 mL of MRS broth medium containing 5% sucrose was inoculated with 10^8 CFU/mL of individually chosen isolates and incubated for 72 h at 37 °C. From each bacterial culture, bacterial cells were separated through centrifugation (4 °C, 5000 rpm for 20 min) using a refrigerated centrifuge. EPSs were obtained by the addition of three volumes of chilled absolute ethanol to the cell-free suspensions (CFSs), and the samples were centrifuged (5000 rpm, 15 min, 4 °C); then, the pellets were dried. The selected bacterial strain with the highest EPS production was subjected to further identification studies [29].

2.2.2. Optimal Growth Temperature of the Isolated Bacterial Strain

The ideal temperature for growth was established using the MRS broth media. Each tube received 1 mL of 10^6 CFU/mL from a culture that had been grown overnight; each tube was incubated for 48 h at specific temperatures: 20, 25, 30, 35, 40 and 45 °C. The negative control tube contained MRS broth. Bacterial proliferation was assessed by measuring the optical density (OD) at 620 nm in three separate trials using a spectrophotometer (Unico, Fairfield, NJ, USA).

2.2.3. NaCl Tolerance Assessment

The tolerance of the chosen bacterial strain to NaCl was evaluated as previously described [30] with some modifications. MRS broth with different concentrations of NaCl of 2, 3, 4, 5, 6 and 8% were prepared. A freshly prepared inoculum, 1 mL of 10^6 CFU/mL, was introduced into the corresponding MRS broth. All cultures were incubated for 48 h at 37 °C. The negative control, consisting solely of MRS broth, exhibited no signs of growth. Bacterial growth was determined by measuring in triplicate the OD at 620 nm.

2.2.4. Acid and Bile Tolerance

The selected bacterial *L. plantarum* B7 was cultured overnight, and then 1 mL of 10^6 CFU/mL of the isolate was inoculated in MRS broth that either had been prepared at pH 3.0 or supplemented with a bile salt (0.3%). Following incubation at 37 °C for 1, 2, 3 and 4 h, 1 mL of this culture medium was mixed with 9 mL of MRS broth and incubated overnight at the same temperature. A control sample was prepared using MRS broth adjusted to pH 7.0. The resistance was measured in triplicate by evaluating the optical density at 620 nm [30]. The formula used to calculate the resistance percentage is as follows:

$$\text{Survival rate (SR\%)} = \text{ODs/ODc} \times 100$$

where ODs is the optical density of the test and ODc is the optical density of the control.

2.2.5. Identification of the Chosen Bacterial Isolate Utilizing 16S rRNA

The bacterial strain chosen was genetically identified by amplifying the 16S rRNA gene from the isolate. The DNA of the selected bacterial isolate was extracted, purified and amplified by polymerase chain reaction (PCR) according to the previously published methods [29]. The 16S rRNA gene was amplified using the universal primers (27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-GGTTACCTTGTTACGACTT-3′). Specific PCR products were confirmed through gel electrophoresis. The PCR product was sequenced at Microgen Sequencing Facilities provided by the website http://dna.macrogen.com, Seoul, Republic of Korea (accessed on 15 September 2024). The sequencing data were matched against the NCBI database using the BLASTN 2.2.6 program. A GenBank accession number was obtained for the chosen B7 isolate. The phylogenetic tree was created using the neighbor-joining method [31].

2.3. Probiotic Properties of the Selected Strain

Cell Surface Hydrophobicity

Bacterial cells were harvested from a culture that had been grown overnight and then rinsed two times with PBS at a pH of 7.0 and resuspended at the level of 10^8 CFU/mL in 10 mL of the same saline solution. The initial absorbance (A0) of the suspension was measured at 620 nm. A clean and dry test tube received 1 mL of the suspension, followed by adding 1 mL of n-hexane, xylene and toluene. The mixture was stirred for 2 min. The tubes were maintained at 37 °C for 1 h. Subsequently, the lower aqueous phase was collected using a sterile Pasteur pipette, and the absorbance (A1) was measured at a wavelength of 600 nm. The percentage of cell surface hydrophobicity was calculated using the formula provided below:

$$\text{Hydrophobicity (\%)} = [\text{A0} - \text{A1})/\text{A0}] \times 100$$

where A0 and A1 are the absorbances of the control reaction and the sample solution in the presence of the test sample, respectively.

2.4. Antimicrobial Potential of the L. plantarum B7

The antimicrobial potential of the *L. plantarum* B7 was assessed using the agar well diffusion method against *Bacillus subtitles*, *Escherichia coli*, *Staphylococcus aureus* NRRL B-767, *Pseudomonas aeruginosa* and *Candida albicans* ATCC as test pathogens. Nutrient agar plates were spread with 100 µL of test pathogen cells, which is about 10^9 CFU/mL. A clean cork hole was used to make wells on the dried agar plate that were all the same size and had a diameter of 6 mm. A total of 100 µL of the CFS from a 24 h growth of *L. plantarum* B7 in MRS broth media was put into each well. The plates were kept at 37 °C for 24 to 48 h. The diameter of the clear zone around each well was measured in mm [32].

2.5. Assessment of the Safety Aspects of L. plantarum B7

2.5.1. Hemolytic Activity

The hemolytic characteristics of *L. plantarum* B7 were assessed by cultivating fresh cultures overnight on blood agar supplemented with 7% sterile sheep blood and incubating them for 48 h at 37 °C. The hemolytic activity was classified into three categories based on the presence of inhibition zones around the colonies: a green zone, indicating α-hemolysis, a clear zone, indicating β-hemolysis, and the lack of an inhibition zone, signifying λ-hemolysis [33].

2.5.2. Antibiotic Susceptibility Test

The disk diffusion procedure was used to assess the antibiotic susceptibility profile of *L. plantarum* B7 [34]. The antibiotics assessed were clindamycin (2 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), imipenem (10 µg), norfloxacin (10 µg), fusidic acid (10 µg), streptomycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), aztreonam (30 µg) and penicillin (10 IU). The culture, which was 24 h old, was sampled on MRS agar plates, and the antibiotic discs were positioned directly on the surface. The plates were incubated at 37 °C overnight, and the diameter of the inhibition zone around each disk was measured and documented in millimeters.

2.6. Crude Exopolysaccharide (EPSc) Isolation, Fractionation and Purification

2.6.1. EPSc Isolation

EPSc was extracted from *L. plantarum* B7 following a previously described method, with certain modifications [28]. *L. plantarum* B7 was cultured in MRS broth enriched with 5% sucrose and incubated at 37 °C for 72 h. The fermented broth was then heated to 100 °C for 20 min to deactivate the enzymes. After cooling, the suspension was centrifuged at 4 °C and 5000 rpm for 20 min to separate the cells. Trichloroacetic acid was subsequently added to the upper liquid to achieve a final concentration of 12% (*w*/*v*) in order to precipitate enzymes, leftover amino acids or proteins, and the mixture was stirred at ambient temperature for 30 min and then centrifuged at 5000 rpm for 30 min at 4 °C. To precipitate the EPS, three volumes of chilled absolute ethanol were mixed in, allowing the mixture to sit at 4 °C for 24 h. Afterward, the precipitate was obtained by centrifuging the solution at 4000 rpm for 10 min at the same temperature. The precipitate was then re-dissolved in distilled water and subjected to dialysis against Milli-Q water with a dialysis membrane that had a 3.5 kDa cut-off at 4 °C for three days while replacing the water twice daily. Finally, the dialyzed solution was freeze-dried to yield the EPSc, and its weight was recorded to determine the amount produced. The phenol-H_2SO_4 method was employed to assess total carbohydrate content [35], while protein levels were measured using bovine serum albumin as a standard, as detailed previously [36].

2.6.2. EPSc Fractionation

The freeze-dried EPSc was subjected to a column (60 × 2.5 cm, i.d) of DEAE-cellulose anion-exchanger resin equilibrated with distilled water and a stepwise elution of 0.0, 0.05, 0.3 and 0.6 M aqueous solutions of NaCl at a flow rate of 1 mL/min. Volumes of 5 mL were gathered and merged based on the total carbohydrate content measured. The combined fractions were concentrated, subjected to dialysis and subsequently freeze-dried.

2.6.3. Fraction Purification

The main two fractions obtained, EPSF1 and EPSF2, were further purified by gel permeation chromatography (GPC) separately over a Sephdex G-100 (90 × 2.4 cm, i.d.) and eluted by 0.1 M NaCl at a flow rate of 0.5 mL/min. The fractions were gathered and analyzed for total sugars, after which the purified polysaccharide fractions, EPSF1 and EPSF2, were obtained, dialyzed and freeze-dried.

2.7. Determination of Monosaccharide Composition and Molecular Weight (MW) of EPSF1 and EPSF2

The purified, freeze-dried EPSF1 and EPSF2 (2 mg) were hydrolyzed in 2 mL of 6 M trifluoracetic acid at 105 °C in a sealed tube for 6 h using a boiling water bath as described before [37], followed by evaporation at 45 °C, co-distillation with water 3 times and dissolving in pure ethanol. The concentration of mono-sugars was analyzed through HPLC separation, accomplished by passing water through the column at a rate of 0.5 mL/min for 30 min. The monitoring involved assessing the variations in refractive indices. The identification and quantification of mono-sugars were performed using external standards [38]. The Agilent PL-aquagel-OH-40 (300 × 7.5 mm, 8 µm) equipped with an MW analyzer and differential refractometer was utilized, with 0.1 M $NaNO_3$, which included 0.2% NaN_3 as the eluent. The EPSF1 and EPSF2 sample solutions were subjected to sonication for 5 min, filtered using 0.2 µm filter units and then injected into the system. Elution took place at a flow rate of 0.6 mL/min at a temperature of 35 °C for a period of 25 min. The standard was introduced first, followed by the sample in duplicate [39].

2.8. FT-IR and 1H NMR Spectroscopy

FT-IR spectroscopy was utilized to identify the main structural groups present in the purified fractions according to the method described previously [40]. EPSF1 and EPSF2 were combined with dry KBr at 1:100 and compressed into a disk under vacuum. The FT-IR spectrum was recorded using a Bruker Tensor 27 FT-IR Spectrometer (Spectra Lab Scientific Inc., Markham, ON, Canada) with a resolution of 4 cm^{-1} over 400–4000 cm^{-1}. To obtain 1H NMR spectra, 50 mg of the sample was placed in 1 mL of DMSO, mixed well and analyzed using a JEOL NMR spectrometer (MA, USA). The chemical shifts were reported in ppm.

2.9. Antioxidant Capacity of the EPSc, EPSF1 and EPSF2

The stock solution of each polysaccharide, EPSc, EPSF1 and EPSF2 (1% w/v) (10 mg/mL), was prepared in saline as a solvent. Different concentrations of each sample were prepared (50, 100, 200, 400, 600, 800 and 1000 µg/mL).

2.9.1. Radical Scavenging by DPPH

The scavenging effect of EPSc, EPSF1 and EPSF2 on the free radical DPPH was estimated as previously described [41]. A 0.2 mM mixture of DPPH in ethanol was prepared. A total of 1 mL of DPPH was added to 1 mL of each sample mixture. The mixture was vigorously shaken and left in the dark to react for 30 min at room temperature. The control sample was prepared by using deionized water instead of EPS samples. Following this, the absorbance was calculated at 517 nm by a spectrophotometer (UV-VIS Milton Roy,

Golden, CO, USA). Ascorbic acid (Vit. C) (5 mg/mL) was used as a reference compound; the experiment was conducted three times. The IC_{50} value indicates the concentration of the substance required to inhibit 50% of the DPPH free radicals, and it was established using a logarithmic dose–response inhibition curve. A decrease in absorbance correlates with an increase in free radical scavenging activity. The DPPH scavenging effect percentage was calculated using the following formula:

$$\text{Radical scavenging (\%)} = (A0 - A1)/A0 \times 100$$

2.9.2. ABTS Assay for Antioxidant Activity Evaluation

Totals of 5 mL of 7 mM ABST and 88 µL of 140 mM $K_2S_2O_8$ were combined to make the ABTS reagent. The mixture was kept at ambient temperature in the dark for 6 h to enable the free radicals' formation. It was then diluted with water (1:44, v/v). A total of 1 mL of each test sample, EPSc, EPSF1 and EPSF2, was combined with 1 mL of the ABST reagent and then incubated at ambient temperature for 6 min. The absorbance was recorded at 734 nm. Vit. C was utilized as a reference compound. Methanol was used as a control [41]. The scavenging activity was determined based on the formula below:

$$\text{Scavenging activity (\%)} = (A0 - A1)/A0 \times 100$$

2.9.3. Hydroxyl Radical Scavenging (HRS) Activity

HRS assesses the antioxidant capacity to neutralize hydroxyl radicals by disrupting their reactions. The HRS of EPSc, EPSF1 and EPSF2 was evaluated based on the method outlined previously [41]. A mixture was prepared by combining 1 mL of 1,10-phenanthroline (0.75 mmol/L), 2 mL of PBS and 1 mL of $FeSO_4$ (0.75 mmol/L). Subsequently, 1 mL of H_2O_2 (0.12%) and 1 mL of the test samples were introduced. The resulting mixtures were incubated at 37 °C for 90 min, while the HRS activity was tracked by measuring the rise in absorbance at 536 nm. The control sample comprised 1,10-phenanthroline, $FeSO_4$ and H_2O_2, while the blank samples consisted of 1,10-phenanthroline, Fe_SO_4 and deionized water. The scavenging activity was represented as a percentage, calculated using the following formula:

$$\text{OH scavenging (\%)} = (As - Ac)/(Ab - Ac) \times 100$$

where As, Ac and Ab represent the absorbance of the test sample, control and blank, respectively.

2.9.4. Superoxide Scavenging Activity

A volume of 0.5 mL of the sample, prepared at various concentrations, was combined with 2.0 mL of 150 mM Tris-HCl buffer at pH 8 and 1.0 mL of 1,2,3-phentriol [42]. The solution was incubated at ambient temperature for 30 min after thorough mixing. For the control, only the Tris-HCl buffer and deionized water were used. The mixture's absorbance was recorded at 325 nm, and the scavenging effect on superoxide radicals was calculated as outlined below:

$$\text{Superoxide scavenging activity (\%)} = (A0 - A1)/A0 \times 100$$

where A0 and A1 represent the absorbance of solutions without sample and the absorbance of the solutions of different concentrations, respectively.

2.9.5. Antioxidant Power Determination

EPSc, EPSF1 and EPSF2 solutions were prepared in a sodium phosphate buffer (0.2 M, pH 6.6). In this procedure, 0.5 mL of each test sample was combined with 0.5 mL of a

1% (*w*/*v*) solution of $K_3Fe(CN)_6$ and incubated at 50 °C for 20 min. Once cooled, 0.5 mL of tri-chloroacetic acid (10%, *w*/*v*) was introduced and mixed thoroughly. The mixture was then centrifuged at 5000 rpm for 5 min to separate the components, and the upper liquid was collected. Following this, 1.0 mL of 0.1% (*w*/*v*) $FeCl_3$ was added to 1.0 mL of the collected liquid and allowed to incubate at the ambient temperature for 10 min. The absorbance was recorded at 700 nm, using a blank for comparison. A higher absorbance reading indicates a greater reducing power. Deionized water was used as the blank, while Vit. C served as the reference sample [43].

2.10. Cytotoxicity and Anticancer Activity of EPSc, EPSF1 and EPSF2

Determination of IC_{50} of EPSc, EPSF1 and EPSF2

A 96-well tissue culture plate was seeded with 100 µL of cell suspension at a concentration of 1×10^5 cells/mL, using either the normal lung WI-38 cells or the HepG2, PC3 and CaCo-2 cell lines. The plate was then incubated at 37 °C for 24 h to allow for the formation of a complete monolayer. The test was completed as performed in our previous study [44]. A total of 100 µL of different doses of EPSc, EPSF1 and EPSF2 (31.25, 62.5, 125, 250, 500 and 1000 µg/mL) in RPMI (maintenance medium) was evaluated in various wells, with three wells designated as controls, receiving only the maintenance medium. The plate was re-incubated for 24 h in a CO_2 incubator with specific conditions: 37 °C, 5% CO_2 and 90% relative humidity. A total of 20 µL MTT solution (5 mg/mL in PBS) (BIO BASIC CANADA INC., Markham, ON, Canada) was added to each well. Shaking at 150 rpm for 5 min was carried out to ensure complete integration of MTT into the media. The plate was then incubated at 37 °C with 5% CO_2 for 3 h to facilitate the MTT reaction. The media were eliminated and formazan, the MTT metabolic product, was resuspended in 200 µL of DMSO. Shaking at 150 rpm for 5 min was carried out to ensure complete integration of the formazan into the solvent. The optical density was measured at 560 nm and the background reading was subtracted at 620 nm using an optima spectrophotometer.

$$\text{Cell viability (\%)} = [1 - (\text{ODt}/\text{ODc})] \times 100.$$

where ODt and ODc represent the mean of the optical density for the samples and for untreated cells, respectively. The IC_{50} value was determined as the concentration that causes cell viability to be 50%.

