

Article

Development and Evaluation of a Bioactive Halophilic Bacterial Exopolysaccharide-Based Coating Material to Extend Shelf Life and Mitigate Citrus Canker Disease in *Citrus limon* L.

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Abstract

Halophilic isolates were screened to mitigate postharvest losses caused by citrus canker disease in lemon fruits. Among all isolates, SWIS03, isolated from the Sambhar Salt Lake in Rajasthan, India, exhibited the highest exopolysaccharide production, with good stability and antibacterial activity against *Xanthomonas citri*. Isolate SWIS03 was identified as halophilic *Bacillus licheniformis* DET601. It produced a very high EPS content in optimized sterilized seawater-based minimal media fortified with 2.5% sucrose and 0.3% beef extract, which was purified through repeated deproteinization and Sephadex G-75 chromatography. HPTLC analysis of composition check indicated the presence of glucose, mannose, and galactose. FTIR analysis also confirmed the presence of sugar and bound water, as well as the presence of hydroxyl, amine, amide, and methyl groups. Rheological characterization revealed a pseudoplastic nature, making it suitable for uniform coating. EPS was reported to have bioactive properties, including antioxidant activity ($84.7 \pm 1.75\%$ DPPH scavenging potential), antimicrobial activity against human pathogens, and a noncytotoxic nature, which are essential for use in edible coatings. The effect of EPS coating formulation on healthy lemon fruits resulted in shelf-life extension of up to 26.6 ± 1.14 days and 18.0 ± 1.41 days at $4\text{ }^{\circ}\text{C}$ and $30\text{ }^{\circ}\text{C}$ in coated lemons, respectively, as demonstrated by physiological parameters such as % weight loss, firmness, titratable acidity, and shelf life of lemons. Also, EPS coating preserved the quality of fruits in terms of phenolic compounds and Vitamin C content, and reduced lipid peroxidation during storage. Biocontrol potential of halophilic EPS coating on lemon fruits revealed an 86.50% and 68.64% reduction in % disease incidence compared to uncoated fruits at $4\text{ }^{\circ}\text{C}$ and $30\text{ }^{\circ}\text{C}$, respectively. Similarly, a significantly lower disease incidence (46.80% at $4\text{ }^{\circ}\text{C}$ and 67.03% at $30\text{ }^{\circ}\text{C}$) was also reported compared to paraffin-coated (positive control) lemons. Consequently, halophilic *Bacillus licheniformis* DET601 EPS is an effective coating material for citrus fruits to prevent canker disease in post-harvest settings for agricultural and food industry applications.

Keywords: biological edible coating; halophiles; shelf-life prolongation; halophilic exopolysaccharide (EPS)



Academic Editors: Mia Kurek, Alena Stupar and Danijela Šuput

Received: 4 August 2025

Revised: 8 September 2025

Accepted: 9 September 2025

Published: 11 September 2025

Citation: Upadhyaya, C.; Patel, H.; Patel, I.; Upadhyaya, T. Development and Evaluation of a Bioactive Halophilic Bacterial Exopolysaccharide-Based Coating Material to Extend Shelf Life and Mitigate Citrus Canker Disease in *Citrus limon* L. *Coatings* **2025**, *15*, 1068. <https://doi.org/10.3390/coatings15091068>

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1. Introduction

Among the key cultivated citrus species, the lemon (*Citrus limon* L.) is highly popular and widely utilized globally due to its nutritional and medicinal properties. It is reported to have numerous health benefits, fortified with a rich content of vitamin C, vitamin B6, phenolic compounds, potassium, iron, and soluble dietary fiber [1]. Post-harvest losses of citrus fruits occur due to weight loss, physical damage, and losses caused by pathogenic diseases, which are instigated by inappropriate handling at packaging houses, during transport, and by thermal decomposition due to high temperatures or pathogens [2]. Citrus canker has been proven to be a ravaging bacterial disease of citrus plants caused by different strains of *Xanthomonas citri*, which can infect various plant parts, viz., leaves, stems, and fruits. The mode of infection involves entry through wounds or stomatal openings on plants, from which bacterial cells invade the intracellular spaces of the apoplast and develop corky, erumpent, necrotic lesions called citrus cankers. The gene responsible for the pathogenicity is *pthA*, whose different variants are found in various strains of *X. citri* [3]. The pathogens responsible for post-harvest losses sometimes remain dormant after infecting plants and entering flowers or fruits, leading to disease later when conditions are favorable. To reduce such losses, applying a coating to their surface and storing them at low temperatures is a common practice. The surface coating protects fruits and vegetables by lowering the rate of respiration, reducing weight loss, and preventing disease onset in stored fruits [4].

For many years, post-harvest preservation techniques for fruits and vegetables have involved using coating materials that contain chemical bactericidal and fungicidal compounds, such as sodium ortho-phenol phenate, thiabendazole, or other synthetic active agents, which help extend the shelf life [4,5]. The unremitting use of such agrochemicals has caused health and environmental hazard issues, as well as the emergence of resistant pathogenic microbes, necessitating nonconventional approaches to using biological alternatives with comparable efficacy in controlling postharvest decay [6]. Natural biological polymeric materials have applicability as a food preserving coating due to their biocompatibility, biodegradability, non-toxic, antimicrobial, as well as antioxidant and other health-beneficial properties. One such polymer derived from the exoskeleton of marine organisms, chitosan, has been thoroughly investigated and reported to be used as a coating material in the citrus fruit industry. Cellulose was also reported to be utilized extensively in the food industry as a food coating material [7]. Moreover, in their pursuit of more efficacious coatings, Ambaye et al. (2022) [7] documented the use of chitosan and cellulose composite materials for food coating. The natural essential oils and secondary metabolites from plants were mixed with such coating materials to enhance the pest and disease control in coated food, plus the edible coating exhibited extra health beneficial attributes upon consumption [8,9]. However, the resources for such coating materials are limited, such as marine organisms for chitosan and plants for cellulose. Therefore, microbial systems are more economical and scalable for producing a diverse range of polymers in a sustained and stable manner.

Tremendous diversity in the microbial world has been explored to produce various products for the betterment of mankind, with industrial applications that offer high sustainability. Diversified microbes have been found to produce biofilms composed of polymeric structures, referred to as exopolysaccharides, as part of their adaptive mechanisms to withstand harsh environments or for competitive survival. Recently, Jiang et al. (2022) developed a food coating material from a novel exopolysaccharide produced by *Pediococcus pentosaceus* E8 and checked its efficacy towards the shelf-life prolongation of strawberry fruits [10]. In the food industry, numerous microbial EPSs, such as xanthan gum, pullulan, dextran, cellulose, and gellan gum, have been reported to be used as preservatives, gelling agents, thickeners, and coating materials. Such extracellular polymeric structures

(EPSs) were reported as excellent alternatives for food packaging and preservation. EPSs possess numerous bioactive properties, including antimicrobial actions against pathogens, antioxidants, anti-inflammatory, and immunomodulatory potential, as well as anticancer activity, which can be exploited for diversified applications across various sectors, such as medicine, agriculture, and the food industry [11]. Xanthan gum was documented as one of the highly economically important EPS, which was used for preparing food coating film by mixing with cinnamic acid and reported to have an anti-browning effect on coated fruits. Such a preserving effect is due to the inhibition of moisture loss and the creation of a barrier to oxygen upon coating, as well as the addition of antioxidants, which avert oxidation and the resulting browning of the coated fruits and vegetables [12]. The flavor and texture enhancer EPS, dextran, was also analyzed for edible coating preparation with plasticizer sorbitol, which was reported to improve elasticity, tensile strength, and stable film formation [13]. The fungal polymer pullulan is utilized in packaging and edible film materials, with its effectiveness against food-borne pathogens, such as *S. aureus* and *S. typhi*, enhanced by incorporating ZnO, silver nanoparticles, and rosemary oil into the film [14]. Hence, various biological coatings prevent food spoilage by preserving quality and becoming an inherent part of sustainable agriculture. Microbial EPSs exhibit an antagonistic potential against the growth of other microbes, resulting from ionic interactions with the cell wall and subsequent inhibition of microbial colonization. Recently, levan-like homopolysaccharides have been utilized as food coatings, and their stability and shelf life-prolonging, as well as health-beneficial actions, can be enhanced by incorporating stabilizers, plasticizers, and antioxidant compounds [15]. The functionality of such polymers is directly dependent on their characteristics, viz., water or moisture permeability, carbon dioxide and oxygen permeability, high tensile strength for withstanding tearing upon stretching, and thermal stability. Microbial EPSs are suitable for preparing edible coating materials due to their safety for consumption, ability to act as a barrier to moisture and oxygen, and good mechanical strength, with physical properties such as adhesion, transparency, solubility, and sustainability. However, the limitations of such biologic coating are low stability, economic production, and effectiveness in different physiological conditions [15,16]. Consequently, the exploration of better alternatives and improvements in existing biologic coating materials has become a recent and extensive area of research.