2.11. In Vitro Anti-Inflammatory Assay (Hypotonicity-Induced Hemolysis)

The prevention of red blood cell (RBC) membrane hemolysis caused by heat or hypotonic conditions has been linked to anti-inflammatory effects. The anti-inflammatory activity of EPSc, EPSF1 and EPSF2 was evaluated according to a previous study [44]. A blood sample was withdrawn from rats with heparinized syringes centrifuged at 3000 rpm for 10 min. After collecting the RBC pellets, they were mixed with normal saline (0.9%) for resuspension. Following this step, the cells were reconstituted in a 10 mM sodium phosphate buffer, resulting in an isotonic suspension of erythrocytes. EPS samples with different concentrations (7.8–1000 µg/mL) were prepared in both hypotonic (in water) and isotonic solutions. Similarly, indomethacin (an anti-inflammatory drug) (0–1000 µg/mL) samples were prepared as a standard. The control tube contained distilled water only. Each tube received 0.1 mL of erythrocyte suspension, followed by slow mixing and incubation at 37 °C for 1 h. All tubes were subjected to centrifugation at 1300 rpm for 3 min. Once this was complete, the absorbance of the hemoglobin present in the supernatant was recorded at 540 nm with a spectrophotometer. The hemolysis percentage was determined by con-

sidering the hemolysis caused by distilled water as 100%. The percentage of hemolysis inhibition for the samples tested was calculated as follows:

$$\text{Inhibition of hemolysis (\%)} = 1 - (OD2 - OD1)/(OD3 - OD1)) \times 100$$

OD1, OD2 and OD3 represent the absorbance of the test sample in an isotonic solution and in a hypotonic solution, and the absorbance of the control tube, respectively. The IC_{50} value refers to the concentration of the sample needed to achieve a 50% inhibition of RBC hemolysis within the defined assay parameters.

2.12. Statistical Analysis

The dataset underwent statistical evaluation using a one-way analysis of variance (ANOVA) to determine significant differences among the mean values. Duncan's multiple range test was applied to assess pairwise variations. The analysis was performed using IBM SPSS software v24. A significance threshold of $p < 0.05$ was set to confirm whether the observed differences were statistically meaningful.

3. Results and Discussion

3.1. Isolation and Purification of L. plantarum

Fifteen unique bacterial colonies, which were round and cream-colored, were grown on the culture medium. These colonies, consisting of both cocci and rod-shaped bacteria, were initially classified as LAB isolates based on Gram-positive staining and catalase-negative tests. The colonies were obtained from ten separate human breast milk samples. The isolates were then purified and preserved in MRS broth enriched with 20% glycerol.

3.1.1. Screening of *L. plantarum* Isolates for EPS Production

Regarding the 15 LAB isolates, the EPSs produced were initially screened based on ropy formation [45]. Out of the 15 isolates, 4 isolates showed a ropy appearance, suggesting EPS production (B1, B5, B7 and B11) on MRS media supplemented with 5% sucrose. The yield of the EPSs produced by B1, B5, B7 and B11 was 2.3 ± 0.14, 2.6 ± 0.16, 5.2 ± 0.47 and 1.9 ± 0.07 g/L, respectively (Figure 1). The isolate *L. plantarum* B7 showed the highest yield (5.2 ± 0.47 g/L) in the MRS medium containing 5% sucrose when compared to the other three isolates; therefore, *L. plantarum* B7 was selected for further analysis. Previous reports indicate that the production of EPSs is largely influenced by the medium's composition, the conditions under which it is cultured and the pH levels [46]. The formation of EPSs is a significant characteristic that improves the stability of probiotics in the intestine and enhances their capacity to inhibit harmful enzymes within the intestinal microflora [47]. Therefore, in this study, the isolate *L. plantarum* B7 isolated from human breast milk was selected due to its ability to synthesize a large quantity of EPSs and its good probiotic properties.

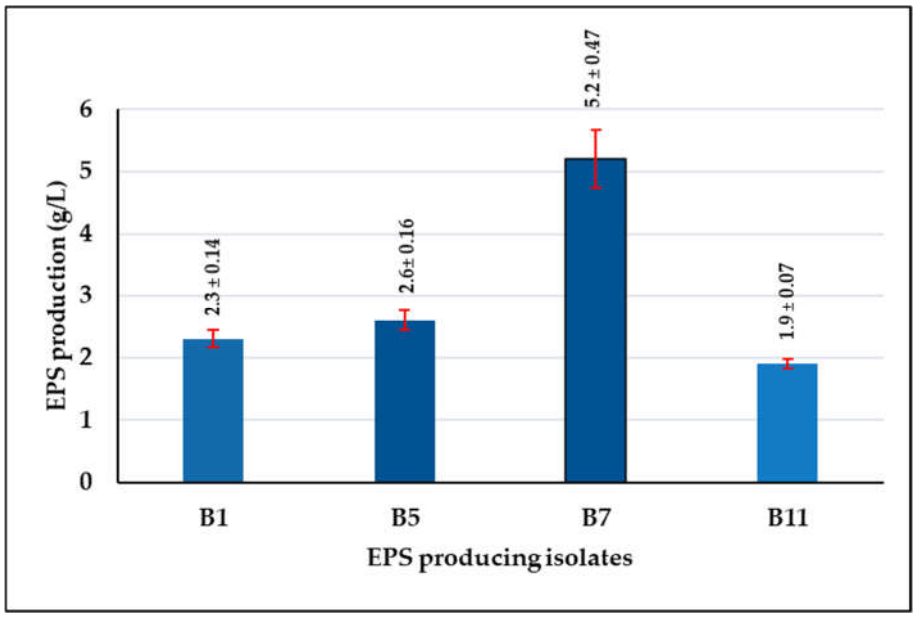


Figure 1. Exopolysaccharide production by different isolates of *L. plantarum* B1, B5, B7 and B11 grown on MRS broth media containing 5% sucrose for 72 h at 37 °C (mean ± SE, n = 3).

3.1.2. Morphological Characters and Molecular Identification of *L. plantarum* B7

Isolate *L. plantarum* B7 was chosen and classified using morphological, biochemical and molecular methods. *L. plantarum* B7 colonies were a Gram-positive, rod-shaped, non-spore-forming bacterium, as shown under the light microscope (Figure 2a,b). The morphological appearance of the selected isolate *L. plantarum* B7 was confirmed using 16S rRNA gene sequencing, which was aligned with NCBI. Sequence alignment analysis through BLAST indicated that the isolate is part of the *Lactiplantibacillus* species, showing a 99% sequence similarity with *L. plantarum* B7, indicating a strong evolutionary connection. This sequence has been submitted to GenBank, and the accession number PV203367 has been assigned. The phylogenetic relationship of isolates B7 and its nearest relatives, as found in the NCBI database, is illustrated in (Figure 2c).

3.1.3. Characteristics of *L. plantarum* B7

L. plantarum B7 exhibited optimal growth, as shown in Figure 3A, at temperatures of 20, 25 and 35 °C, with OD600 nm values of 1.82 ± 0.05, 1.96 ± 0.03 and 1.98 ± 0.1, respectively. A decline in growth rate was observed at 40 and 45 °C. At the same time, the results indicate that *L. plantarum* B7 tolerated various NaCl concentrations (5.0% and 6.0%), as shown in Figure 3B, and a broad pH range (4.0–8.0), with optimal growth observed at pH 5.0 (OD600 nm = 2.15). The glucose fermentation test indicated that *L. plantarum* B7 is a homofermentative bacterium, generating lactic acid as its only main by-product during the fermentation of glucose, and no gas production was detected. The quantity of EPSs produced varied among bacterial strains, indicating that EPS yield is affected by both the strains used and the nutritional and non-nutritional conditions [48,49].

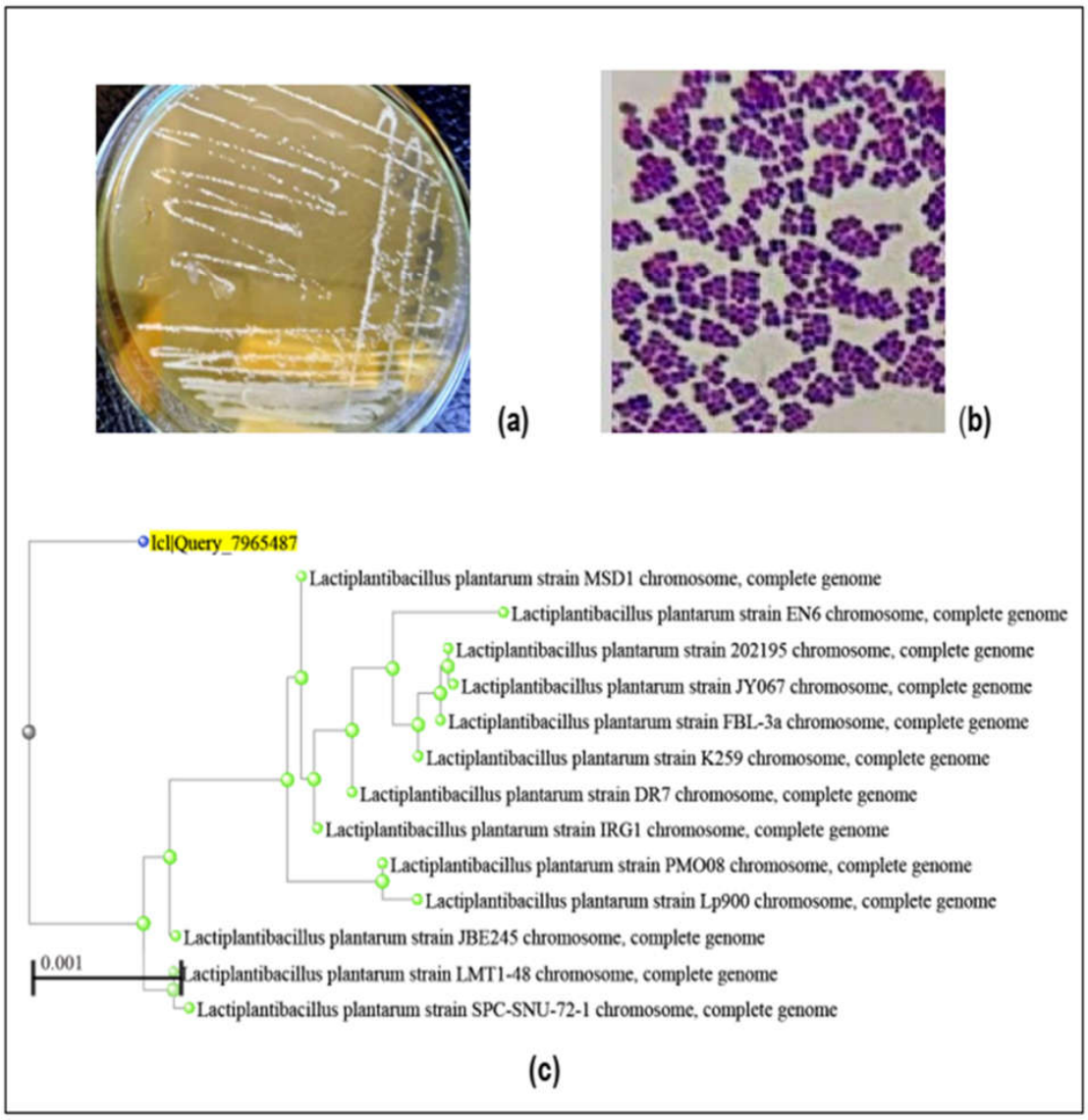
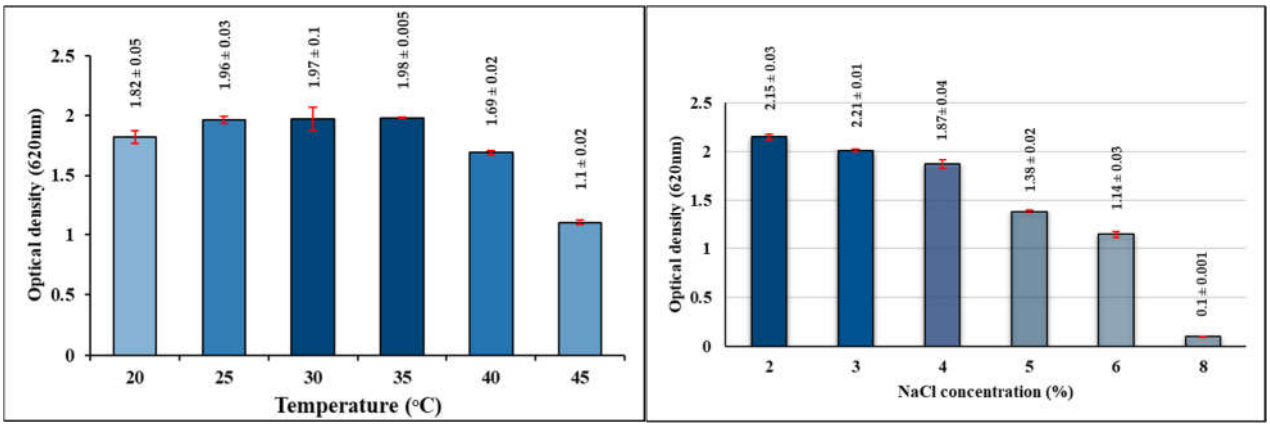


Figure 2. Culture characteristics (**a**), microscopic examination under light microscope (×1500) (**b**) and phylogenetic tree of gene sequences of *L. plantarum* B7 isolate with respect to closely related sequences retrieved from the NCBI GenBank databases (**c**).



(**A**) (**B**)

Figure 3. Effect of temperature (**A**) and NaCl concentration (%) (**B**) on the growth of *L. plantarum* B7 after 24 h incubation period (mean ± SE, n = 3).

3.2. Probiotic Characteristics of L. plantarum B7

3.2.1. Low pH and High Bile Salt Tolerance

The survival rates were 95.82 ± 1.37% after 3 h at pH 3.0 and 97.23 ± 1.78% after 4 h at 0.3% bile salt (Table 1). The tolerance of probiotic bacteria to varying pH levels and bile salt concentrations, prevalent in food processing, represents a significant advantage. This tolerance enables the bacteria to initiate metabolism and produce acids that inhibit undesirable bacteria growth. *L. plantarum* B7 demonstrates the potential capacity to withstand various growth-inhibiting conditions, including elevated NaCl concentrations and a wide pH range. These results are like those reported on isolates from yogurt [50] and camel milk [51], which were exposed to low pH, where a similar declining trend was noticed. One of the key factors for probiotics to offer therapeutic benefits is their capacity to endure and grow in acidic environments. Since gastric pH typically falls between 2.5 and 3.5, this acidity serves as a barrier, preventing bacteria from entering the intestinal tract [52].

Table 1. Low pH and high bile salt tolerance of *L. planturm* B7.

Time (h)	pH (3)	Bile Salt (0.3%)
	Survival rate (%)	
1	98.56 ± 2.65	99.25 ± 3.15
2	97.83 ± 1.76	98.11 ± 2.35
3	95.82 ± 1.37	97.66 ± 1.85
4	79.12 ± 1.71	97.23 ± 1.78
5	47.36 ± 1.63	81.35 ± 1.65

3.2.2. Cell Surface Properties

The assessment of bacterial cell surface characteristics is a laboratory test aimed at elucidating the biological properties of bacteria, which significantly differ among probiotic Lactobacillus strains. The findings indicate that it exhibited the highest hydrophobic properties, with values of 12.3 ± 0.97%, 23.4 ± 1.52% and 19.8 ± 1.25% for n-hexane, toluene and xylene, respectively. Compared with the previous studies, the hydrophobicity of several bacteria, including *L. plantarum*, *L. pentosus*, *L. casei* and *L. delbrueckii*, was measured at 5.5%, 6.5%, 6.2% and 3.7%, respectively, all of which are significantly lower than that of *L. plantarum* B7. The low hydrophobicity observed in certain isolates may account for the ability of these strains to produce EPSs. The existence of EPS fractions is expected to decrease the adhesion of probiotic strains [53,54] significantly. EPSs can bind directly to mucus, leading to competition with probiotics for adhesion. Differences in hydrophobicity can be linked to species-specific variations in the expression cell surface proteins' density [55]. Bacterial surface hydrophobicity can influence adherence to various surfaces; however, it does not facilitate strong adhesion to human gastrointestinal cells [56].