Extremophiles are an excellent source of a wide range of biological materials, including EPSs of industrial importance. Moreover, as they produce such polymers in response to harsh environments, the stability of these polymers is naturally higher [17]. Halophiles are salt-tolerant extremophiles with widespread industrial applications. Halophiles require a saline climate for their growth and are categorized as mild ($\leq 3\%$ (w/v) salt), moderate (3 to 15% (w/v) salt), and extreme ($>15\%$ (w/v) salt) halophiles based on salt concentration required for their optimal growth. They adapt to produce EPS, which forms a protective shield surrounding cells, and also interacts with Na^{+2} ions via electrostatic interaction, thereby sequestering and reducing their content from the environment. It also enhances biofilm formation, which is responsible for improved cell viability in a saline niche. Additionally, they possess unique enzymes that remain active in high salinity, and they employ either a salting-in strategy, which accumulates K^{+} ions, or a salting-out strategy, which accumulates compatible osmolytes, to maintain osmotic balance in saline conditions [18]. However, their application in the food industry is less explored and unfamiliar. Furthermore, the main limitations of biological coating are cost, stability, and their ability to effectively preserve food under various storage conditions, which necessitate ongoing improvement and exploration of better alternatives in the field. Therefore, the research gap addressed in this study focused on obtaining the halophilic exopolysaccharide that can create a stable food coating to reduce post-harvest losses in lemon fruits caused by

citrus canker disease. Exopolysaccharide was tested for its capacity to inhibit pathogenic bacteria, characterized, and evaluated for its potential to extend the post-harvest shelf life of coated fruit products. At the same time, the anti-phytopathogenic effect of EPS against citrus canker, caused by *Xanthomonas citri*, was analyzed, which, to the authors' knowledge, had not been reported previously.

2. Materials and Methods

The isolation of halophiles was previously carried out using water and soil samples from various sites, including beaches such as Kutch (23.7° N, 69.9° E), Tithal (20.6° N, 72.9° E), Khambhat (22.3° N, 72.6° E), Dumas (21.1° N, 72.7° E) and Sambhar Salt Lake (26.9° N, 75.2° E) of India [11]. Zobell marine agar (HiMedia, Mumbai, Maharashtra, India) plates were used for cultivation, and slimy colonies were selected. These colonies were further purified through repeated subculturing using the Quadrant streak method and then used for future analysis.

2.1. Primary and Secondary Screening of Halophiles for Selection of an Isolate

The primary screening was governed by evaluating EPS production capacity, for which a loop full of each isolate was inoculated in separate 100 mL Zobell marine broth, added with 3% sucrose and NaCl. After incubating at 28 ± 2 °C for 72 h, the liquid broth was measured for cell density by recording optical density (OD) at 595 nm. It was then centrifuged at 7000 rpm for 20 min to remove cell mass. EPS was collected through chilled acetone precipitation using a double volume. The recovered EPS was quantified on a precision scale after lyophilization (Bionics Scientific Technologies, Delhi, India). Based on EPS quantity, halophilic isolates were selected for secondary screening, which evaluated the antagonistic effect of recovered EPSs against the procured *Xanthomonas citri* phytopathogen, and their stability was assessed. The stability of EPS was tested by incubating samples under various conditions, including different temperatures—from room temperature (RT) (28 ± 2 °C) to 100 °C—pH levels from 4 to 10, and for a storage period of up to 30 days. This was measured by carbohydrate quantification at 490 nm [11]. The steady carbohydrate content indicates stability, while increased variability reveals the unstable nature of EPS. To determine the anti-phytopathogenic potential, EPSs of selected isolates were dissolved in water at a concentration of 5 mg/mL and tested for antibacterial activity using the agar well diffusion method. Nutrient agar plates were inoculated with 0.1 mL of *X. citri* inoculum (5.0×10^8 CFU/mL), and 10 µL of EPS solutions were added to wells for diffusion. For comparison, a streptomycin control (5 mg/mL, 10 µL) was used. After inoculation, plates were incubated at 30 °C, and the zone of inhibition diameter was determined, which exemplified the characteristic antibacterial potential of EPS, based on which the producer halophilic isolate was selected.

2.2. Characterization of Marine Isolate

The chosen isolate, whose colony characteristics and biochemical profiles were assessed using standard protocols. Molecular identification by 16S rRNA was done by using universal primers 1492R (5'-GG TTA CCT TGT TAC GAC TT-3') & 8F (5'-AGAGTTTGATCATGGCTCAG-3') and an ABI Veriti PCR machine according to [19]. The sequence data obtained were aligned with GenBank data using a multiple alignment tool to maximize the identity score for isolate strain identification. The phylogenetic tree was then built using the neighbor-joining method with 500 bootstrap replicates in MEGA 6.0 version.

2.3. EPS Recovery and Purification

2.3.1. EPS Biosynthesis and Crude-EPS Recovery

EPS was produced by utilizing the minimal media in a 5 L bioreactor inoculated with 0.5% inoculum (3×10^8 CFU) with an OD of 1.0 at 600 nm at a temperature of 30 °C for 144 h. For recovery, 100 mL of broth was centrifuged at 7000 rpm for 35 min, followed by filtration through a 0.45 µm Millipore cellulose nitrate filter under pressure after each 24 h interval. The broth, devoid of cell mass, was further supplemented with chilled acetone after incubation at 4 °C for 24 h to facilitate precipitation. The crude EPS was freeze-dried and weighed to determine its amount. Before recovery, the absorbance of the broth at 600 nm was measured to assess cell density. This information was then used to establish the growth curve and identify the optimal time point for EPS production recovery. The EPS precipitates were recovered by centrifugation, lyophilized, and stored at −20 °C for purification and subsequent use.

2.3.2. EPS Purification by Deproteinization

EPS was suspended in Distilled water (20 mg/mL) and deproteinized by adding an equal volume of 20% TCA (*w/v*) (Trichloroacetic acid) and incubating overnight at 4 °C. It was followed by centrifugation at 8000 rpm for 20 min to pellet the precipitated impurities. The supernatant was collected and mixed with a sewage reagent (chloroform: butanol [2:1 *v/v*] in equal volume) and incubated on a shaker at room temperature (28 ± 2 °C). Then, centrifugation at 3500 rpm for 20 min was performed to remove denatured protein (at the intermediate phase), and the upper aqueous phase was collected and dialyzed against MilliQ water using a dialysis membrane with a 14 kDa cut-off (HiMedia, Mumbai, Maharashtra, India). The dialysis setup was incubated under controlled conditions of 4 °C for 48 h, after which the purified EPS was collected by acetone precipitation followed by centrifugation.

2.3.3. Column Chromatography Refinement and Yield Determination

Recovered EPS was fractionated by column chromatography using a Sephadex G75 column (1.5×100 cm). A 5 mL dialyzed EPS sample (10 mg/mL) was inoculated onto the column, and Milli-Q water was utilized as the eluent at a flow rate of 3 mL/min. Fractions of 3 mL were recovered in separate tubes and analyzed for carbohydrate content using the phenol-sulfuric acid method with standard glucose [20]. The highest EPS-containing fractions were collected, concentrated by alcohol precipitation, lyophilized, and used further. Crude, deproteinized, and column-purified EPS samples were quantified to determine the % yield after each purification step.

2.4. EPS Characterization

2.4.1. Composition and HPTLC Analysis

A purified and lyophilized EPS sample was assessed for carbohydrate and protein contents using the phenol-sulfuric acid method (glucose standard) and the Folin-Lowry assay (BSA standard), respectively [21]. The monomeric composition of the sample was also checked by HPTLC analysis. EPS was hydrolyzed to release the monomers by 2 M TFA (Trifluoroacetic acid) at 100 °C for four hours. The EPS hydrolysate (7 µL) was applied to an HPTLC gel plate (30×20 cm) using a sample applicator (CAMAG Linomat 5, CAMAG, Muttenz, Switzerland). With sample EPS, standards like glucose, galactose, mannose, xylose, fructose, sucrose, and glucuronic acid were applied on the plate via HPTLC sample loader (CAMAG, Muttenz, Switzerland). The mobile phase used for separation was a mixture of butanol, ethanol, and water (5:3:1), and plate development was performed using ethanolic p-anisaldehyde and concentrated ethanolic H₂SO₄. The plates were dried and

kept under white light within the CAMAG Developing Chamber for taking images whose scanning and analysis were governed by the CAMAG visualizer system and CAMAG visionCATS [22].

2.4.2. Spectrophotometric and Spectral Analysis of EPS

EPS (0.3 mg/mL) was spectrophotometrically evaluated by taking absorbance from 200 to 600 nm to determine lambda max. The structural groups were identified using Fourier Transform Infrared Spectroscopy (FTIR). A 0.2 mg lyophilized EPS was combined with 5 mg of KBr (potassium bromide), then pressed into pellets for analysis. These samples were examined with an Alpha II compact FTIR spectrophotometer. The IR spectra, covering the range of 400–5000 cm⁻¹, were recorded using a Hewlett-Packard plotter [23].

2.4.3. Rheological Behavior of EPS

Rheology analysis was performed using a stress-controlled rheometer (TA Instruments, Dallas, TX, USA). EPS samples at concentrations of 0.10%, 0.25%, and 0.50% (*w/v*) underwent viscometric testing across a shear stress range of 10 to 800 s⁻¹ at 25 °C. Additionally, dynamic strain sweep measurements were conducted at 1 rad/s, as described in [24].

2.4.4. Molecular Weight Determination

EPS's intrinsic viscosity (0.01 g/mL) was deduced via the Ostwald viscometer and the following equations:

$$\text{Relative viscosity} = \eta_{rel} = \frac{t}{t_0} \quad (1)$$

$$\text{Specific activity} = \eta_{sp} = \frac{t}{t_0} - 1 \quad (2)$$

$$\text{Intrinsic viscosity} = [\eta] = \frac{\eta_{sp}}{C} \quad (3)$$

where *t* is the flow time of test sample, *t*₀ is the solvent flow time, *c* is polymer content.

The intrinsic viscosity was measured to determine the molecular weight by the Houwink–Sakurada formula, as follows [25]:

$$\text{Molecular weight} = [\eta] = KM^\alpha \quad (4)$$

where *M* is the molecular weight, *K* and α are predefined constants

2.4.5. Solubility Index and Water Holding Capacity Analysis

100 mg of purified sample EPS was suspended in 5 mL of D/W and mixed until a uniform solution. The suspension was centrifuged at 10,000 rpm for 25 min, and the supernatant was collected in a separate tube [26]. From supernatant, EPS was reprecipitated, dried, and weighed. Following equation was used for calculating WSI:

$$WSI = \frac{\text{Weight of dry solid in supernatant}}{\text{Weight of dry sample}} \times 100 \quad (5)$$

Water holding capacity was determined by uniform dispersion of 200 mg EPS within 10 mL of distilled water, and centrifuged at high speed for 25 min, and the pellet was plunged on pre-weighed paper for determination of weight. % WHC calculation is as follows:

$$WHC = \frac{\text{Total sample weight after water absorption}}{\text{Total dry sample weight}} \times 100 \quad (6)$$

2.4.6. Antimicrobial and Antioxidant Potential of EPS

Agar well diffusion assay was performed to check antimicrobial potential against clinical pathogens, whose working inoculum was prepared by setting broth OD 1.0 at 600 nm (3×10^8 cells) [27]. 0.1 mL of inoculum was inoculated in agar media to which wells were inoculated with 5 mg/mL EPS and streptomycin, following 24 h incubation at 30 ± 2 °C, after which the zone of growth inhibition diameter was recorded. The antioxidant potential of EPS was checked by % DPPH radical scavenging action of EPS. The assay was performed according to our previously published work [11]. The assay was performed by taking 50, 100, and 200 µg/mL EPS and the same concentration of standard ascorbic acid for comparison.

2.4.7. Cytotoxic Effect Analysis of EPS for Using It in Edible Coating

The simple cytotoxic assay was performed using a Trypan blue dye-based method, in which only viable cells do not turn blue, while damaged cells take up the dye and turn blue. For the presented analysis, 10-day-old chick embryonic liver cells were taken by homogenization of the dissected liver and incubated with PBS buffer inoculated with different concentrations of EPS, viz., 0.05, 0.1, and 0.25 mg/mL. 2 drops of TB dye were added following 30 min incubation, and the control tubes had distilled water. Both control and test homogenate drops were taken and microscopically inspected for viable cell counting versus total cell counting, and % cell viability was determined by the following equation [28]:

$$\% \text{ viable cells} = \frac{\text{Total no. of cells per ml of aliquot}}{\text{Total no. of counted cells per ml of aliquot}} \times 100 \quad (7)$$

2.5. Development of Biological Coating Material

The coating material was developed by a modified method [11] in which glycerol was used as a plasticizer, oleic acid as a surfactant, and sunflower oil was used as an emulsifying agent that increases flexibility, better adhesion, and even coating of the formulation over the fruit surface. The hydrophilic phase was prepared by mixing 1% Tween 80 & 0.5% oleic acid, while the hydrophobic phase was made by mixing 2% EPS with 0.5% glycerol. 70 mL of distilled water was vortexed with the hydrophilic phase for 10 min. Then, the hydrophobic phase was added with continuous stirring until the total volume reached 100 mL, and the mixture was stored at -20 °C for further use [29].

2.6. Uniform Coating over Lemon Fruits

The lemon fruits, of the same size, texture, and ripening stage (colour), were procured from a local farm (Gujarat, India), disinfected by immersing them in 2% (*v/v*) sodium hypochlorite, followed by three times rinsing with distilled water, and then dried to remove excess water. Fruits were divided into three groups: exopolysaccharide coating (EC), paraffin wax coating (PC), and uncoated (UC) fruits. (Table 1) Lemon fruits were immersed in EPS or paraffin wax for 1 min for uniform coating and kept at a laminar airflow for one hour to dry. All three groups of fruits were shelved at two temperatures, viz., 30 °C and 4 °C, and were periodically evaluated for shelf-life prolonging action and % disease incidence [29].

Table 1. Coating treatment for lemon fruits.

Treatment	Composition	No. of Replication	Total Number of Lemons Treated
EPS coating (EC)	EPS of <i>Bacillus tequilensis</i> (2%) + 0.5% glycerol + 1% Tween 80 + 0.5% Oleic acid	5	100
Paraffin wax coating (PC)	Paraffin wax (1%) + 0.5% glycerol + 1% Tween 80 + 0.5% Oleic acid	5	100
Uncoated fruits (UC)	Uncoated lemons (Immersed in D/W)	5	100

2.7. Effects of EPS Coating (EC) on Shelf Life and Quality of Lemon Fruits Compared to Uncoated (UC) and Paraffin Wax (PC) Coated Fruits

2.7.1. Investigation of % Weight Loss, Firmness, and Titratable Acidity

The reduction in weight of the fruits with the increase in storage time was determined by using AUX220 weighing machine (Shimadzu, Columbia, MD, USA) via which the initial and post-incubation weight of fruits was determined, and the % weight loss was defined as follows:

$$\text{Weight loss} = \frac{W_i - W_f}{W_f} \times 100 \quad (8)$$

where W_i is the initial weight, W_f is the weight after incubation

The firmness of the fruits was determined by the Shimadzu EZ food texturometer, as described in our previous study [11]. The titratable acidity (TA) of lemon fruit juice was measured according to Kayesh et al. (2018) [30]. The method involves titration with phenolphthalein indicator and 0.1 N NaOH using the following equation:

$$M = \frac{V_{\text{NaOH}} \times N \times \text{meq}}{V_m} \times 10^3 \quad (9)$$

where M = mg citric acid present in 100 g of fruit, V_{NaOH} = volume of titratable NaOH, N = NaOH solution (0.1 N), meq = citric acid weight (0.064 meq) and V_m = volume of sample.

2.7.2. Assessment of Shelf Life of Lemon Fruits

All groups of fruits were assessed by manual evaluation for browning, sogginess (unpleasant texture), taste, odor-like properties and the onset of mold or other infections. A highly trained five-person panel (three females and two males) evaluated each fruit twice and reached a consensus decision regarding its quality. The assessment was conducted in a standard laboratory testing room with a temperature of 28 ± 2 °C and a relative humidity of $50 \pm 5\%$. The fruits that deteriorated and were unacceptable for consumption were discarded, and the percentage was calculated from the total number of fruits [31]. The time point at which 50% fruits were left, that time point (day) was considered as the shelf life of the fruits. Five replicates ($5 \times 50 = 250$ fruits), each comprising 50 fruits (i.e., 10 fruits in one container = one replicate), were evaluated daily for the analysis.

2.7.3. Determination of the Quality of Lemon Fruits

The quality of lemon fruits was assessed by determining biochemical parameters, such as vitamin C, phenolic compounds, and lipid peroxidation, as indicated by the Malondialdehyde (MDA) concentration in all fruit groups stored at two different temperatures, 30 °C and 4 °C. The methodology for measuring vitamin C was adopted from Ayrañci

et al. (2004) [32]. The standard Folin–Ciocalteu method was used to determine phenolic content [33]. MDA content was estimated according to Chen et al. (2019) [34].

2.8. Analysis of % Disease Incidence of Citrus Canker Disease

Lemon fruits treated with EPS, paraffin wax, and uncoated controls were inoculated with *X. citri*. The inoculum was prepared in nutrient broth by CFU counting, with a working cell concentration of 3×10^8 cells per mL. 3 mL of inoculum was sprayed on each lemon fruit, and the fruits were incubated at 30 °C for 10 to 15 days, after which they were checked for bacterial colonization and citrus canker development. The analysis was performed as five replicates, including 100 fruits ($5 \times 100 = 500$) in each treatment, and the % disease incidence was determined by following equation [35]:

$$\% \text{ disease incidence} = \frac{\text{Total no. of fruits} - \text{diseased fruits}}{\text{Total fruits}} \times 100 \quad (10)$$

2.9. Statistical Analysis

All experiments in the study were conducted with a minimum of three replicates. The effect of coating on fruits was analyzed using a random selection of fruits from both coated and uncoated groups. Outcomes were statistically evaluated and represented as Mean $\pm$ SD. A comparative analysis between treatments was performed using ANOVA analysis via GraphPad Prism 8.3, and significant variation ($p < 0.05$) was determined for the efficacy check of the coating.