3.3. Antimicrobial Potential of L. plantarum B7

The study of the EPSs' antimicrobial activity confirmed that the crude polysaccharide affects the growth of bacteria and yeast, as shown in Figure 4. In general, various compounds, including metabolites, bacteriocins, organic acids, hydrogen peroxide and peptides generated by LAB during their growth, may contribute to the antimicrobial properties of LAB strains. The inhibition mechanism could be through surface hydrophobicity and autoaggregation, as suggested in a previous study [57], or by the destructive effect of EPSs on the bacterial cell envelope, especially the peptidoglycan layer [58]. Another reported mechanism was that EPSs may obstruct the receptors or channels present on the outer membrane of Gram-negative bacteria [59]. In various ways, the functional groups within the EPS structure can engage with the bacterial cell envelopes. The higher inhibitory effect

of EPSc in this study may be related to the prevention of fungal adhesion and reduction in hyphal induction, as suggested in a previous study [60].

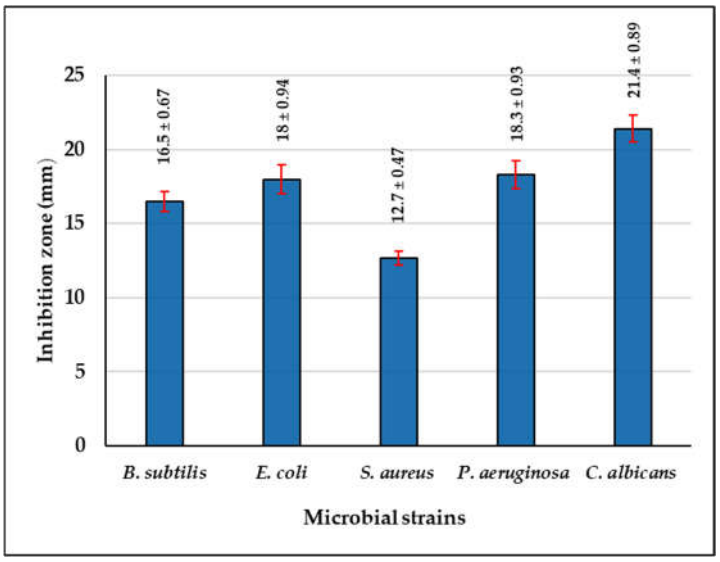


Figure 4. Antimicrobial potential of *L. plantarum B7* CFS *against B. subtilis*, *E. coli*, *S. aureus* NRRL B-767, *P. aeruginosa* and *C. albicans* ATCC after incubation period of 34–48 h at 37 °C (mean ± SE, n = 3).

3.4. Safety Aspects of L. plantarum B7

3.4.1. Hemolytic Properties of *L. plantarum* B7

The European Food Safety Authority (EFSA) strongly advises assessing hemolytic activity, particularly for isolated bacteria that are meant for inclusion in food products, regardless of their GRAS or QPS status (FAO/WHO 2006). The hemolytic activity of the *L. plantarum* B7 was estimated on blood agar plates. The results did show that no hemolytic activity was observed when cultured on blood agar. The absence of hemolytic activity is a crucial factor in determining the safety of probiotics when choosing specific strains, as it indicates that these strains are non-harmful. Furthermore, the absence of hemolysin helps to prevent any potential virulence in the bacterial strains (FAO/WHO 2006). Through biological screening of the tested strain *L. plantarum* B7, it was found that it has no γ-hemolytic potential and cannot secrete cytotoxins such as cytolysin or/and streptolysin, which present a significant risk to the immune system due to their ability to increase virulence in animal models [61].

3.4.2. Antibiotic Susceptibility of *L. plantarum* B7

One of the key factors in choosing probiotics, particularly for human consumption, is antibiotic susceptibility, as the ability to withstand certain antibiotics can be an advantageous characteristic [62]. This property may help them to survive in the intestine for a enough period of time, especially when used to restore the balance of intestinal bacteria and a healthy environment after the use of doses of antibiotics. They may function as a reservoir for antibiotic resistance genes within the intestines or food products as they can possess plasmids and transferable elements that harbor these resistance genes. Consequently, there is a possibility that these genes could be transmitted to other harmful microbes in the intestinal environment or throughout the food chain [63,64]. Therefore, before using any strains of LAB bacteria as antibiotics, antibiotic resistance tests should be performed to ensure their safety for human use. The disk diffusion method was applied to assess the sensitivity of *L. plantarum* B7 toward 11 antibiotics from a specific group. The results indicate sensitivity to imipenem, ciprofloxacin, fusidic acid, tetracycline, chloramphenicol, penicillin, ofloxacin and clindamycin, while resistance to streptomycin, aztreonam

and norfloxacin was observed (Table 2). Although Lactobacillus shows a significant level of resistance to norfloxacin, gentamicin, streptomycin and trimethoprim, this does not raise major safety concerns, as intrinsic resistance is considered to have a minimal risk of horizontal transfer [65]. In contrast, Lactobacilli are typically sensitive to antibiotics that disrupt protein synthesis, as well as to those that lead to cell wall synthesis inhibition, like β-lactamase inhibitors [66]. The phenomenon of antibiotic resistance can be associated with factors such as cell wall composition and mechanisms of efflux [67,68].

Table 2. The antibiotic susceptibility of *L. planturm* B7.

Antibiotic	Concentration (μg)	Inhibition Zone (mm)	Sensitivity
Streptomycin	10	0.0	R
Fusidic acid	10	10.8 ± 0.65	L
Tetracycline	30	8.3 ± 0.37	L
Chloramphenicol	30	18.6 ± 0.74	H
Penicillin	10	23.5 ± 1.33	H
Aztreonam	30	0.0	R
Imipenem	10	25.7 ± 1.65	H
Ciprofloxacin	5	9.7 ± 0.35	L
Norfloxacin	10	0.0	R
Ofloxacin	5	8.4 ± 0.41	L
Clindamycin	2	6.7 ± 0.47	L

R: resistant, L; low; H; high.

3.5. EPS Production and Isolation

Several previous studies have indicated that there is genetic evidence confirming the production of EPSs by the LAB strain, as its genomic analysis indicated the genes' existence specific to EPS synthesis in a group. The total amounts of carbohydrates and proteins in the isolated EPS were measured, showing a notably high carbohydrate-to-protein ratio (6.32 mg/mL of carbohydrate and 0.24 mg/mL of protein). This result agrees with published work that indicated low protein levels obtained from different lactic acid bacteria derived from cow's milk while exhibiting significantly high carbohydrate levels [69]. This observation is particularly significant, as products from microbes with a lower protein content are thought to be more suitable for medical applications because of higher immunogenicity generally linked to proteins [70]. Additionally, the protein concentration in EPSs can affect several of their biophysical features [71].

3.6. Purification and Characterization of EPSc

EPSc was isolated from *L. plantarum* B7 by ethanol precipitation from CFS, then deproteinized by TCA precipitation and finally dried by lyophilization. Exopolysaccharide was purified using anion exchange chromatography. The carbohydrate levels in each 5 mL aliquot of eluent were assessed. A subsequent elution was only performed when the final aliquot of the previous one contained no carbohydrates. Deionized water was used to elute the nearly neutral fraction that does not bind to the tertiary amine of the DEAE-cellulose, whereas the acidic EPSs that were attached were released using a NaCl solution. The three fractions, eluted with 0.02, 0.15 and 0.3 M NaCl, were designated as EPSF0, EPSF1 and EPSF2, respectively (Figure 5A). EPSF0 was a small quantity of EPSs, and was eluted with 0.02 M NaCl, while EPSF1 and EPSF2, which were eluted with 0.15 and 0.3 M NaCl solutions, respectively, are acidic exopolysaccharides. Each of the two fractions (EPSF1 and EPSF2) was further purified by Sephadex G-100 gel filtration chromatography using 0.1 M NaCl as the eluting agent. The emulsified liquid (5 mL) was collected, yielding pure EPSF1 and EPSF2 (Figure 5B,C). The recovery rates of the two fractions were 35.6% and 42.5%, respectively, based on the original amount of EPSc. There may be an approximately 22%

loss of EPSc due to adsorption to the column. Each of these elution curves had a single symmetrical peak, indicating that all the purified products were homogeneous EPSs. The total sugar content of EPSF1 and EPSF2 was 89.53% and 95.83%, while the protein content was 1.85% and 3.93%, respectively, indicating that EPSF2 had a higher purity and higher protein content than EPSF1.

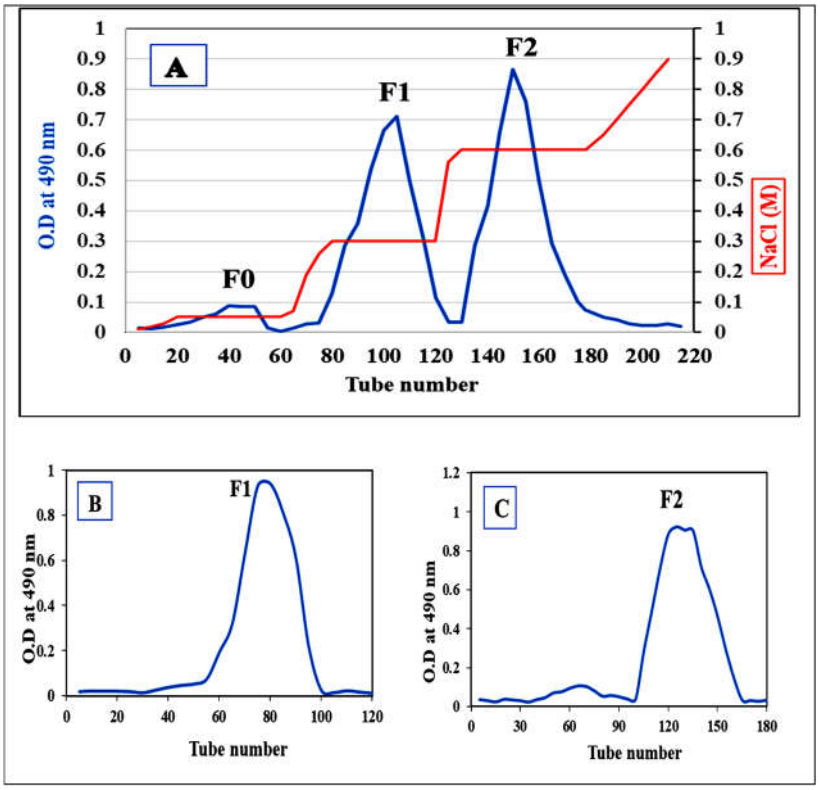


Figure 5. Elution profile of EPS fractionations on DEAE-cellulose column. F0, F1 and F2 represent the fractions that were eluted with 0.02, 0.15 and 0.3 M NaCl, respectively (**A**), and fractions purification on Sephadex G-100 column using 0.1 M NaCl as eluting agent (**B**,**C**).

3.7. Monosaccharide Composition and MW Determination of EPSF1 and EPSF2

EPSF1 and EPSF2 produced by *L. plantarum* B7 were completely hydrolyzed and analyzed by HPLC; three monosaccharides, mannuronic acid, mannose and glucose, were identified. The results indicate that EPSF1 and EPSF2 were rich in mannuronic acid and mannose. The molar ratios of mannuronic acid/mannose/glucose in EPSF1 and EPSF2 were 2.76:2.15:1.00 and 3.92:2.65:1.00, respectively. The results indicate that mannuronic acid and mannose constituted the predominant monosaccharide composition in EPSF1 and EPSF2. The monosaccharide composition of the two fractions differed in the proportions of mannuronic acid and mannose: EPSF2 is more acidic than EPSF1 (Figure 6a,b). Previous studies indicated that LAB-EPSs contain a high percentage of carbohydrates, with the most common monosaccharides. Additionally, certain monosaccharide derivatives, including N-acetyl galactosamine and N-acetyl glucosamine, may be present [29,72,73]. The EPS derived from *L. plantarum* WLPL04, isolated from human breast milk, consists of xylose, glucose and galactose in a ratio of 3.4:1.8:1.0, respectively [74]. It was previously reported that the strains used, culture conditions and media composition can influence the EPSs [75].

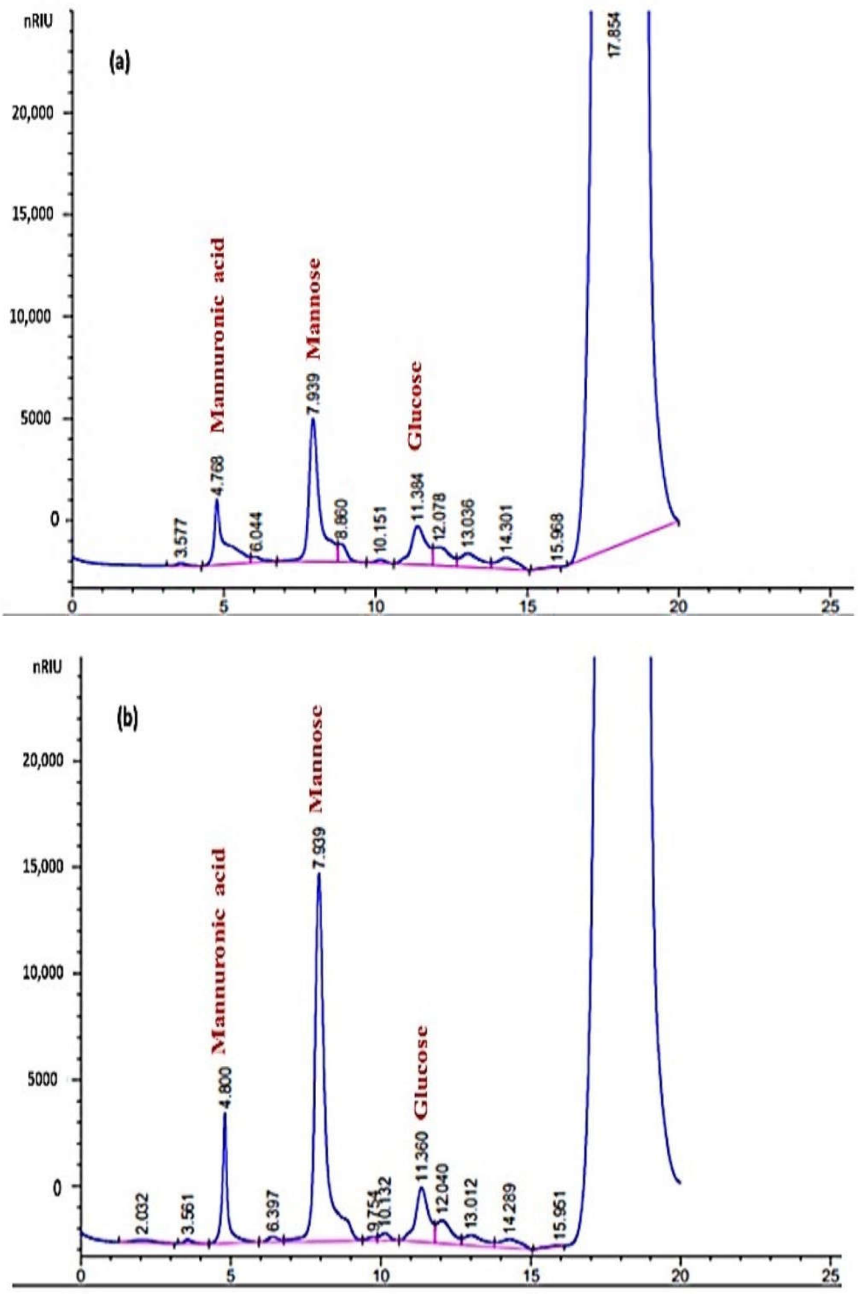


Figure 6. HPLC chromatogram of monosaccharide composition of EPSF1 (**a**) and EPSF2 (**b**) hydrolysate.

The MW distributions of the EPSF1 and EPSF2 were estimated using the GPC technique. EPSF1 had a single major fraction with an Mw of 4.36×10^4, Mn of 3.51×10^4 g/mol and PI of 1.24. Also, EPSF2 had one major fraction with an Mw of 5.27×10^5 Da and Mn 3.18×10^5 Da with a PI of 1.65 (Table 3). The MW results of EPSF1 and EPSF2 were similar to that of EPS from *L. plantarum* EP56 (4.4×10^4 Da) [76], while they were higher than that from *L. plantarum* BC-25 (1.83×10^4 and 1.33×10^4 Da) [77] and (EPS-ETOH) (3.35×10^4 Da) of *L. plantarum* C7 [78] and lower than the EPS of *L. plantarum* YW11 (1.1×10^5 Da) [6] and EPS of *L. plantarum* C88 (1.15×10^6 Da) [79]. The polydispersity index (PI) indicates the degree of heterogeneity in the chain lengths of EPSF1 and EPSF2, with values of 1.24 and 1.65, respectively, demonstrating a diverse population regarding polysaccharide chain sizes. Comparable findings of PI were documented for AEPS derived from *L. acidophilus* and EPS from *L. plantarum* AR307, which were 1.54 and 1.57, respectively [80,81], revealing a narrow

MW distribution of the EPSs. It could be interesting to explore distinct EPSs with different MW levels for use in various applications.

Table 3. Monosaccharide composition and MW of EPSF1 and EPSF2.

EPS	Monosaccharide Composition	Molar Ratio	MW (Da)	Mn (Da)	PI (Mw/Mn)
EPSF1	manuronic acid/mannose/glucose	2.76:2.15:1.00	4.36×10^4	3.51×10^4	1.24
EPSF2	manuronic acid/mannose/glucose	3.92:2.65:1.00	5.27×10^5	3.18×10^5	1.65

3.8. FT-IR and 1HNMR Spectroscopy of EPSF1 and EPSF2

The FT-IR spectrum of the EPSF1 and EPSF2 exhibited numerous peaks within the 400–4000 cm^{-1} range, confirming its polymeric structure (Figure 7A,B). The infrared spectra revealed the presence of typical EPS functional groups, such as O—H, the vibration of C—H_2, C—H, C=O, COO^- stretching and glycoside linkages. The spectra of EPSF1 and EPSF2 showed some differences in some bands' intensities, but the main characteristic bands showed broad stretching peaks in the region of 3500–3200 cm^{-1} and a small band at around 2927 cm^{-1} that can be assigned to O—H and C—H stretching vibrations of the sugar ring, respectively [82]. The relatively strong absorption peak at 1659 cm^{-1} in EPSF2 and 1648 cm^{-1} in EPSF1 reflected the absorption of the carbonyl (C=O) and carbonyl (COO^-) groups in each fraction, which may prove the presence of mannuronic acid [83]. The moderate absorption peaks at 1250 cm^{-1} in EPSF2 and the weak peak at 1028 cm^{-1} in EPSF1 indicated a pyranose form of sugars [84]. The comparison of the FT-IR spectra of EPSF1 and EPSF2 with those of other PSs analyzed in previous studies confirmed that EPSF1 and EPSF2 are acidic exopolysaccharides containing mannuronic acid, consistent with the HPLC results [85,86].

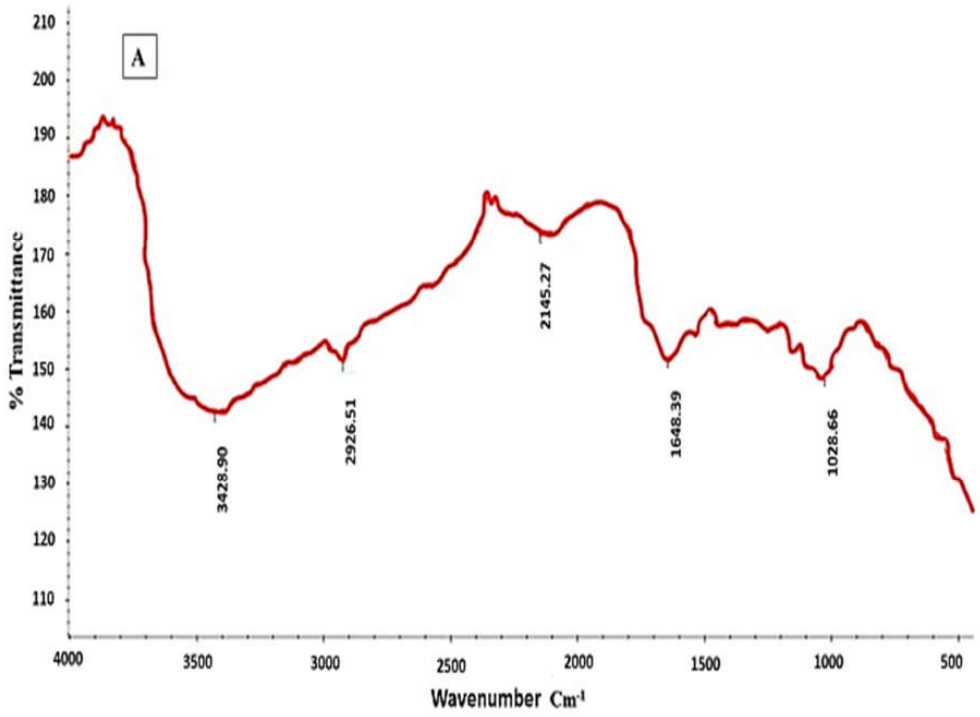


Figure 7. *Cont.*

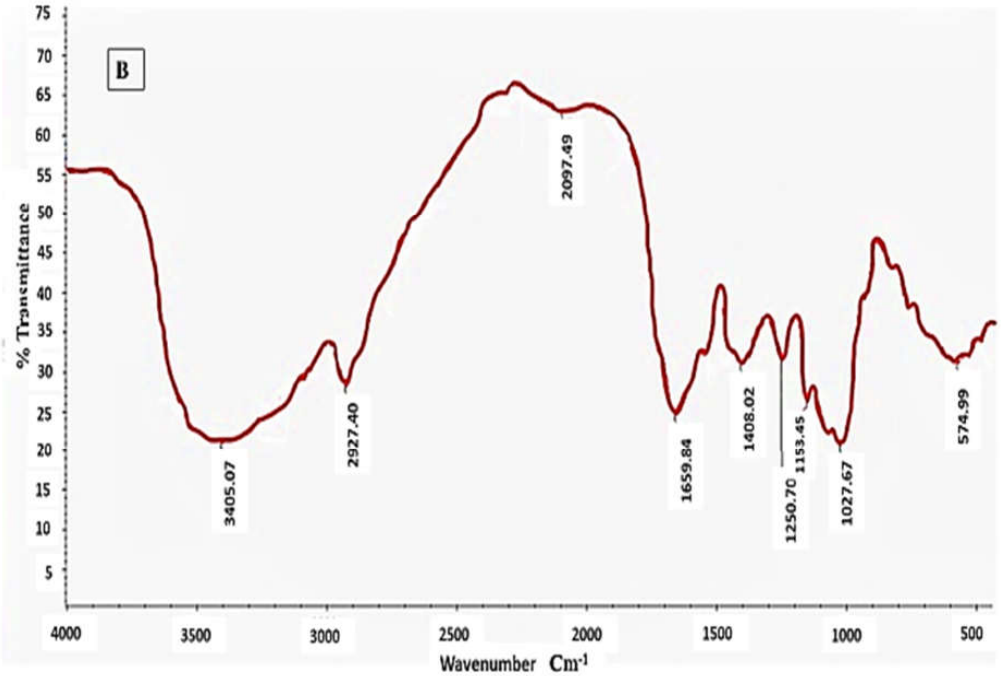
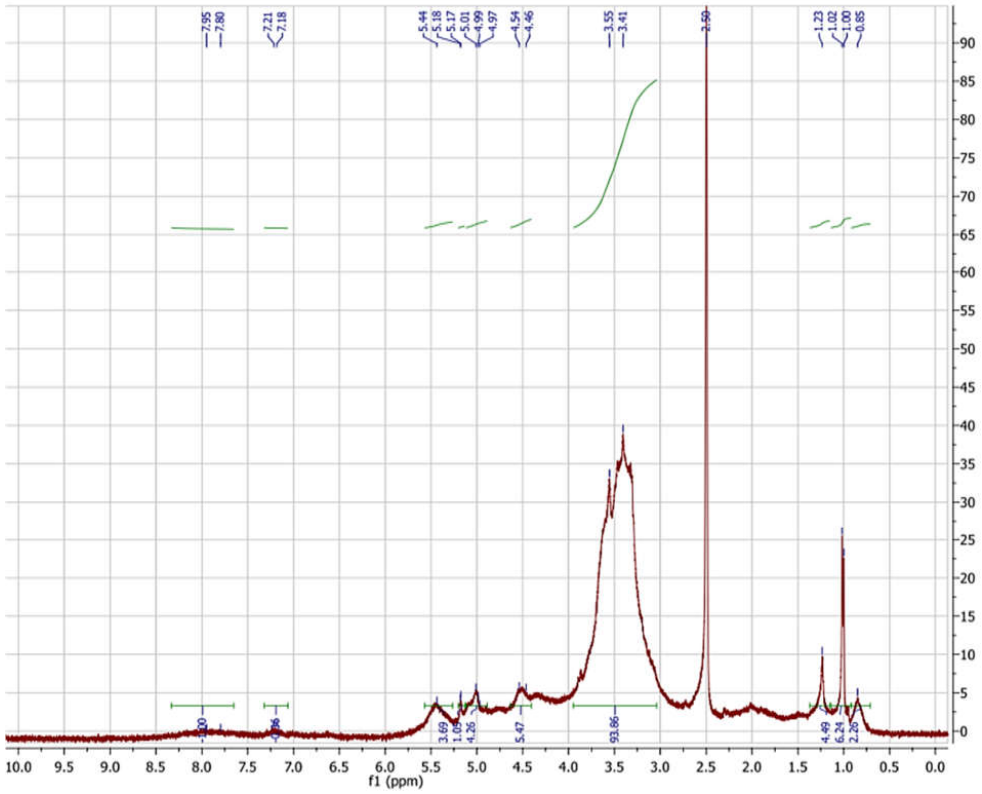
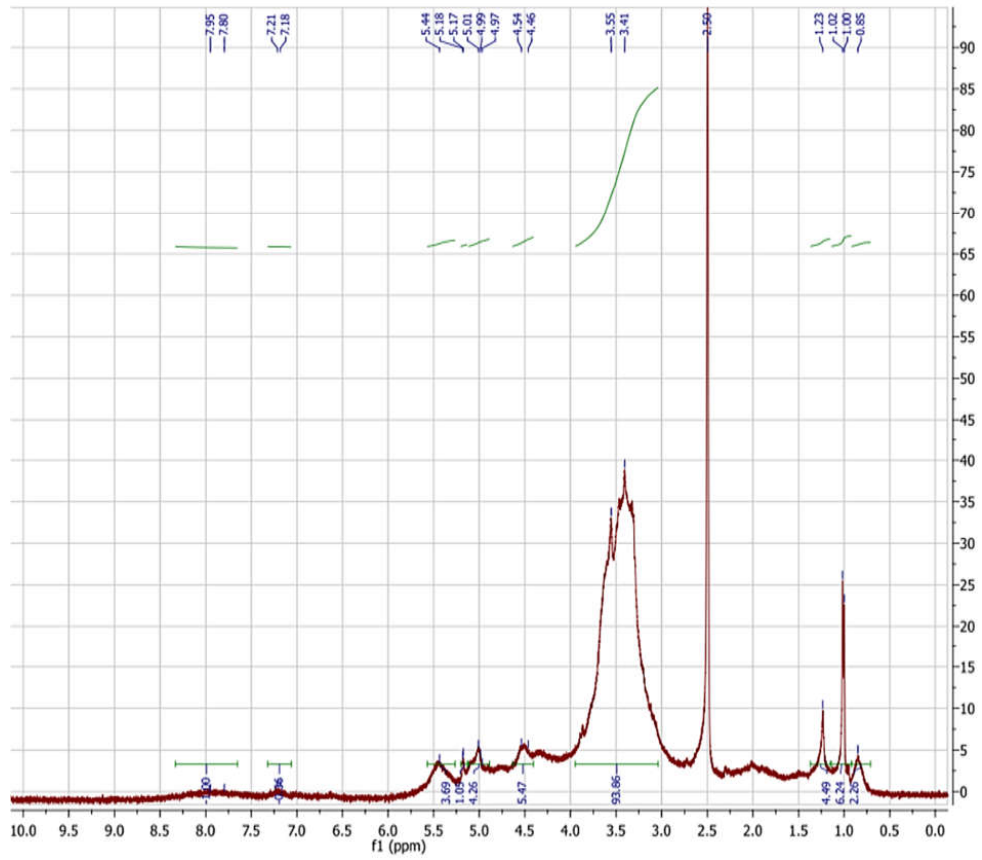


Figure 7. FTIR spectrum of EPSF1 (**A**) and EPSF2 (**B**).

The 1H NMR spectra for EPSF1 and EPSF2 demonstrate a complex EPS structure, showing pronounced signals in the upper ring proton region at δ = 3.12–4.35 ppm and a variety of signals in the lower anomeric region spanning δ = 4.40–5.50 ppm. The predominant signal range for the hydrogen spectrum of both EPSF1 and EPSF2 fell within δ 3.00–5.50 ppm. In the anomeric region, which ranges from δ 4.30 to 5.40 ppm, numerous coupling peaks were present, suggesting the presence of sugar residues associated with the anomeric hydrogen's chemical shift at δ 4.25, 4.75, 5.17, 5.32 and 5.44 ppm (Figure 8a, b). Referring to the previously reported results, the primary signals for non-anomeric hydrogen were observed in the range of δ 3.15–4.27 ppm. Although overlapping signals made precise assignments challenging, a signal at δ = 1.98 ppm was identified as corresponding to the C—H_3 group protons in the EPS, suggesting that some sugars exist in an O-acetylated form [87]. The presence of several signals within the chemical shift range of δ = 5.0–5.50 ppm, in contrast to those at δ = 4.40–4.80 ppm, implies that the majority of monosaccharides are linked via α-glycosidic bonds rather than β-glycosidic bonds [87,88]. A similar structural pattern was noted in both dextrins derived from *W. confusa* [89,90]. In this research, the prominent peak at δ 4.93 ppm suggested that the isolated EPSF1 and EPSF2 were pyranoses composed of α-(1→6)-glycosidic linkages, while the less intense peak at δ 5.17–5.44 ppm suggested the α-(1→3)-glycosidic bonds' existence. Additionally, EPSs derived from *L. plantarum* W1 exhibited six unusual proton signals at δ 5.76, 5.67, 5.59, 5.59, 5.56, 5.55 and 5.37 ppm within the anomalous range of δ 4.5–5.5 ppm, indicating that these EPSs were made up of various monosaccharides [91], which is different from the results of this study.



(**a**)



(**b**)

Figure 8. ^{1}H NMR spectra of EPSF1 (**a**) and EPSF2 (**b**).

3.9. Antioxidant Activity of EPSc, EPSF1 and EPSF2

The EPSs' antioxidant assessment demonstrated their capacity to shield cells from oxidative harm induced by free radicals, metal ions, oxidizing agents and lipid peroxidation, which cause several diseases, such as inflammation, aging and cancer [92–94]. In recent years, the free radical scavenging activity of bacterial EPSs has garnered increasing interest due to its safety profile. The free radical scavenging activities of EPSc and purified EPSF1 and EPSF2 were tested using different methods. The inhibitions of the radical DPPH at a concentration of 800 µg/mL were 62.9 ± 0.02%, 81.3 ± 0.01% and 88.0 ± 0.12, respectively, while, for ABTS at the same concentration, they were 65.7 ± 0.03%, 79.8 ± 0.05% and 84.0 ± 0.13%, respectively (Figure 9a,b). The EC_{50} value of EPSc, EPSF1 and EPSF2 and Vit. C was calculated to be 600 ± 0.01, 430 ± 0.2, 385 ± 0.05 and 65 ± 0.1 µg/mL, respectively, for DPPH, while it was 591 ± 0.03, 395 ± 0.013, 282 ± 0.02 and <50 ± 0.05 µg/mL, respectively, for ABTS. These results confirm the promising antioxidant properties of the two fractions. Higher concentrations of EPSc from various other LAB isolates have reported similar levels of antioxidant effectiveness [95,96].