3. Results and Discussion

3.1. Isolation, Screening, and Selection of Halophiles Having High Antagonism Against the *Xanthomonas citri*

Primary screening of marine samples leads to the isolation of 13 different halophilic EPS producers, out of which 7 were Gram-negative and 6 were Gram-positive. The higher EPS production was reported in the case of three Gram-negative isolates, viz. KMIS01, KMIS02, LWIS03, and two Gram-positive isolates, viz. KSIS03, SWIS03, and further selected for secondary screening based on EPS stability and anti-phytopathogenic potential of EPS against *Xanthomonas citri* (Figure 1). The outcome exemplified the fact that in most microbial isolates, cell density and EPS production are inversely proportional. Higher cell growth and division are signs of active primary metabolism, whereas EPS is a secondary metabolite, and its production occurs at a lower cell density. However, some bacterial isolates are fast-growing and attain high cell density alongside EPS production.

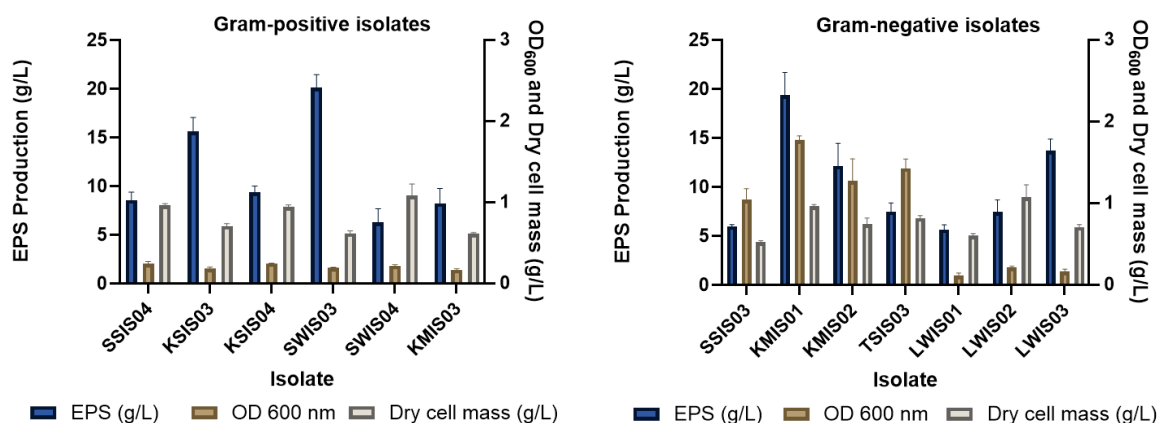


Figure 1. EPS production by Gram-positive and Gram-negative halophiles upon primary screening [Results are represented as Mean $\pm$ SD].

Secondary screening of selected isolates, specifically high EPS producers, was conducted based on the stability of the exopolysaccharide and the growth antagonism potential of halophilic EPS. For formulating a biological coating, the microbial EPS should be sturdy enough to withstand harsh incubation conditions, including temperature, pH, and prolonged incubation times [36]. The outcome of the stability analysis revealed that out of the selected five isolates, the EPS of three isolates—KSIS03, SWIS03, and KMIS01—did not exhibit a substantial reduction ($p < 0.05$) in carbohydrate concentration and were therefore stable at all selected parameters (Figure 2). The carbohydrate content of EPS of KMIS02 and LWIS03 was reduced from $127.1 \pm 0.38 \mu\text{g}/\text{mg}$ to $98.7 \pm 1.29 \mu\text{g}/\text{mg}$ (22.48%) and 140.3 ± 0.58 to $85.7 \pm 2.36 \mu\text{g}/\text{mg}$ (38.92%), respectively, at prolonged incubation time. Compared to room temperature, a similar reduction of 33.56% and 15.07% was observed in the carbohydrate content of KMIS02 and LWIS03. A significant decline in carbohydrate concentration ($p < 0.05$) exemplified the low stability of EPS under selected parameters. A study by Li et al. (2014) reported a similar carbohydrate content analysis to assess the stability of EPS, a crucial parameter for the real-time usage of such biopolymers [36].

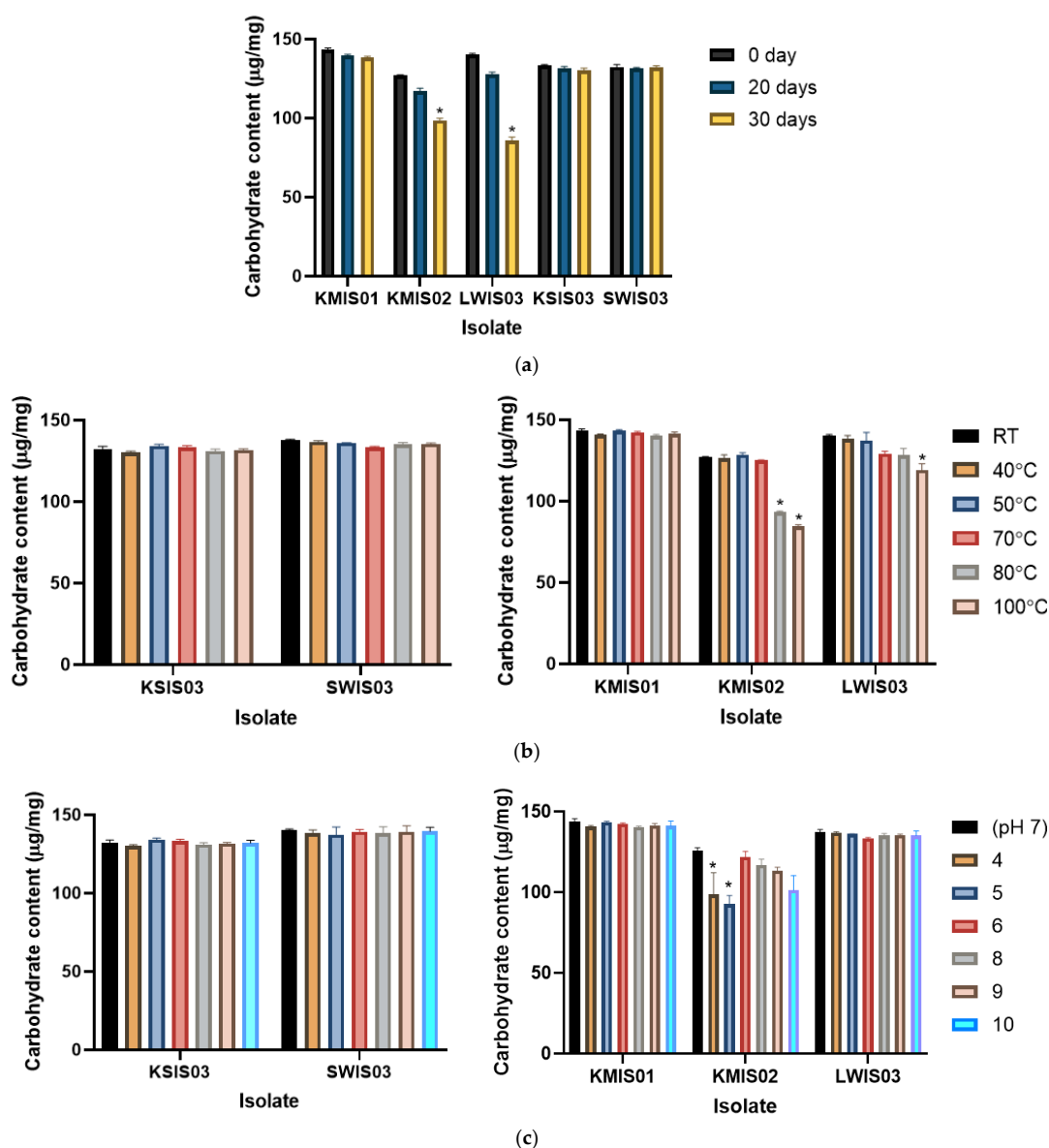


Figure 2. Assessment of stability of EPS produced by five halophilic isolates at (a) different incubation time, (b) different temperature and (c) different pH [Results are represented as Mean $\pm$ SD and significant variations are denoted as * for $p < 0.05$].

For the selection of a halophilic isolate, the anti-phytopathogenic potential of all three stable EPSs was analyzed by the agar diffusion method. The maximum zone of inhibition of 3.50 ± 0.50 cm against *Xanthomonas citri* was achieved with SWIS03-EPS (EPSSW), which was comparable to the standard (3.56 ± 0.25 cm). Based on the outcome, the SWIS03 isolate was selected, characterized, and further utilized in the investigation (Figure 3). The potential mode of action responsible for the antagonistic effect on phytopathogenic growth is the loss of adhesion between pathogenic cells and the food surface, which is a primary need for nutrient uptake, development, and ultimate successful colonization. A similar growth-inhibitory effect by ‘dextran’ against fungal phytopathogens was documented [37].

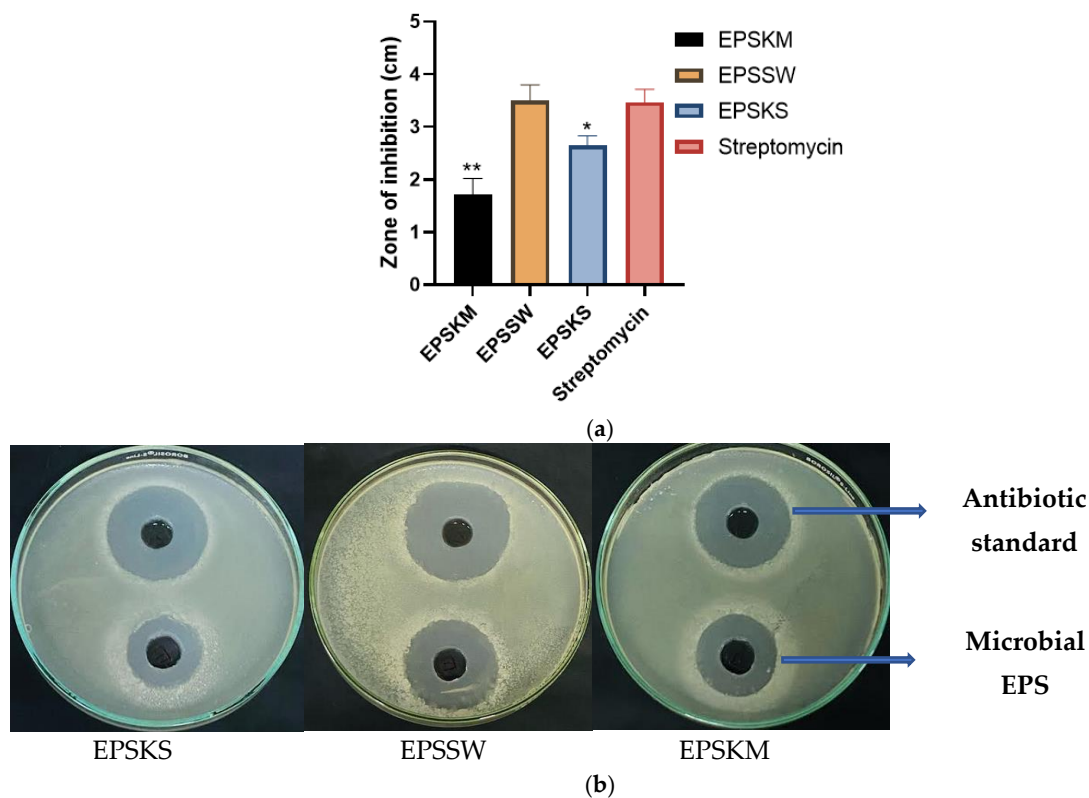


Figure 3. Analysis of antagonistic or growth-inhibitory potential of selected halophilic EPS against phytopathogenic bacteria *Xanthomonas citri*. (a) Agar diffusion assay; (b) Zone of inhibition of halophilic EPSs. [Results are represented as Mean $\pm$ SD, and significant variations are denoted as * for $p < 0.05$ and ** for $p < 0.01$].

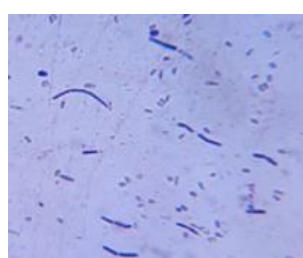
3.2. Identification of SWIS03

SWIS03 colonies were mucoid, large, wrinkled, rough, and elevated, with pale yellow pigmentation (Table 2). The microscopic analysis illustrated in Figure 4 shows that the isolate is Gram-positive, consisting of large rods that exhibit motility and produce large capsules. Moreover, the isolate can withstand temperatures of 45 °C and 15% NaCl and is therefore moderately halophilic. Biochemical characterization of SWIS03 revealed a close identity with *Bacillus licheniformis*, as reported by Makowski et al. (2021), which showed positive results for VP, citrate utilization, catalase, and the triple sugar iron test [38]. *B. licheniformis* was reported to utilize starch, casein, glucose, lactose, mannitol, and sucrose, as well as produce cellulase in the case of certain strains (Table 2). A 16S rRNA gene sequence determination reported a maximum (100%) homology with *Bacillus licheniformis* strain DET601. The maximum alignment score of SWIS03 designated it as *Bacillus licheniformis* DET601.

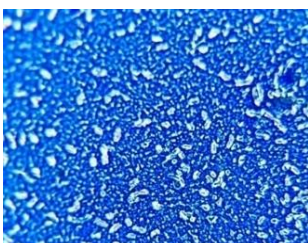
Table 2. Characterization of SWIS03 isolate.

	Characteristics	SWIS03
Colony characteristics	Size	Medium
	Shape	Round
	Margin	Irregular
	Texture	Rough, wrinkled
	Elevation	Convex
	Opacity	Opaque
	Consistency	Mucoid (sticky)
Morphological characteristics	Gram's nature	Gram-positive
	Size and shape	Large rods
	Motility	Motile
	Capsule production	Large capsule forming
	Spore formation	Spore forming
Biochemical characteristics	Indole production	-
	MR	-
	VP	+
	Citrate	+
	Oxidase	-
	Catalase	+
	Urease test	-
	Starch Hydrolysis	+
	Casein Hydrolysis	+
	Glucose	+
	Xylose	+
	Mannitol	+
	Lactose	+
	Sucrose	+
	Maltose	+

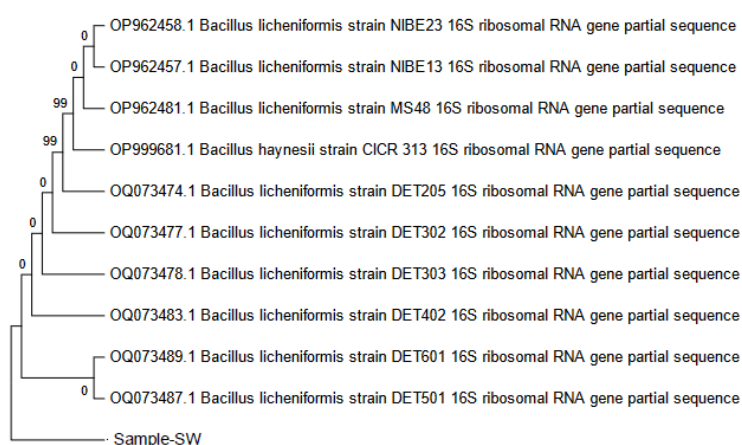
MR: Methyl red test, VP: Voges-Proskauer test.



(a)



(b)

Phylogenetic Tree:

(c)

Figure 4. Characterization of SWIS03 (a) Gram's staining, (b) capsule staining, and (c) phylogenetic analysis.

3.3. Production, Purification, and Recovery of Exopolysaccharide

The production of exopolysaccharide was governed by inoculating the working inoculum of *B. licheniformis* (SWIS03) within minimal media production broth fortified with 3% sucrose and 10% NaCl.

The crude EPS was recovered by alcohol precipitation and deproteinized by multiple steps of TCA (Trichloroacetic acid) and sewage reagent treatments. The deproteinized EPS sample was dialyzed and purified using Sephadex G75 column chromatography, and the recovered fractions were selected based on their high carbohydrate content (Figure 5b). The purification profile of the produced EPS is illustrated in Table 3. For the complete recovery of EPS from production broth, determining the optimal cell mass harvesting time following EPS precipitation is crucial. The growth pattern of SWIS03 revealed that the exponential growth phase continued from 12 to 72 h, and the stationary phase was attained from 72 to 144 h. The EPS accumulation was maximum at 96 h incubation time, which was deduced as the optimum for maximum recovery, as a decline in EPS quantity was reported after 96 h (Figure 5a). Thus, EPS synthesis was initiated during the exponential phase and maximized in the stationary phase. The decline in EPS production was reported, accompanied by a plateau in the growth cycle of microbial cells. However, EPS decline before the plateau during the late stationary phase is due to the nutrient deprivation and utilization of accumulated EPS as a carbon source for the metabolism of microbial cells [39]. Moreover, EPS production was synchronized with microbial growth, a phenomenon observed in previous studies [40]. However, some findings revealed the growth-independent production of EPS in the late stationary phase, which may be due to extracellular synthesis mediated by released glycosidases whose activity is not dependent on cell growth or viability [41].