Superoxide free radicals ($O_2^{\bullet-}$) are the most damaging reactive oxygen species to the living cell as they act as precursors to other oxygen species, thus reacting with large biomolecules inside the living cell, causing cell damage by stimulating lipid peroxidation and oxidative damage; therefore, $O_2^{\bullet-}$ must be removed [97]. Accordingly, we tested the ability of EPSc, EPSF1 and EPSF2 compared with Vit. C to scavenge $O_2^{\bullet-}$, and the results show the maximum removal (65.9 ± 0.012, 71.2 ± 0.05, 78 ± 0.13. and 100%) at a concentration of 1000 µg/mL (Figure 9c). The removal of $O_2^{\bullet-}$ was found to be dependent on the concentration of the tested substances. In contrast, the EPSs from *L. plantarum* YW32 had a removal capacity of 66.5% at a concentration of 5 mg/mL [94]. Also, the removal capacity of EPS-1, EPS-2 and EPS-3 from *L. helveticus* MB2-1 was 35.73, 51.60 and 49.26%, respectively, at a concentration of 4 mg/mL [72]. In this study, EPSc, EPSF1 and EPSF2 showed higher removal activity compared to these two EPSs from *L. plantarum*, indicating that EPSc, EPSF1 and EPSF2 could be utilized as a free radical scavenger ($O_2^{\bullet-}$). The EC_{50} value of EPSc, EPSF1 and EPSF2 and Vit. C was calculated to be 610 ± 0.02, 400 ± 0.016, 230 ± 0.01 and <50 ± 0.02 µg/mL, respectively.

Hydroxyl radicals ($OH^{\bullet}$) react readily and rapidly with biomolecules such as DNA, lipids and proteins, thus causing severe oxidative damage to biomolecules inside living cells, leading to cancer and aging [72]. Different bacterial EPSs have shown a high capacity to scavenge these $OH^{\bullet}$. The capacity of EPSc, EPSF1 and EPSF2 to eliminate OH● enhanced as the concentration increased, demonstrating a greater effectiveness compared to Vit. C (Figure 9d). The results show a notable rise ($p < 0.05$) in the scavenging% as the concentrations of EPSc, EPSF1 and EPSF2 were elevated from 50 to 1000 µg/mL. The highest free radical scavenging percentage was noticed for EPSc, EPSF1 and EPSF2 (65.9 ± 0.02, 71.2 ± 0.01 and 78.5 ± 0.04%, respectively) and Vit. C (100%) at 1000 µg/mL. A similar type of $OH^{\bullet}$ scavenging pattern was observed in EPSs from *L. helveticus* MB2-1, *L. plantarum* C88 and *L. plantarum* YW32, with scavenging percentages of 62.93, 85.21 and 77.5%, respectively [72,94]. The EC_{50} value of EPSc, EPSF1 and EPSF2 and Vit. C was found to be 860 ± 0.014, 715 ± 0.02, 620 ± 0.02 and 82 ± 0.01 µg/mL, respectively. It is proposed that the optimal $OH^{\bullet}$ removal capacity of EPSs is a result of the active transfer of hydrogen atoms from the hydroxyl groups present in the EPSs [72].

The reducing power of EPSs is a significant measure of their antioxidant potential. The reducing capacity of EPSc, EPSF1 and EPSF2 compared with Vit. C increased with an increasing concentration (Figure 9e). The maximum reducing power of EPSc, EPSF1, EPSF2 and Vit. C at 1000 µg/mL was 0.69, 0.61, 0.58 and 1.82, respectively. The reducing power of EPSc, EPSF1 and EPSF2 was observed to be higher than that of EPSs isolated from *B.*

amyloliquefaciens C-1 and *L. plantarum* [98,99]. It was observed that the antioxidant activity and total antioxidant values were comparable to findings from earlier studies on EPSs derived from various species of *Enterococcus* and *Streptococcus* [95,100–102]. The results indicate that all antioxidant properties exhibited a dose-dependent increase.

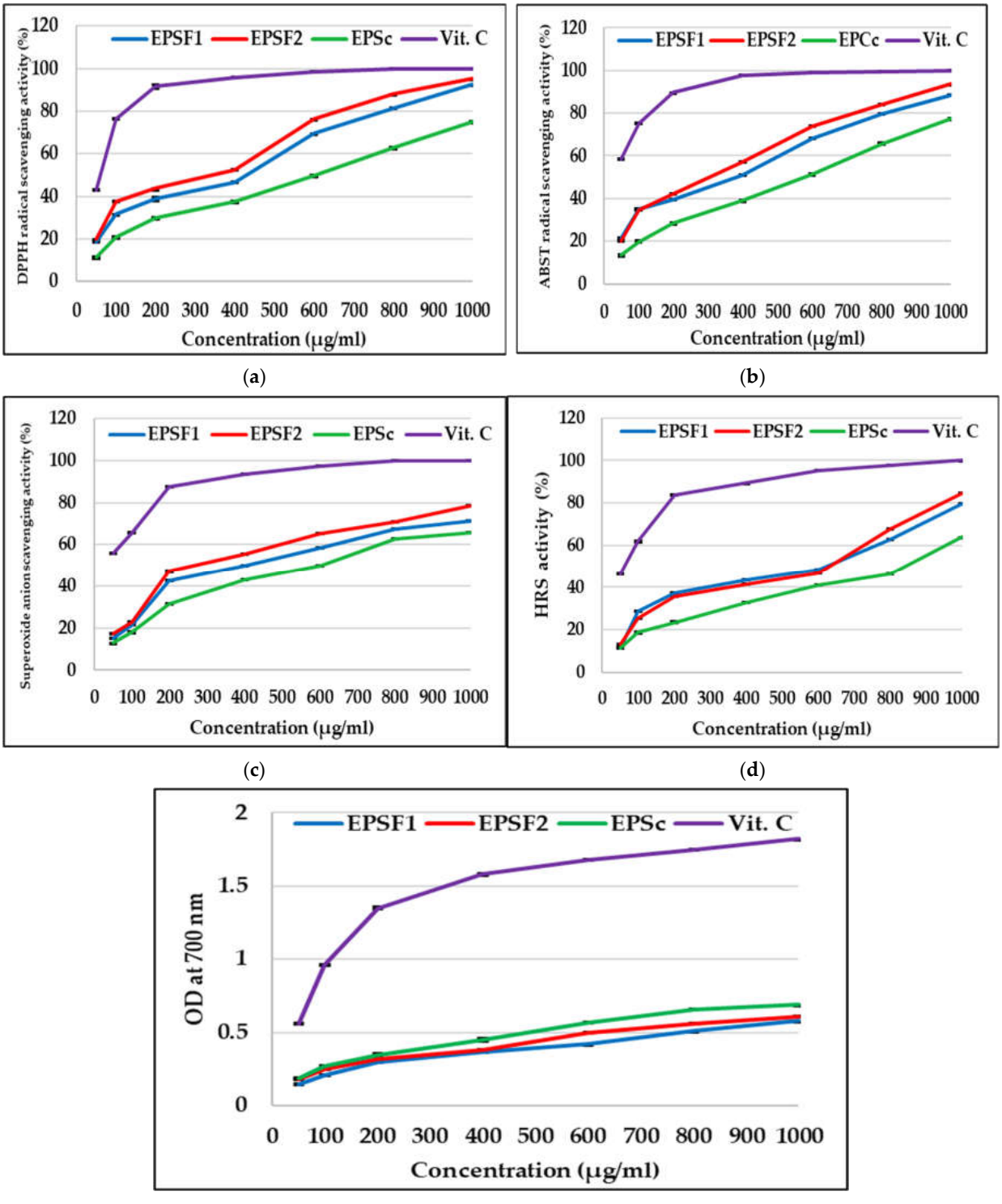


Figure 9. Scavenging activity of EPSc, EPSF1 and EPSF2 compared to Vit. C on DPPH (**a**), ABTS (**b**), superoxide radical (**c**), hydroxy radical (**d**) and reducing power (**e**). Results are presented as means ± SE for triplicate. Black bars indicate standard errors.

3.10. Cytotoxicity and Antitumor Activity

Several potential antitumor mechanisms have been identified for LAB-EPSs. Firstly, they might suppress the growth of cancer cells, thereby reducing tumor growth and progression and inducing cell cycle arrest [10,103,104]. LAB-EPSs have the potential to promote apoptosis by activating enzymes like caspases or by initiating signaling pathways associated with apoptosis [105,106]. Thirdly, they might enhance immune regulatory abilities alongside their antioxidant activity [107,108]. In most assays, EPS-MLB10 demonstrated greater antioxidant activity compared to EPS-LB13. This difference could be attributed to variations in certain physicochemical properties, including MW and the presence of specific functional groups. Overall, the robust antioxidant properties of EPSs highlight their promising applications in the food and health sectors [92,109].

HepG2, PC3 and Caco-2 were chosen to detect the EPSc, EPSF1 and EPSF2; WI-38 was utilized to determine the cytotoxicity of samples. Cells were incubated with varying concentrations of EPSc, EPSF1 and EPSF2 for 72 h. Subsequently, cell viability was assessed using an MTT assay. The IC_{50} values of these cell lines for EPSc, EPSF1 and EPSF2 were 837.4 ± 0.03, 860.7 ± 0.01 and 895.3 ± 0.02 μg/mL for the WI-38 cell line; 122.13 ± 0.01, 72.5 ± 0.012 and 81.6 ± 0.1 μg/mL for the Caco-2 cell line; 109.6 ± 0.03, 65.7 ± 0.01 and 70.3 ± 0.04 μg/mL for PC3; and 112.5 ± 0.01, 60.3 ± 0.1 and 62.0 ± 0.03 μg/mL for HepG2, respectively (Figure 10a–d). The cell morphology of cancer cells after treatment confirmed the cytotoxicity results, as shown in Figure 11. All samples do not adversely affect WI-38 cell viability and morphology up to 500 μg/mL. Morphological and quantitative assessments indicate that EPSc, EPSF1 and EPSF2 are well tolerated and non-toxic on WI-38. However, EPSF1 demonstrated the highest antitumor activity, while EPSF2 exhibited significantly greater antitumor activity than ESPs. The variations in antitumor activity among ESPs, EPSF1 and EPSF2 might be associated with the differences in their composition and MW. EPSF2 contained 51.78% uronic acid and EPSF1 contained 46.70% uronic acid, while the MW of EPSF1 and EPSF2 was 4.36×10^4 and 5.27×10^5, respectively. Previous studies have shown that the chemical properties, sugar composition and MW of EPSs play crucial roles in their antitumor activity [110,111]. For instance, EPSR5 isolated from marine *Kocuria* sp. demonstrated a suppressive effect on cancer cell growth [112], with the highest IC_{50} value of 1691.00 μg/mL against the MCF-7 cell line and the lowest value of 453.46 μg/mL against HepG-2. Additionally, EPSs isolated from *B. albus* DM-15 exhibited an IC_{50} value of 20.0 μg/mL against A549 cells, with cell staining revealing necrotic and apoptotic characteristics [113]. Moreover, the strain of *B. subtilis* produced EPSR4, which showed antiproliferative impacts on different cancer cell lines [14]. The antitumor properties of LEP-2b, isolated from *Lachnum* YM405, were enhanced after modification with PO_4^- and SO_3^-, leading to significant improvements in cytotoxic activity against liver, colon and lung cells [114]. Furthermore, EPS-A and EPS-B from *P. aeruginosa* were found to be cytotoxic against HT-29 cells, with IC_{50} values of 44.8 and 12.7 mg/mL, respectively, highlighting their potential as natural and effective anticancer agents [115]. Interestingly, there can be considerable variation in the ability of EPSs to hinder cancer cell proliferation, even among different types within the same species [116]. Additionally, EPSs were discovered to affect or suppress the activity of genes associated with carcinogenesis, including p53 [103]. The cytotoxic activity of EPSs has been attributed to MW properties. Low-MW EPSs might penetrate more through cell membranes, leading to suppressive functions like cell cycle arrest [117]. The antitumor effects of EPSs may involve modulating some carcinogenic genes like BCL2, p53 and β-catenin [103]. Furthermore, the anti-proliferation properties of EPSs can be attributed to the existence of unique structures, including COO^- and SO_3^{2-} [118].

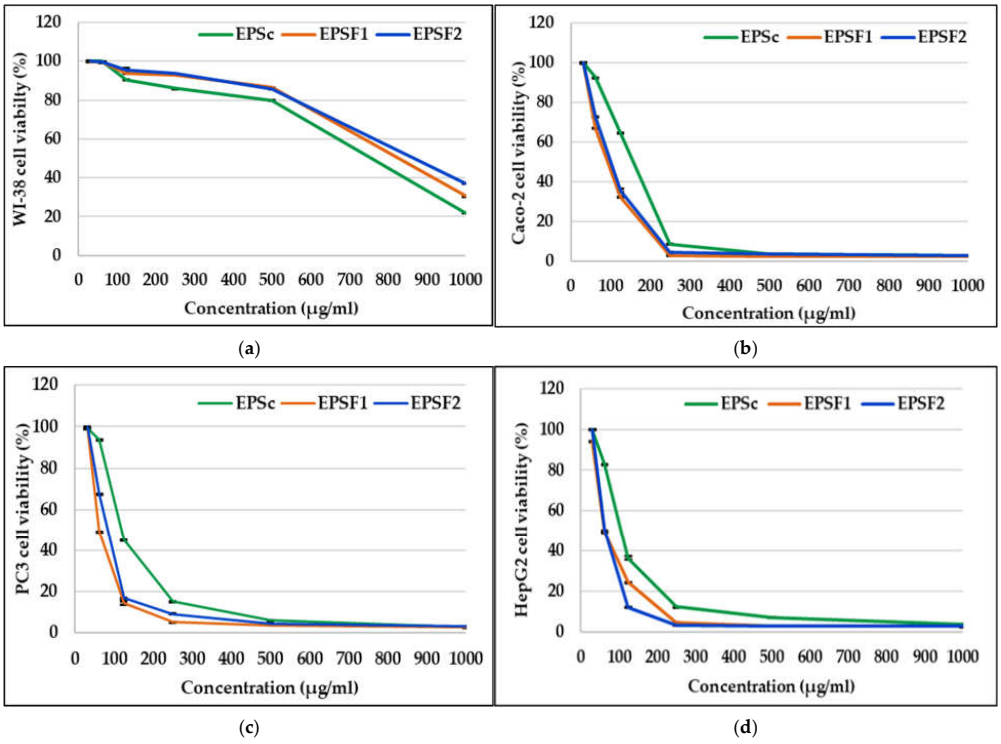


Figure 10. Cytotoxicity effect of EPSc, EPSF1 and EPSF2 at different concentrations on the viability of normal lung cell line WI-38 (**a**), Caco-2 (**b**) and PC3 (**c**) and human cancer cell line HepG2 (**d**). The values are the means ± SE for triplicate.