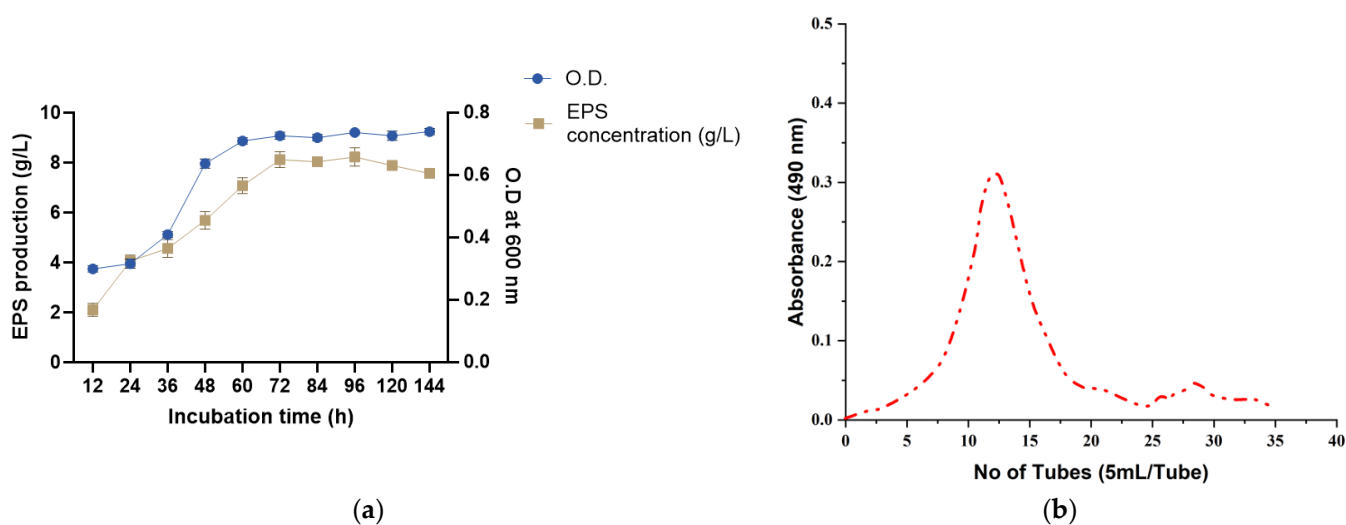


Figure 5. Recovery and chromatographic purification of EPS (a) determination of time for harvesting cell mass and recovery of EPS for maximum quantity gain (b) Carbohydrate quantification exemplified Sephadex G75 purification of EPS [Results are represented as Mean $\pm$ SD].

Table 3. Purification profile of exopolysaccharide.

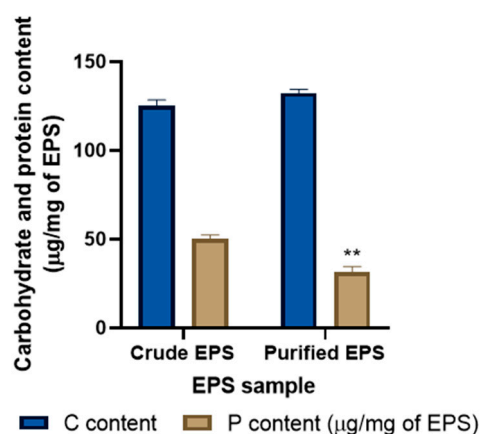
Isolate	Stepwise Purified EPS	Total Volume (mL)	EPS Recovered (mg/mL)	Total EPS (mg/mL)	% Recovery/Yield
SWIS03	Crude EPS	50	40.50	2025	100
	Deproteinized and dialyzed EPS	33.5	41.00	1373.5	67.82
	Gel exclusion chromatographic purification (Sephadex G-75)	5	53.70	268.5	13.25

3.4. Characterization and Analysis of Properties of Halophilic EPS for Biological Coating Development

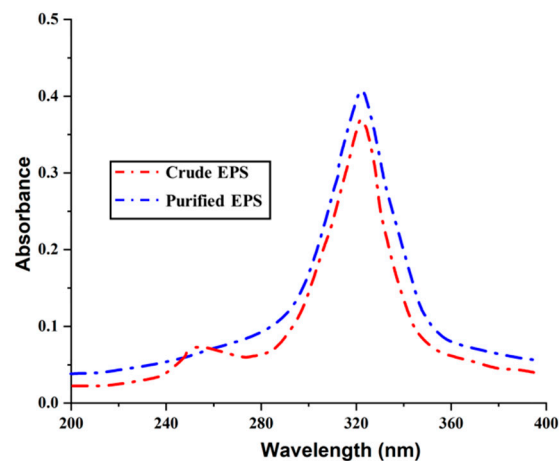
3.4.1. Determination of Composition and Spectral Study of EPS

The carbohydrate and protein concentrations were determined (Figure 6a), revealing higher carbohydrate levels ($125.33 \pm 3.05 \mu\text{g}/\text{mg}$) than protein ($50.03 \pm 2.28 \mu\text{g}/\text{mg}$) in crude EPS. However, upon purification, the protein concentration was significantly reduced (62.80% reduction) with a slight increase in carbohydrates ($132.33 \pm 2.08 \mu\text{g}/\text{mg}$) due to the concentrating effect. The presence of structural proteins in *Bacillus* EPS was also confirmed from the former outcome, whose role is to provide stability to such polymers.

UV–visible spectrophotometric analysis of the purified EPS confirmed the presence of a carbohydrate polymer, as indicated by the peak or λ_{max} at 323 nm, as illustrated in Figure 6b. The analogous peak of 320 nm was obtained in the case of EPS levan produced by *B. licheniformis*, whereas the EPS of *Bacillus albus* DM-15 gave a peak at 195 nm [42,43].



(a)



(b)

Figure 6. Cont.

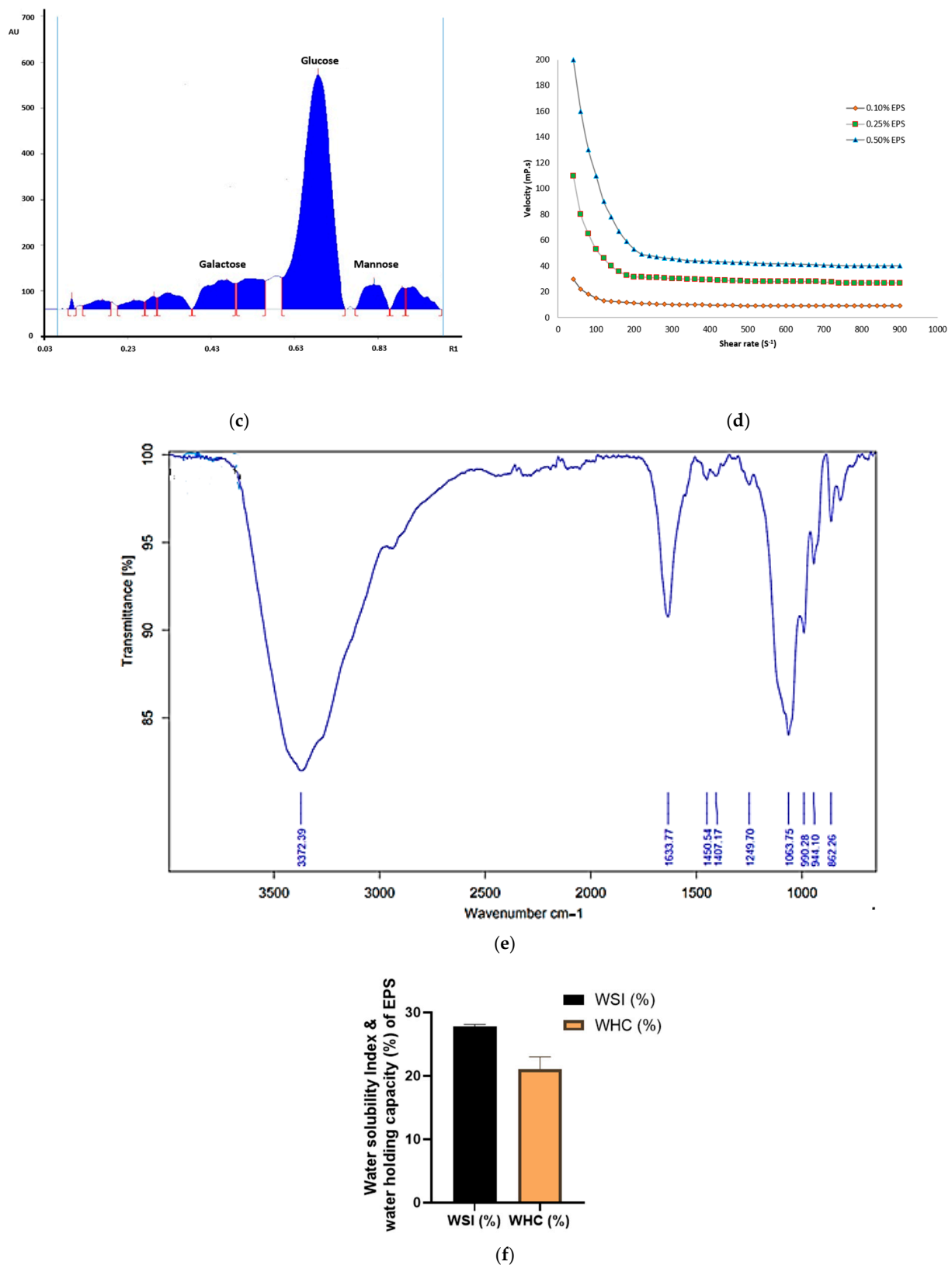


Figure 6. Characterization of EPS (a) Determination of composition, (b) $\lambda_{\max}$ determination, (c) HPTLC analysis, (d) rheological behavior, and (e) FTIR spectral analysis (f) Water solubility and Water holding capacity [Results are represented as Mean $\pm$ SD and significant variations are denoted as ** for $p < 0.01$].

3.4.2. HP-TLC Analysis

The TFA-hydrolyzed EPS sample formed two spots on the HPTLC plate, which showed the presence of glucose and mannose when read on the TLC reader. Based on comparison with R_f of standards, the sugar spots were recognized as glucose (76%), galactose (21.3%) and mannose (11.5%). Consequently, the EPS has a heteropolymer structure (Figure 6c). Asgher et al. (2020) [43] reported similar heteropolymeric EPS production by *B. licheniformis* mutant strain (exposed briefly to UV light). However, the monomeric composition shows the presence of glucose, mannose, and fructose [43]. The monomeric composition of EPS is influenced by several parameters, including the strain of microbes, type of carbon source, and physicochemical growth environment, resulting in the diversification of exopolysaccharides [44].

3.4.3. FTIR

FTIR spectra showed the presence of carboxyl, carbonyl, and hydroxyl-like reactive groups (Figure 6e). The broad peak at 3372.29 cm^{-1} indicated the presence of N-H or O-H stretches, consistent with the existence of amide, amine, or carboxylic acid groups. It is a peculiar peak of polysaccharides [45]. A stretching vibration of 1633.77 cm^{-1} confirms the presence of carboxylate bonds [46]. Moreover, the discrete band at $1200\text{--}1000\text{ cm}^{-1}$, viz. 1063.75 cm^{-1} revealed coinciding stretching vibrations of glycosidic bond (C-O-C), and the peak at 862.26 cm^{-1} indicated the presence of α -mannose monomers [37]. Hamada et al. (2022) [46] reported similar FTIR characteristics of levan produced by *B. subtilis*. Its IR spectrum showed a broad stretched peak of OH around 3417 cm^{-1} , a C=O stretching vibration peak at 1639 cm^{-1} , and peaks between 1126 and 900 cm^{-1} indicating a fingerprint of polysaccharide [46]. The absorption band at 1450 cm^{-1} showed the presence of water. Similar IR spectra were documented from *Bacillus* EPS [47].

3.4.4. Rheological Behavior and Molecular Weight Determination

The rheological behavior was determined at three EPS concentrations, namely 0.10%, 0.25%, and 0.50% (w/v), revealing that the viscosity was concentration-dependent, due to polymeric intermolecular entanglement. As the shear stress rate increases, the viscosity of the polymer decreases due to the uncoiling of the polymeric structure. The analogous shear thinning of EPS polymeric solutions was reported by Izadi et al. (2021) [48]. The presented outcome showed a similar flow behavior of EPS for the selected concentrations, with a constant decline in viscosity observed at high shear stress. However, the viscosity was restored upon the elimination of shear stress, which exemplified the pseudoplastic nature and consequent suitability of the presented EPS for use as a food coating material. The molecular weight was calculated from the intrinsic viscosity, and for a concentration of 0.10% (w/v), it was approximately $1.32 \times 10^5\text{ Da}$. The molecular weight of such biopolymers is an important characteristic, as noted by Ye et al. (2019) [49].

3.4.5. Water Holding Capacity and Solubility Index Analysis

The water-holding capacity of biological coatings is a crucial feature, as increased water permeability leads to greater dehydration and consequent shrinkage of the coated fruits or vegetables [50]. The water solubility index of EPS was $27.8 \pm 0.03\%$ while its water holding capacity was $21.0 \pm 2.0\%$ (21 g water per 100 g of EPS). The reported solubility was lower, and water holding capacity (WHC) was higher than the reported standard xanthan gum-like exopolysaccharides, which help retain moisture within coated fruits. The polymeric material, which has lower WHC and stability, is supplemented with stabilizers such as corn starch, which enhances the % WHC of the coating material [51], a factor not explored in this research.

3.4.6. Bioactive Properties of EPS

Antimicrobial Activity Against Human Pathogens

The antimicrobial activity against human pathogens demonstrated the growth-inhibitory potency of the presented EPS against both Gram-positive and Gram-negative isolates. In the case of standard streptomycin, a higher antimicrobial action was observed against Gram-negative isolates compared to Gram-positive isolates, which is consistent with its inherent action. EPS also exhibited the highest antimicrobial action against *E. coli* (3.38 ± 0.44 cm) and *S. typhi* (3.27 ± 0.35 cm), like Gram-negative human pathogens. However, it can better inhibit *P. vulgaris* and antibiotic-resistant *Nocardia* sp. compared to standard antibiotics (Figure 7a). Moreover, EPS yielded a comparable outcome to the standard in the case of *B. cereus* and *S. aureus*, like Gram-positive bacterial pathogens, demonstrating broad-spectrum antibacterial potential that can be further utilized to gain an added advantage in the form of an edible coating. Similar antimicrobial property of EPS was reported by former investigators [40,41,52]. The antibacterial attributes of bacterial exopolysaccharides are diverse, viz., inhibiting the cell-cell as well as cell-host interaction and communication, raising competitiveness due to adhesion and resulting auto-aggregation of other organisms, adverse effect on the growth of pathogens upon accumulation, and presence of the bioactive functional group in media, etc., refs. [53,54].

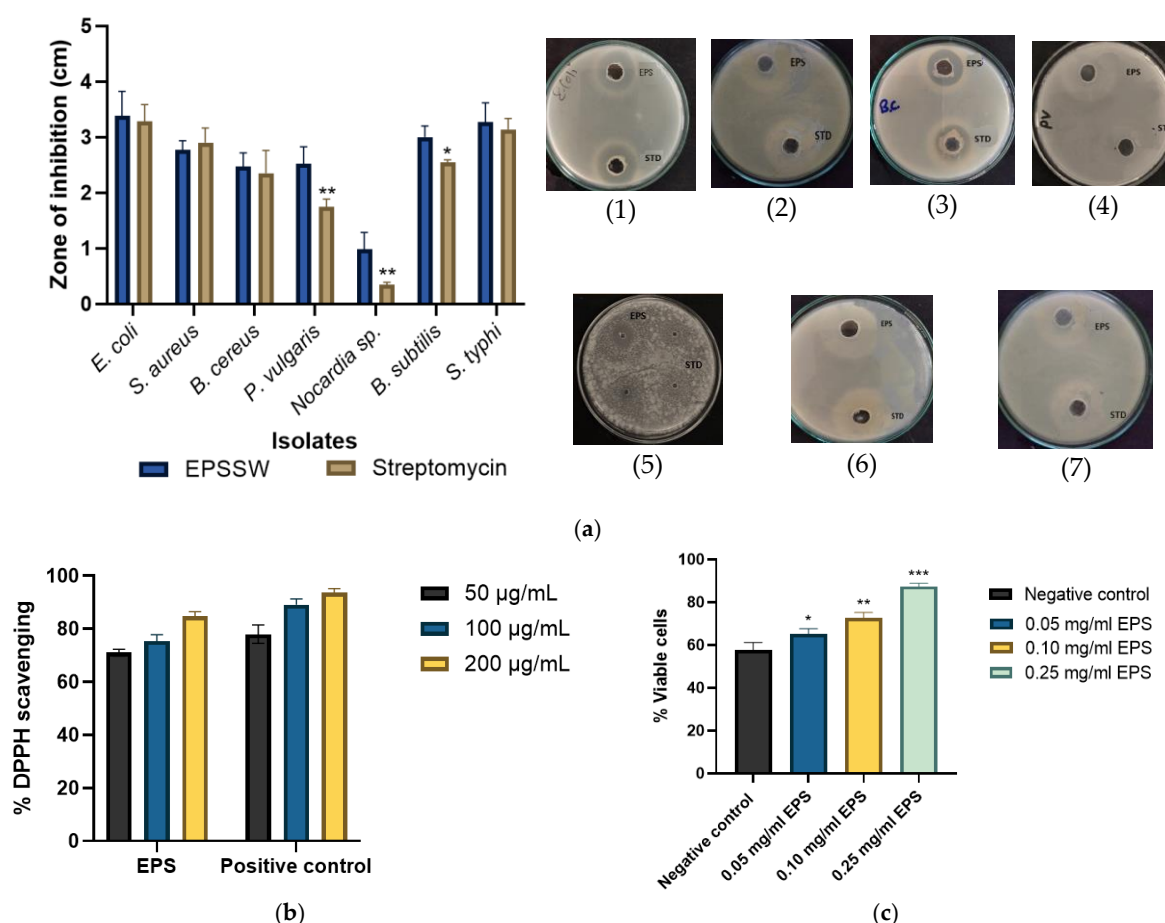


Figure 7. Bioactive properties of EPS (a) Antimicrobial activity of EPS against human pathogens viz. (1) *E. coli*, (2) *S. aureus*, (3) *B. cereus*, (4) *P. vulgaris*, (5) *Nocardia* sp., (6) *B. subtilis*, (7) *S. typhi*; (b) Antioxidant potential of EPS; (c) Cytotoxicity assay of EPS against 10-day old chick embryo liver cells [Results are represented as Mean $\pm$ SD and significant variations are denoted as * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$].

Antioxidant Potential of EPS

Antioxidant potential is an essential bioactive feature of secondary metabolites or products produced by microbes. Our EPS was tested for % DPPH radical scavenging potential compared to standard ascorbic acid. A concentration-dependent increase in DPPH scavenging activity was reported in both EPS and the standard (Figure 7b). The respective DPPH scavenging percentages were $71.0 \pm 1.32\%$, $73.4 \pm 2.43\%$, and $84.7 \pm 1.75\%$ for 50, 100, and 200 $\mu\text{g/mL}$ EPS, which were comparable to the standard with no significant difference. The results were similar to Bomfim [47], who reported a comparable concentration-dependent increase in the antioxidant potential of *Lactobacillus* EPS. These findings were equivalent to our previous investigation of EPS from *B. tequilensis*, which showed $76.3 \pm 1.69\%$ DPPH radical scavenging [11]. Similar antioxidant activity was reported in exopolysaccharides of different microbes, which neutralize the free radicals and reactive oxygen species produced due to various stresses on tissue cells [55,56]. Hence, the current EPS with high antioxidant potential offers an additional advantage as an edible coating material.