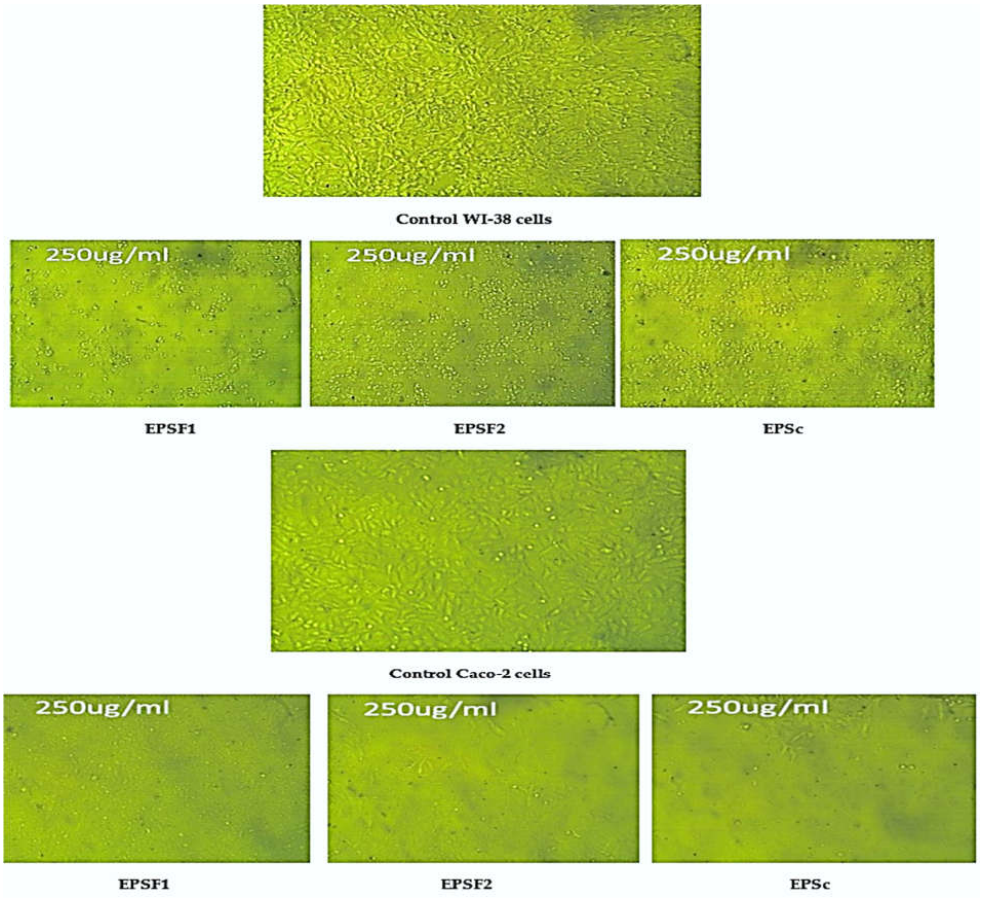


Figure 11. *Cont.*

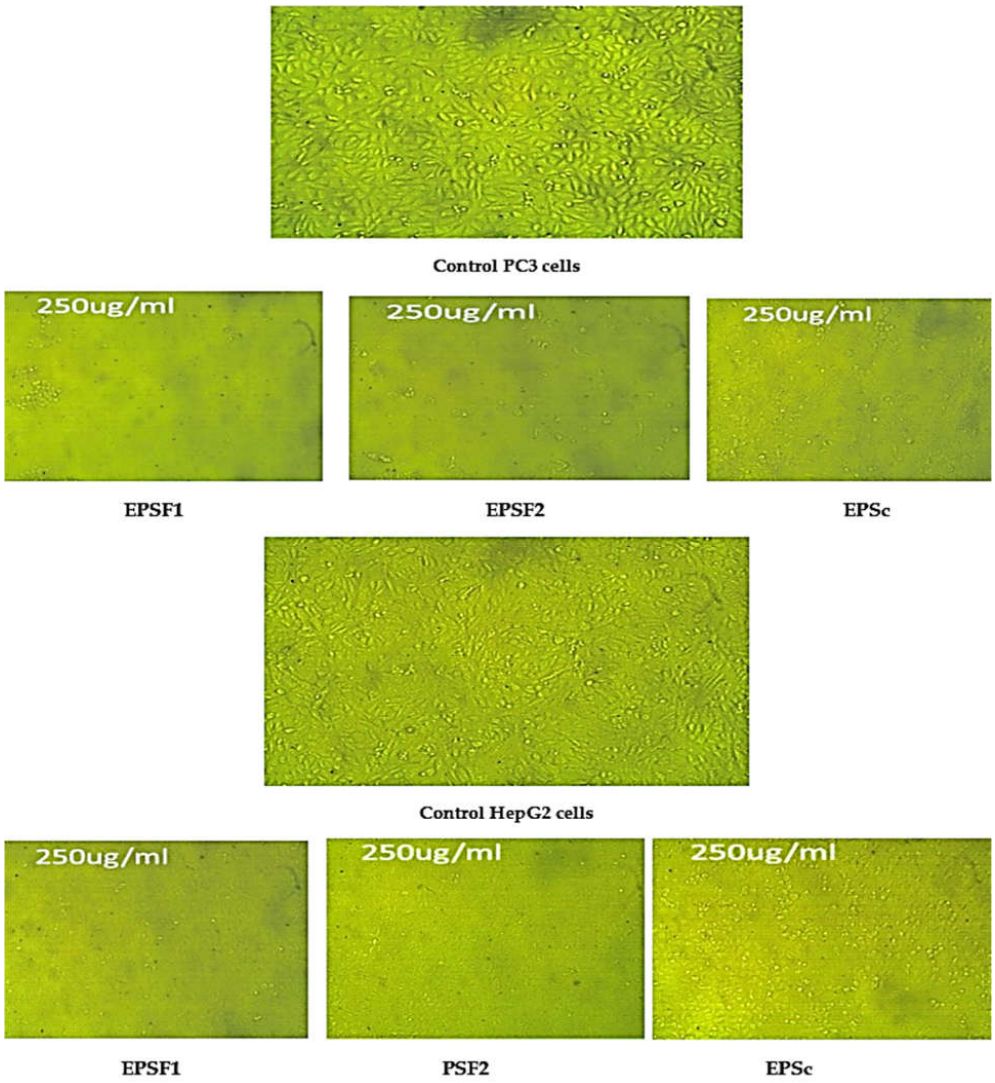


Figure 11. Morphology of control cells of WI-38, Caco-2, PC3 and HepG2 and the corresponding cells after treatment with SPSF1, ESPF2 and EPSc at the same concentration of 250 μg/mL, which represents the condition at which cell viability highly drops in the different cancer cell lines.

3.11. In Vitro Anti-Inflammatory Activity of EPSc, EPSF1 and EPSF2 (Hemolysis Inhibition)

Stabilization of RBC membranes is a well-established test to investigate the anti-inflammatory effect of different biomaterials. It is known that inflammation is caused by the release of various enzymes from degradative lysosomal vesicles. Therefore, stabilization of RBC membranes may prevent the leakage of these enzymes and thus prevent inflammation [119]. The hemolysis test is undoubtedly an especially important test for evaluating anti-inflammatory biomaterials, especially for those that will be in contact with blood. As shown in Figure 12, the hemolysis inhibition activity of EPSc, EPSF1 and EPSF2 at 500 μg/mL was 79.3 ± 0.05, 93.5 ± 0.05 and 87.8 ± 0.03, respectively. From these results, EPSc, EPSF1 and EPSF2 are promising anti-inflammatory materials. Inflammation is a protective response by the body aimed at removing harmful stimuli and initiating the healing process [120–122]. During inflammation, lysis of the lysosomal membrane occurs, releasing its constituent enzymes that produce a variety of disorders. When RBCs are subjected to damaging factors like a hypotonic environment or elevated temperatures, their membranes can become compromised, leading to cell lysis and hemoglobin oxidation [123]. Since RBC membranes share similarities with lysosomal membrane components, researchers have examined the inhibition of RBC membrane lysis induced by hypotonic conditions

and heat as a potential indicator of the anti-inflammatory effects of EPSs. In hypotonic environments, excessive water intake by RBCs leads to membrane rupture. Damage to the RBC membrane increases its vulnerability to subsequent injury, particularly through lipid peroxidation initiated by free radicals [124]. Stabilizing the membrane is crucial as it helps to prevent the leakage of serum proteins and fluids into tissues during episodes of heightened permeability associated with inflammatory mediators. The anti-inflammatory properties of EPSs may stem from their ability to inhibit enzymes that play a role in the synthesis of inflammatory mediators and the metabolism of arachidonic acid [125,126]. Numerous studies have demonstrated that certain antioxidants derived from various sources, such as EPSs from *Bifidobacterium*, effectively inhibit erythrocyte hemolysis induced by free radicals in laboratory settings [127–130]. Moreover, EPSs sourced from *Lactobacillus plantarum* and *Bifidobacterium bifidum* provide protective effects against damage to normal mouse erythrocytes, and this protection is dose-dependent [72].

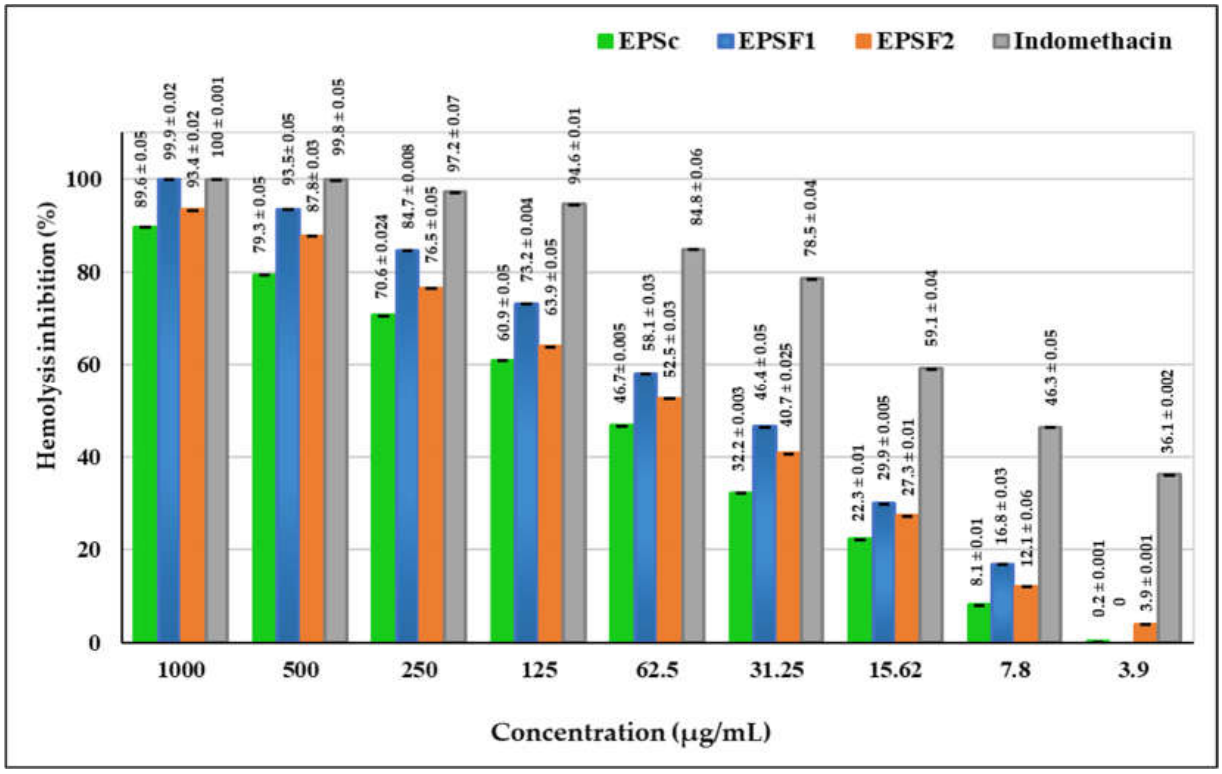


Figure 12. In vitro, the anti-inflammatory activity of EPSc, EPSF1 and EPSF2 compared to indomethacin. Results are presented as means ± SE for triplicate. Black bars indicate standard errors.

4. Conclusions

The *L. plantarum* B7, which was isolated from human breast milk, has demonstrated a high level of safety without any hemolytic activity and could be recommended as a safe probiotic. Highly yielded exopolysaccharides EPSF1 and EPSF2 extracted and characterized from L. plantarum B7 showed excellent antioxidant activity, which could guarantee its use in the food industry. The polymers showed negligible toxicity against human normal lung cells WI-38; at the same time, EPSF1 showed high in vitro antitumor activity against several human cancer cell lines. Also, EPSF1 exhibited more potent anti-inflammatory activity than EPSF2. These characteristics make it a valuable biopolymer for a variety of biomedical applications.

Author Contributions: Conceptualization, A.A.A. and S.F.M.; bacterial isolation and characterization, S.F.M.; polysaccharide purification and characterization, A.A.A.; biochemical analysis, data curation and writing—original draft preparation A.A.A.; writing—review and editing, S.F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The datasets generated and/or analyzed during the current study are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Rahman, M.S.; Emon, D.D.; Toma, M.A.; Nupur, A.H.; Karmoker, P.; Iqbal, A. Recent advances in probiotication of fruit and vegetable juices. *J. Adv. Vet. Anim. Res.* **2023**, *10*, 522–537. [PubMed]
2. Sharifi-Rad, J.; Rodrigues, C.F.; Stojanović-Radić, Z.; Dimitrijević, M.; Aleksić, A.; Neffe-Skocińska, K.; Zielińska, D.; Kołożyn-Krajewska, D.; Salehi, B.; Milton Prabu, S.; et al. Probiotics: Versatile Bioactive Components in Promoting Human Health. *Medicina* **2020**, *56*, 433. [CrossRef] [PubMed]
3. Khushboo, K.A.; Malik, T. Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. *Front. Microbiol.* **2023**, *14*, 1170725.
4. Ren, D.; Li, C.; Qin, Y.; Yin, R.; Du, S.; Ye, F.; Liu, C.; Liu, H.; Wang, M.; Li, Y.; et al. In vitro evaluation of the probiotic and functional potential of *Lactobacillus* strains isolated from fermented food and human intestine. *Anaerobe* **2014**, *30*, 1–10. [PubMed]
5. Saha, S.; Alam, N.N.; Rahman, S.M.N. The Role of Probiotic Supplementation on Insulin Resistance in Obesity-Associated Diabetes: A Mini-Review. *Biomedicine* **2022**, *42*, 651–656. [CrossRef]
6. Wang, J.; Zhao, X.; Yang, Y.; Zhao, A.; Yang, Z. Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. *Int. J. Biol. Macromol.* **2015**, *74*, 119–126. [CrossRef]
7. Bernal, P.; Llamas, M.A. Promising biotechnological applications of antibiofilm exopolysaccharides. *Microb. Biotechnol.* **2012**, *5*, 670–673.
8. Caggianiello, G.; Kleerebezem, M.; Spano, G. Exopolysaccharides produced by lactic acid bacteria: From health-promoting benefits to stress tolerance mechanisms. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 3877–3886.
9. El-Deeb, N.M.; Yassin, A.M.; Al-Madboly, L.A.; El-Hawiet, A. A novel purified *Lactobacillus acidophilus* 20079 exopolysaccharide, LA-EPS-20079, molecularly regulates both apoptotic and NF-KB inflammatory pathways in human colon cancer. *Microb. Cell Factories* **2018**, *17*, 29. [CrossRef]
10. Di, W.; Zhang, L.; Yi, H.; Han, X.; Zhang, Y.; Xin, L. Exopolysaccharides produced by *Lactobacillus* strains suppress HT-29 cell growth via induction of G0/G1 cell cycle arrest and apoptosis. *Oncol. Lett.* **2018**, *16*, 3577–3586. [CrossRef]
11. Tang, Q.; Huang, G.; Zhao, F.; Zhou, L.; Huang, S.; Li, H. The antioxidant activities of six (1→3)-β-d-glucan derivatives prepared from yeast cell wall. *Int. J. Biol. Macromol.* **2017**, *98*, 216–221. [PubMed]
12. Shahid, M.R.R.; Mehwish, M.M.; Kitazawa, H.; Barba, F.J.; Berthelot, L.; Umair, M.; Zhu, Q.; He, Z.; Zhao, L. Techno-functional properties and immunomodulatory potential of exopolysaccharide from *Lactiplantibacillus plantarum* MM89 isolated from human breast milk. *Food Chem.* **2022**, *377*, 131954.
13. Zou, S.; Zhang, X.; Yao, W.; Niu, Y.; Gao, X. Structure characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Lycium barbarum* L. *Carbohydr. Polym.* **2010**, *80*, 1161–1167.
14. Abdel-Wahab, B.A.; Abd El-Kareem, H.F.; Al Zamami, A.; Fahmy, C.A.; Elesawy, B.H.; Mahmoud, M.M.; Ghareeb, A.; El Askary, A.; Abo Nahas, H.H.; Attallah, N.G.M.; et al. Novel Exopolysaccharide from Marine Bacillus subtilis with Broad Potential Biological Activities: Insights into Antioxidant, Anti-Inflammatory, Cytotoxicity, and Anti-Alzheimer Activity. *Metabolites* **2022**, *12*, 715. [CrossRef]
15. Tarannum, N.; Ali, F.; Khan, M.S.; Alhumaidan, O.S.; Zawad, A.N.M.; Hossain, T.J. Bioactive exopolysaccharide from *Limosilactobacillus fermentum* LAB-1: Antioxidant, anti-inflammatory, antibacterial and antibiofilm properties. *Bioact. Carbohydr. Diet. Fibre* **2024**, *31*, 100409.
16. Oleksy, M.; Klewicka, E. Exopolysaccharides produced by *Lactobacillus* sp.: Biosynthesis and applications. *Crit. Rev. Food Sci Nut.* **2018**, *58*, 450–462.
17. Liu, C.T.; Chu, F.J.; Chou, C.C.; Yu, R.C. Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. *Mutat. Res.* **2011**, *721*, 157–162.
18. Liu, A.; Liu, D.; Zhao, S.; Zheng, J.; Cao, D.; Zhang, H. Up regulation of annexin A2 on murine H22 hepatocarcinoma cells induced by cartilage polysaccharide. *Cancer Epidemiol.* **2011**, *35*, 490–496.

19. Reuben, R.; Roy, P.; Sarkar, S.; Alam, A.R.U.; Jahid, I. Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *J. Dairy Sci.* **2020**, *103*, 1223–1237.
20. Wang, Y.; Wang, S.X.; Song, R.Z.; Cai, J.J.; Xu, J.J.; Tang, X.Z.; Li, N. Ginger polysaccharides induced cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells. *Int. J. Biol. Macromol.* **2018**, *123*, 81–90.
21. Angelin, J.; Kavitha, M. Exopolysaccharides from probiotic bacteria and their health potential. *Int. J. Biol. Macromol.* **2020**, *1*, 853–865.
22. Aziz, T.; Naveed, M.; Jabeen, K.; Shabbir, M.A.; Sarwar, A.; Zhennai, Y.; Alharbi, M.; Alshammari, A.; Alasmari, A.F. Integrated genome-based evaluation of safety and probiotic characteristics of *Lactiplantibacillus plantarum* YW11 isolated from Tibetan kefir. *Front. Microbiol.* **2023**, *14*, 1157615. [CrossRef] [PubMed]
23. Aziz, T.; Naveed, M.; Sarwar, A.; Makhdoom, S.I.; Mughal, M.S.; Ali, U.; Yang, Z.; Shahzad, M.; Sameeh, M.Y.; Alruways, M.W.; et al. Functional Annotation of *Lactiplantibacillus plantarum* 13-3 as a Potential Starter Probiotic Involved in the Food Safety of Fermented Products. *Molecules* **2022**, *27*, 5399. [CrossRef] [PubMed]
24. Aziz, T.; Naveed, M.; Shabbir, M.A.; Sarwar, A.; Naseeb, J.; Zhao, L.; Yang, Z.; Cui, H.; Lin, L.; Albekairi, T.H. Unveiling the whole genomic features and potential probiotic characteristics of novel *Lactiplantibacillus plantarum* HMX2. *Front. Microbiol.* **2024**, *15*, 1504625.
25. Hossain, T.J. Functional genomics of the lactic acid bacterium *Limosilactobacillus fermentum* LAB-1: Metabolic, probiotic and biotechnological perspectives. *Heliyon* **2022**, *8*, e11412.
26. Logan, N.A.; De, V.P.; Genus, I. Bacillus Cohn 1872, 174AL. In *Bergey's Manual of Systematic Bacteriology*, 2nd ed.; De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H., Whitman, W.B., Eds.; Springer: New York, NY, USA, 2009; Volume 3, pp. 21–128.
27. Kanmani, R.; Kumar, S.; Yuvaraj, N.; Paari, K.A.; Pattukumar, V.; Arul, V. Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* PI80 and its functional characteristics activity in vitro. *Bioresour. Technol.* **2011**, *102*, 74827–74833.
28. Hu, S.M.; Zhou, J.M.; Zhou, Q.Q.; Ping, L.; Yuan-Yuan, X.; Tao, Z.; Qing, G. Purification, characterization and biological activities of exopolysaccharides from *Lactobacillus rhamnosus* ZFM231 isolated from milk. *LWT* **2021**, *147*, 111561.
29. Zaghloul, E.H.; Ibrahim, M.I.A.N. Production and Characterization of Exopolysaccharide from Newly Isolated Marine Probiotic *Lactiplantibacillus plantarum* EI6 with in vitro Wound Healing Activity. *Front. Microbiol.* **2022**, *13*, 903363.
30. Chen, Z.; Leng, X.; Zhou, F.; Shen, W.; Zhang, H.; Yu, Q.; Meng, X.; Fan, H.; Qin, M. Screening and identification of probiotic *Lactobacilli* from the infant gut microbiota to alleviate lead toxicity. *Probiot. Antimicrob. Proteins* **2022**, *21*, 9895.
31. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
32. Mulaw, G.; Sisay, T.T.; Muleta, D.; Tesfaye, A. In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. *Int. J. Microbiol.* **2019**, *2019*, e7179514.
33. Mogrovejo, D.C.; Perini, L.; Gostinčar, C.; Sepčić, K.; Turk, M.; Ambrožič-Avguštin, J.; Brill, F.H.H.; Gunde-Cimerman, N. Prevalence of Antimicrobial Resistance and Hemolytic Phenotypes in Culturable Arctic Bacteria. *Front. Microbiol.* **2020**, *3*, 570.
34. Sadeghi, M.; Panahi, B.; Mazlumi, A.; Hejazi, M.A.; Komi, D.E.A.; Nami, Y. Screening of potential probiotic lactic acid bacteria with antimicrobial properties and selection of superior bacteria for application as biocontrol using machine learning models. *LWT* **2022**, *162*, 113471.
35. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
36. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
37. Sudhamani, S.R.; Tharanathan, R.N.; Prasad, M.S. Isolation and characterization of an extracellular polysaccharide from *Pseudomonas caryophylli* CFR 1705. *Carbohydr. Polym.* **2004**, *56*, 423–427.
38. Adebayo-Tayo, B.; Fashogbon, R. In vitro antioxidant, antibacterial, in vivo immunomodulatory, antitumor and hematological potential of exopolysaccharide produced by wild type and mutant *Lactobacillus delbureckii* subsp. *bulgaricus*. *Heliyon* **2020**, *6*, e03268.
39. Zheng, Z.M.; Huang, Q.L.; Ling, C.Q. Water-soluble yeast β-glucan fractions with different molecular weights: Extraction and separation by acidolysis assisted-size exclusion chromatography and their association with proliferative activity. *Int. J. Biol. Macromol.* **2019**, *123*, 269–279.
40. Kogan, G.; Sandula, J.; Korolenko, T.A.; Falameeva, O.; Poteryaeva, O.; Zhanaeva, S.; Levina, O.; Filatova, T.; Kaledin, V. Increased efficiency of Lewis lung carcinoma chemotherapy with a macrophage stimulator-yeast carboxylmethylglucan. *Int. Immunopharmacol.* **2002**, *2*, 775–781.
41. Wang, J.; Ji, H.F.; Wang, S.X.; Zhang, D.Y.; Liu, H.; Shan, D.C.; Wang, Y.M. *Lactobacillus plantarum* ZLP001: In vitro Assessment of Antioxidant Capacity and Effect on Growth Performance and Antioxidant Status in Weaning Piglets. *Asian-Australas J. Anim. Sci.* **2012**, *25*, 1153–1158.

42. Zhang, S.; Liu, L.; Su, Y.; Li, H.; Sun, Q.; Liang, X.; Lv, J. Antioxidative activity of lactic acid bacteria in yogurt. *Afr. J. Microbiol. Res.* **2011**, *5*, 5194–5201.
43. Liu, J.; Luo, J.; Ye, H.; Sun, Y.; Lu, Z.; Zeng, X. Production, characterization and antioxidant activities in vitro of exopolysaccharides from endophyticbacterium *Paenibacillus polymyxa* EJS-3. *Carbohydr. Polym.* **2009**, *78*, 275–281.
44. Abeer, A.A.; Sahera, F.M. New Carrageenan/2-Dimethyl Aminoethyl Methacrylate/Gelatin/ZnO Nanocomposite as a Localized Drug Delivery System with Synergistic Biomedical Applications. *Processes* **2024**, *12*, 2702. [CrossRef]
45. Guérin, M.; Silva, C.R.; Garcia, C.; Remize, F. Lactic acid bacterial production of exopolysaccharides from fruit and vegetables and associated benefits. *Fermentation* **2020**, *6*, 115. [CrossRef]
46. Korcz, E.; Varga, L. Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. *Trends. Food Sci. Technol.* **2021**, *110*, 375–384.
47. Sornplang, P.; Piyadeatsoontorn, S. Probiotic isolates from unconventional sources: A review. *J. Anim. Sci. Technol.* **2016**, *58*, 26.
48. Khalil, E.S.; Abd Manap, M.Y.; Mustafa, S.; Alhelli, A.M.; Shokryazdan, P. Probiotic Properties of Exopolysaccharide-Producing *Lactobacillus* Strains Isolated from Tempoyak. *Molecules* **2018**, *23*, 398. [CrossRef]
49. Aarti, C.; Khusro, A. Functional and technological properties of exopolysaccharide producing autochthonous Lactobacillus plantarum strain AAS3 from dry fish based fermented food. *LWT* **2019**, *114*, 108387.
50. Tarique, M.; Abdalla, A.; Masad, R.; Al Sbiei, A.; Kizhakkayi, J.; Osaili, T.; Olaimat, A.; Liu, S.Q.; Cabezudo, F.M.; al-Ramadi, B.; et al. Potential probiotics and postbiotic characteristics including immunomodulatory effects of lactic acid bacteria isolated from traditional yogurt-like products. *LWT* **2022**, *159*, 113207.
51. Ayyash, M.; Abushelaibi, A.; AlMahadin, S.; Enan, M.; El-Tarabily, K.; Shah, N. In-vitro investigation into probiotic characterisation of Streptococcus and Enterococcus isolated from camel milk. *LWT* **2018**, *87*, 478–487.
52. Yamada, T.; Shimizu, K.; Ogura, H.; Asahara, T.; Nomoto, K.; Yamakawa, K.; Hamasaki, T.; Nakahori, Y.; Ohnishi, M.; Kuwagata, Y.; et al. Rapid and Sustained Long-Term Decrease of Fecal Short-Chain Fatty Acids in Critically Ill Patients with Systemic Inflammatory Response Syndrome. *JPEN J. Parenter. Enter. Nutr.* **2015**, *39*, 569–577.
53. Ruas-Madiedo, P.; Hugenholtz, J.; Zoon, P. An overview of the functionalityof exopolysaccharides produced by lactic acid bacteria. *Int. Dairy J.* **2002**, *12*, 163–171.
54. Čuljak, N.; Bellich, B.; Pedroni, A.; Butorac, K.; Pavunc, A.L.; Novak, J.; Banić, M.; Šušković, J.; Cescutti, P.; Kos, B. *Limosilactobacillus fermentum* strains MC1 and D12: Functional properties and exopolysaccharides characterization. *Int. J. Biol. Macromol.* **2024**, *273*, 133215.
55. Dos, S.L.E.; Ginani, V.C.; de Alencar, E.R.; Pereira, O.G.; Rose, E.C.P.; do Vale, H.M.M.; Pratesi, R.; Hecht, M.M.; Cavalcanti, M.H.; Tavares, C.S.O. Isolation, identification, and screening of lactic acid bacteria with probiotic potential in silage of different species of forage plants, cocoa beans, and artisanal salami. *Probiotics Antimicrob. Proteins* **2021**, *13*, 173–186.
56. De Souza, B.M.S.; Borgonovi, T.F.; Casarotti, S.N.; Todorov, S.D.; Penna, A.L.B. *Lactobacillus casei* and *Lactobacillus fermentum* strains isolated from mozzarella cheese: Probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. *Probiotics Antimicrob. Proteins* **2019**, *11*, 382–396.
57. Dertli, E.; Mayer, M.J.; Narbad, A. Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. *BMC Microbiol.* **2015**, *15*, 8.
58. Sivasankar, P.; Seedevi, P.; Poongodi, S.; Sivakumar, M.; Murugan, T.; Sivakumar, L.; Balasubramanian, T. Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by Streptomyces violaceus MM72. *Carbohydr. Polym.* **2018**, *181*, 752–759.
59. Salachna, P.; Mizieliñska, M.; Soból, M. Exopolysaccharide gellan gum and derived oligo-gellan enhance growth and antimicrobial activity in Eucomis plants. *Polymers* **2018**, *10*, 42. [CrossRef]
60. Matsubara, V.H.; Wang, Y.; Bandara, H.; Mayer, M.P.A.; Samaranayake, L.P. Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6415–6426.
61. Asadi, A.; Lohrasbi, V.; Abdi, M.; Mirkalantari, S.; Esghaei, M.; Kashanian, M. The probiotic properties and potential of vaginal *Lactobacillus* spp. isolated from healthy women against some vaginal pathogens. *Lett. Appl. Microbiol.* **2022**, *74*, 752–764.
62. Onyibe, J.E.; Oluwole, O.B.; Ogunbanwo, S.T.; Sanni, A.I. Antibiotic Susceptibility Profile and Survival of *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum* of Human and Avian Origin in Stored Yoghurt. *Niger. Food* **2013**, *31*, 73–83. [CrossRef]
63. Huys, G.; D'Haene, K.; Swings, J. Genetic basis of tetracycline and minocycline resistance in potentially probiotic *Lactobacillus plantarum* strain CCUG 43738. *Antimicrob. Agents Chemother.* **2006**, *50*, 1550–1551.
64. Tao, S.; Chen, H.; Li, N.; Wang, T.; Liang, W. The Spread of Antibiotic Resistance Genes In Vivo Model. *Can. J. Infect. Dis. Med. Microbiol.* **2022**, *18*, 3348695.
65. Fraqueza, M.J. Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. *Int. J. Food Microbiol.* **2015**, *212*, 76–88. [CrossRef]

66. Federic, S.; Ciarrocchi, F.; Campana, R.; Ciandrini, E.; Blasi, G.; Baffone, W. Identification and functional traits of lactic acid bacteria isolated from Ciauscolo salami produced in Central Italy. *Meat Sci.* **2014**, *98*, 575–584.
67. Uddin, T.M.; Chakraborty, A.J.; Khusro, A.; Zidan, B.M.; Mitra, S.; Emran, T.B. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *J. Infect. Public Health* **2021**, *14*, 1750–1766.
68. Lerner, A.; Benzvi, C.; Vojdani, A. The Potential Harmful Effects of Genetically Engineered Microorganisms (GEMs) on the Intestinal Microbiome and Public Health. *Microorganisms* **2024**, *12*, 238. [CrossRef]
69. Tarannum, N.; Hossain, T.J.; Ali, F.; Das, T.; Dhar, K.; Nafiz, I.H. Antioxidant, antimicrobial and emulsification properties of exopolysaccharides from lactic acid bacteria of bovine milk: Insights from biochemical and genomic analysis. *LWT* **2023**, *186*, 115263.
70. Dwivedi, M. Exopolysaccharide (EPS) producing isolates from sugarcane field soil and antibacterial activity of extracted EPSs. *Acta Sci. Microbiol.* **2018**, *1*, 6–13.
71. Santschi, P.H.; Xu, S.C.; Lin, K.A.; Sun, P.; Chin, L.; Kamalanathan, W.C.; Bacosa, M.P.; Quigg, A. Can the protein/ carbohydrate (P/C) ratio of exopolymeric substances (EPS) be used as a proxy for their 'stickiness' and aggregation propensity? *Mar. Chem.* **2020**, *218*, 103734.
72. Li, W.; Ji, J.; Chen, X.; Jiang, M.; Rui, X.; Dong, M. Structural elucidation and antioxidant activities of exopolysaccharides from *Lactobacillus helveticus* MB2-1. *Carbohydr. Polym.* **2014**, *102*, 351–359.
73. Rajoka, M.S.R.; Mehwish, H.M.; Fang, H.; Padhiar, A.A.; Zeng, X.; Khurshid, M.; He, Z.; Zhao, L. Characterization and anti-tumor activity of exopolysaccharide produced by *Lactobacillus kefiri* isolated from Chinese kefir grains. *J. Funct. Foods* **2019**, *63*, 103588.
74. Liu, Z.; Zhang, Z.; Qiu, L.; Zhang, F.; Xu, X.; Wei, H.; Tao, X. Characterization and ioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. *J. Dairy Sci.* **2017**, *100*, 6895–6905.
75. Bamigbade, G.; Ali, A.H.; Subhash, A.; Tamiello-Rosa, C.; Al Qudsi, F.R.; Esposito, G.; Hamed, F.; Liu, S.Q.; Gan, R.Y.; Abu-Jdayil, B.; et al. Structural characterization, biofunctionality, and environmental factors impacting rheological properties of exopolysaccharide produced by probiotic *Lactococcus lactis* C15. *Sci. Rep.* **2023**, *13*, 17888. [CrossRef]
76. Tallon, R.; Bressollier, P.; Urdaci, M.C. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. *Res. Microbiol.* **2003**, *154*, 705–712.
77. Zhou, K.; Zeng, Y.; Yang, M.; Chen, S.; He, L.; Ao, X.; Zou, L.; Liu, S. Production, purification and structural study of an exopolysaccharide from *Lactobacillus plantarum* BC-25. *Carbohydr. Polym.* **2016**, *144*, 205–214.
78. Ziadi, M.; Bouzaiene, T.; Sana, M.H.; Kaouther, Z.; Mokhtar, F.; Hamdi, M.; Boisset-Helbert, C. Evaluation of the Efficiency of Ethanol Precipitation and Ultrafiltration on the Purification and Characteristics of Exopolysaccharides Produced by Three Lactic Acid Bacteria. *BioMed Res. Int.* **2018**, *2018*, e1896240. [CrossRef]
79. Zhang, L.; Liu, C.; Li, D.; Zhao, Y.; Zhang, X.; Zeng, X.; Yang, Z.; Li, S. Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. *Int. J. Biol. Macromol.* **2013**, *54*, 270–275. [CrossRef]
80. Yang, X.; Ren, Y.; Zhang, L.; Wang, Z.; Li, L. Structural characteristics and antioxidant properties of exopolysaccharides isolated from soybean protein gel induced by lactic acid bacteria. *LWT* **2021**, *150*, 111811.
81. Feng, X.; Zhag, H.; Lai, P.F.; Xiong, Z.; Ai, L. Structure characterization of a pyruvated exopolysaccharide from *Lactobacillus plantarum* AR307. *Int. J. Biol. Macromol.* **2021**, *178*, 113–120.
82. Khan, B.M.; Qiu, H.M.; Wang, X.F.; Liu, Z.Y.; Zhang, J.Y.; Guo, Y.J.; Chen, W.Z.; Liu, Y.; Cheong, K.L. Physicochemical characterization of *Gracilaria chouae* sulfated polysaccharides and their antioxidant potential. *Int. J. Biol. Macromol.* **2019**, *134*, 255–261.
83. Cui, L.; Butler, H.J.; Martin-Hirsch, P.L.; Martin, F.L. Aluminium foil as a potential substrate for ATR-FTIR, transflection FTIR or Raman spectrochemical analysis of biological specimens. *Anal. Methods* **2016**, *8*, 463–690.
84. Li, J.; Niu, D.; Zhang, Y.; Zeng, X.A. Physicochemical properties, antioxidant and antiproliferative activities of polysaccharides from *Morinda citrifolia* L. (Noni) based on different extraction methods. *Int. J. Biol. Macromol.* **2020**, *150*, 114–121.
85. Min, W.H.; Fang, X.B.; Wu, T.; Fang, L.; Liu, C.L.; Wang, J. Characterization and antioxidant activity of an acidic exopolysaccharide from *Lactobacillus plantarum* JLAU103. *J. Biosci. Bioeng.* **2019**, *127*, 758–766.
86. Amiri, S.; Mokarram, R.R.; Khiabani, M.S.; Bari, M.R.; Khaledabad, M.A. Exopolysaccharides production by *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis subsp. lactis* BB12: Optimization of fermentation variables and characterization of structure and bioactivities. *Int. J. Biol. Macromol.* **2019**, *12*, 3752–3765.
87. Yuan, Q.; Zhang, J.; Xiao, C.; Harqin, C.; Ma, M.; Long, T.; Li, Z.; Yang, Y.; Liu, J.; Zhao, L. Structural Characterization of a Low-Molecular-Weight Polysaccharide from *Angelica pubescens* Maxim. F. Biserrata Shan Et Yuan Root and Evaluation of Its Antioxidant Activity. *Carbohydr. Polym.* **2020**, *236*, 116047.
88. Yao, H.Y.Y.; Wang, J.Q.; Yin, J.Y.; Nie, S.P.; Xie, M.Y. A Review of NMR Analysis in Polysaccharide Structure and Conformation: Progress, Challenge and Perspective. *Food Res. Int.* **2021**, *143*, 110290.
89. Du, R.; Yu, L.; Yu, N.; Ping, W.; Song, G.; Ge, J. Characterization of exopolysaccharide produced by *Levilactobacillus brevis* HDE-9 and evaluation of its potential use in dairy products. *Int. J. Biol. Macromol.* **2022**, *217*, 303–311.

90. Du, R.; Yu, L.; Sun, M.; Ye, G.; Yang, Y.; Zhou, B.; Qian, Z.; Ling, H.; Ge, J. Characterization of Dextran Biosynthesized by Glucansucrase from *Leuconostoc pseudomesenteroides* and Their Potential Biotechnological Applications. *Antioxidants* **2023**, *12*, 275. [CrossRef]
91. Do, T.B.T.; Tran, B.K.; Tran, T.V.T.; Le, T.H.; Cnockaert, M.; Vandamme, P.; Nguyen, T.H.C.; Nguyen, C.C.; Hong, S.H.; Kim, S.Y.; et al. Decoding the capability of *Lactobacillus plantarum* W1 isolated from soybean whey in producing an exopolysaccharide. *ACS Omega* **2020**, *5*, 33387–33394.
92. Andrew, M.G. Jayaraman Structural features of microbial exopolysaccharides in relation to their antioxidant activity. *Carboh. Res.* **2020**, *487*, 107881. [CrossRef]
93. Chaudhary, P.; Janmeda, P.; Docea, A.O.; Yeskaliyeva, B.; Abdull Razis, A.F.; Modu, B.; Calina, D.; Sharifi-Rad, J. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Front. Chem.* **2023**, *11*, 1158198.
94. Yang, H.; Deng, J.; Yuan, Y.; Fan, D.; Zhang, Y.; Zhang, R.; Han, B. Two novel exopolysaccharides from *bacillus amyloliquefaciens* C-1: Antioxidation and effect on oxidative stress. *Curr. Microbiol.* **2015**, *70*, 298–306.
95. Lobo, R.E.; Gómez, M.I.; de Valdez, G.F.; Torino, M.I. Physicochemical and antioxidant properties of a gastro protective exopolysaccharide produced by *Streptococcus thermophilus* CRL1190. *Food Hydrocoll.* **2019**, *96*, 625–633.
96. Sharma, P.; Sharma, A.; Lee, H.J. Antioxidant potential of exopolysaccharides from lactic acid bacteria: A comprehensive review. *Int. J. Biol. Macromol.* **2024**, *281*, 135536.
97. Dai, Y.; Guo, Y.; Tang, W.; Chen, D.; Xue, L.; Chen, Y.; Guo, Y.; Wei, S.; Wu, M.; Dai, J.; et al. Reactive oxygen species-scavenging nanomaterials for the prevention and treatment of age-related diseases. *J. Nanobiotechnol.* **2024**, *22*, 252.
98. Ali, A.H.; Bamigbade, G.; Tarique, M.; Esposito, G.; Obaid, R.; Abu-Jday, B.; Ayyash, M. Physicochemical, rheological, and bioactive properties of exopolysaccharide produced by a potential probiotic *Enterococcus faecalis* 84B. *Int. J. Biol. Macromol.* **2023**, *240*, 124425.
99. Trabelsi, I.; Ktari, N.; Ben, S.S.; Triki, M.; Bardaa, S.; Mnif, H.; Ben, S.R. Evaluation of dermal wound healing activity and in vitro antibacterial and antioxidant activities of a new exopolysaccharide produced by *Lactobacillus* sp. Ca6. *Int. J. Biol. Macromol.* **2017**, *103*, 194–201.
100. Tan, X.; Ma, B.; Wang, X.; Cui, F.; Li, X.; Li, J. Characterization of Exopolysaccharides from *Lactiplantibacillus plantarum* PC715 and Their Antibiofilm Activity Against Hafnia alvei. *Microorganisms* **2024**, *12*, 2229. [CrossRef]
101. Tan, X.; Wang, X.; Cui, F.; Zeshan, A.; Wang, D.; Li, X.; Li, J. Effect of *Enterococcus hirae* GS22 Fermentation-Assisted Extraction on the Physicochemical and Bioactivities of Sea Cucumber Intestinal Polysaccharides. *Molecules* **2024**, *29*, 5800. [CrossRef]
102. Nguyen, P.T.; Nguyen, H.T. Antioxidant property and structural characteristics of exopolysaccharides derived from *Lactiplantibacillus plantarum* VAL6. *Discov. Bact.* **2024**, *1*, 6. [CrossRef]
103. Wu, B.; Cui, J.; Zhang, C.; Li, Z. A polysaccharide from Agaricus blazei inhibits proliferation and promotes apoptosis of osteosarcoma cells. *Int. J. Biol. Macromol.* **2012**, *50*, 1116–1120. [CrossRef]
104. Wu, J.; Zhang, Y.; Ye, L.; Wang, C. The anti-cancer effects and mechanisms of lactic acid bacteria exopolysaccharides in vitro: A review. *Carbohydr. Polym.* **2021**, *253*, 117308. [CrossRef]
105. Mahmoudi, M.; Khomeiri, M.; Saeidi, M. Anticancer potential of fermented milk with autochthonous lactic acid bacteria. *J. Appl. Microbiol.* **2023**, *134*, lxad041. [CrossRef]
106. Tukenmez, U.; Aktas, B.; Aslim, B.; Yavuz, S. The relationship between the structural characteristics of lactobacilli-EPS and its ability to induce apoptosis in colon cancer cells in vitro. *Sci. Rep.* **2019**, *9*, 8268. [CrossRef]
107. Deepak, V.; Ramachandran, S.; Balahmar, R.M.; Pandian, S.R.; Sivasubramaniam, S.D.; Nellaiah, H.; Sundar, K. In vitro evaluation of anticancer properties of exopolysaccharides from *Lactobacillus acidophilus* in colon cancer cell lines. *Vitr. Cell Dev. Biol. Anim.* **2016**, *52*, 163–173. [CrossRef]
108. Kawanabe-Matsuda, H.; Takeda, K.; Nakamura, M. Dietary Lactobacillus-derived exopolysaccharide enhances immune-checkpoint blockade therapy. *Cancer Discov.* **2022**, *12*, 1336–1355. [CrossRef]
109. Tiwari, S.; Kavitake, D.; Devi, P.B.; Halady, S.P. Bacterial exopolysaccharides for improvement of technological, functional and rheological properties of yoghurt. *Int. J. Biol. Macromol.* **2021**, *183*, 1585–1595. [CrossRef]
110. Zaidman, B.Z.; Yassin, M.; Mahajna, J.; Wasser, S.P. Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 453–468. [CrossRef]
111. Yousef, R.H.; Baothman, A.S.; Abdulaal, W.H.; Golayel, K.A.; Darwish, A.A.; Moselhy, S.S.; Ahmed, Y.M.; Hakeem, K.R. Potential antitumor activity of exopolysaccharide produced from date seed powder as a carbon source for *Bacillus Subtilis*. *J. Microbiol. Methods* **2020**, *170*, 105853. [CrossRef]
112. Alshawwa, S.Z.; Alshallash, K.S.; Ghareeb, A.; Elazzazy, A.M.; Sharaf, M.; Alharthi, A.; Abdelgawad, F.E.; El-Hossary, D.; Jaremko, M.; Emwas, A.H. Assessment of Pharmacological Potential of Novel Exopolysaccharide Isolated from Marine *Kocuria* sp. Strain AG5: Broad-Spectrum Biological Investigations. *Life* **2022**, *12*, 1387. [CrossRef] [PubMed]

113. Vinothkanna, A.; Sathiyanarayanan, G.; Rai, A.K.; Mathivanan, K.; Saravanan, K.; Sudharsan, K.; Kalimuthu, P.; Ma, Y.; Sekar, S. Exopolysaccharide Produced by Probiotic *Bacillus albus* DM-15 Isolated from Ayurvedic Fermented *Dasamoolarishta*: Characterization, Antioxidant, and Anticancer Activities. *Front. Microbiol.* **2022**, *13*, 832109. [CrossRef] [PubMed]
114. He, Y.; Ye, M.; Jing, L.; Du, Z.; Surhio, M.M.; Xu, H.; Li, J. Purification, characterization and promoting effect on wound healing of an exopolysaccharide from *Lachnum* YM405. *Carbohydr. Polym.* **2014**, *105*, 169–176. [PubMed]
115. Tahmourespour, A.; Ahmadi, A.; Fesharaki, M. The anti-tumor activity of exopolysaccharides from Pseudomonas strains against HT-29 colorectal cancer cell line. *Int. J. Biol. Macromol.* **2020**, *149*, 1072–1076.
116. Slïzewska, K.; Markowiak-Kopeć, P.; Ślïzewska, W. The Role of Probiotics in Cancer Prevention. *Cancers* **2020**, *13*, 20. [CrossRef]
117. Wasser, S.P. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol.* **2002**, *60*, 258–274.
118. Li, S.; Shah, N.P. Characterization, Anti-Inflammatory and Antiproliferative Activities of Natural and Sulfonated Exopolysaccharides from *Streptococcus thermophilus* ASCC 1275. *J. Food Sci.* **2016**, *81*, 1167–1176.
119. Anosike, C.A.; Obidoa, O.; Ezeanyika, L.U. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *DARU J. Pharm. Sci.* **2012**, *20*, 76.
120. Fehervari, Z.; Philips, J.M.; Dunne, D.W.; Cooke, A. Parasitic worms and inflammatory disease. *Parasite Immunol.* **2005**, *28*, 515–523.
121. Cunha, T.M.; Verri, W.A.; Schivo, I.R.; Napimoga, M.H.; Parada, C.A.; Poole, S.; Teixeira, M.M.; Ferreira, S.H.; Cunha, F.Q. Crucial role of neutrophils in the development of mechanical inflammatory hypernociception. *J. Leukocyte Biol.* **2008**, *83*, 824–832.
122. Cunha, T.M.; Verri, W.A.; Poole, S.; Parada, C.A.; Cunha, F.Q.; Ferreira, S.H. *Pain Facilitation by Proinflammatory Cytokine Actions at Peripheral Nerve Terminals*; Immune and Glial Regulation of Pain; Sorkin, L., DeLeo, J., Watkins, L.R., Eds.; IASP: Seattle, WA, USA, 2007; pp. 67–83.
123. Mounnissamy, V.M.; Kavimani, S.; Balu, V.; Drlin, Q.S. Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Canjera rehedi*. *Iranian J. Pharmacol. Therapeut.* **2008**, *6*, 235–237.
124. Halliwell, B.; Whiteman, M. Measuring reactive species and oxidative damage in vivo and in cell culture: How should you do it and what do the results mean? *Br. J. Pharmacol.* **2004**, *142*, 231–255. [CrossRef] [PubMed]
125. Oweyele, B.; Oloriegbe, Y.Y.; Balaogun, E.A.; Soladoye, A.O. Analgesis and anti-inflammatory properties of Nelsonia Canescens leaf extract. *J. Ethnopharmacol.* **2005**, *99*, 153–156.
126. Metowogo, K.; Agbonon, A.; Eklu-Gadegbeku, K.; Aklikokou, A.K.; Gbeassor, M. Anti-ulcer and anti-inflammtory effects of Hydro-alcohol extract of *Aloe buettneri* A. Berger (Lilliaceae). *Trop. J. Pharmaceut. Res.* **2008**, *7*, 907–912.
127. Ng, T.B.; Liu, F.; Wang, Z. Antioxidative activity of natural products from plants. *Life Sci.* **2000**, *66*, 709–723. [CrossRef]
128. Zhu, Q.Y.; Holt, R.R.; Lazarus, S.A.; Orozco, T.J.; Keen, C.L. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. *Exp. Biol. Med.* **2002**, *227*, 321–329.
129. Saengkhae, C.; Arunnopparat, W.; Sungkhajorn, P. Antioxidative activity of the leaf of *Nelumbo nucifera* Gaertn. on oxidative stress-induced erythrocyte hemolysis in hypertensive and normotensive rats. *Thai. J. Physiol. Sci.* **2007**, *20*, 70–78.
130. Ajila, C.M.; Prasada, U. Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. *Food Chem. Toxicol.* **2008**, *46*, 303–309.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.