3.4.7. Cytotoxic Assay

To rule out the possibility of side effects and toxicity upon consumption, microbial products are routinely assessed for their safety. Ten-day-old chick embryonic liver cells were used to evaluate different concentrations of EPS and determine their effect on cell viability. The outcome revealed a positive impact of EPS on cell viability, rather than cytotoxicity. As the EPS concentration increased, the count of viable cells also increased. The maximum cell viability of $85.0 \pm 3.00\%$ was achieved at an EPS content of 0.25 mg/mL , which was 51.43% higher than the control (Figure 7c). Former investigations have also shown the similar nontoxic and safe nature of microbial exopolysaccharides [54,55]. Although a few microbial EPSs have been reported to have cytotoxic effects on cancer cells. A similar study, using the most reliable MTT assay, revealed a significant decline in cell damage and a protective effect on normal fibroblastic cells upon application of microbial EPS [56]. The former constructive effect of *Bacillus tequilensis* P21 exopolysaccharide on RBC and WBC counts, as determined by a haematological study, was reported by Sutthi et al. (2023) [52]. Consequently, based on the obtained outcome, the presented EPS was found to be safe, non-cytotoxic, and a potent bioactive polymer suitable for use in edible coating formulations.

3.5. Analysis of EPS Edible Coating on Lemon Fruits

3.5.1. Effect of EPS Coating on % Weight Loss, Firmness, and Titratable Acidity (TA) of Lemon Fruits

The % weight loss of lemon fruits increased upon prolonged storage, though the rate of increase was faster at 30 °C than in refrigerated conditions at 4 °C. All groups of fruits were assessed at 5-day intervals up to 30 days of storage at two selected temperatures. The result revealed less weight loss (%) in EPS-coated (EC) and paraffin wax (PC)-coated fruits compared to uncoated lemons (UC) (Figure 8a,b). A significant weight loss (%) was reported after a 15-day storage period, which became more pronounced at a 20- to 30-day storage period. The lowest weight loss (%) at a 30-day storage time was reported in the case of EC ($7.9 \pm 1.08\%$), which occurred at 4 °C. The positive control PC also revealed a reduction in weight loss (%) at 4 °C, which was 60% less than UC at the 30-day time point. Though compared to UC, EC revealed 74.41% less weight loss at 4 °C. A similar protective effect was reported at 30 °C. However, the EPS coating was reported to show a 61.9% reduction in weight loss (%) at 30 days of storage time compared to UC. The EPS coating showed a respective 48.22% and 34.33% reduction in weight loss (%) at 30 °C and 4 °C compared to the positive control, paraffin wax coating. A significant decline ($p < 0.01$) in weight loss (%) of lemons coated with the presented EPS proved it to be an efficacious

protective coating material, superior to paraffin wax coating. The prominent causes of weight loss deterioration in fruits are water loss due to transpiration and respiration under diverse storage conditions. The EPS coating, possessing a hydrophobic nature, acts as a barrier to moisture loss, as well as to oxygen and carbon dioxide, which slows down oxidation, respiration, and water loss via transpiration, ultimately slowing down the weight loss of coated fruits [57,58].

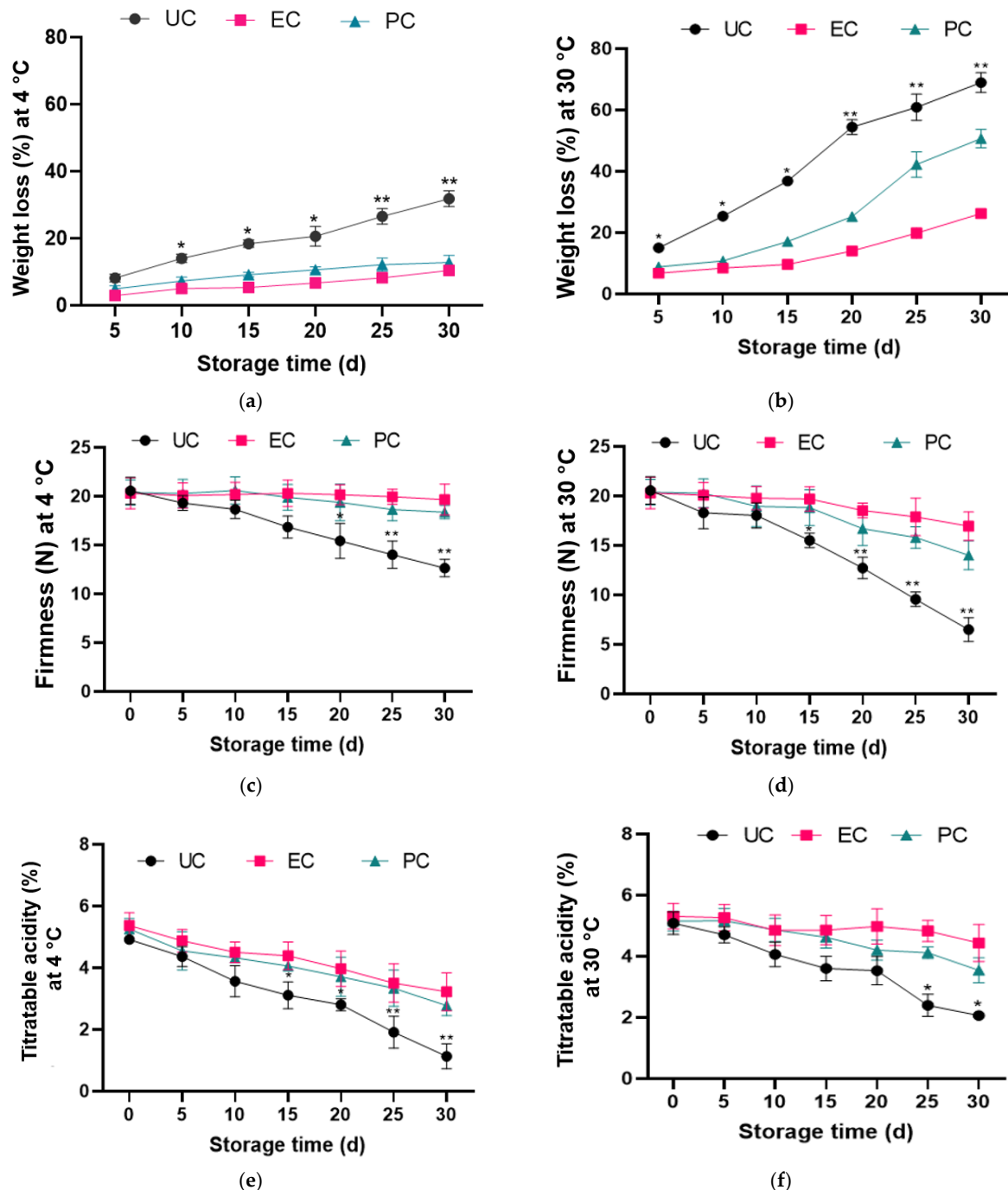


Figure 8. Analysis of effect of coating on lemon fruits weight loss (%) at (a) 4 °C & (b) 30 °C, firmness (N) at (c) 4 °C & (d) 30 °C and titratable acidity of lemon juice at (e) 4 °C & (f) 30 °C [Results are represented as Mean $\pm$ SD and significant variations are denoted as * for $p < 0.05$, ** for $p < 0.01$].

Firmness of the fruit is another prominent characteristic that indicates quality. The presented analysis revealed a decline in firmness with prolongation of storage time in both 30 °C and 4 °C conditions, which was more noticeable in fruits incubated at 30 °C.

A comparative study with 0 day showed a significant reduction ($p < 0.01$) in firmness in uncoated fruits upon 30 days of storage.

There was a decline in extent of reduction in firmness of coated lemons, viz., firmness of EPS-coated lemons only declined 3.20% at 30-day storage time at 4 °C compared to 0 day. At 4 °C, the paraffin wax-coated lemon fruits showed a 2.45% reduction up to 15 days, which increased by 9.91% at 30 days (Figure 8c). The comparable lowering by PC might be due to the higher strength of paraffin wax at lower temperatures. The respective decline in firmness at 30 °C was 68.48%, 16.59%, and 31.20% in the UC, EC, and PC groups of fruits at 30 days of storage time. EPS-coated fruits revealed 51.89% and 14.61% less decline in firmness than the uncoated and paraffin wax-coated fruits (Figure 8d). Hence, the presented EPS coating confirmed the preserving effect on the firmness of coated lemons at both selected temperatures. Post-harvest storage of fruits leads to the degradation of pectin and other cell wall components, resulting in the softening of the fruits [59]. Temperature is a major affecting factor that promotes the softening of fruits and is reflected in the presented outcome as well. At lower temperatures, storage spoilage is reduced, primarily due to the inhibition of polygalacturonase, pectinase, and other similar enzymes involved in the ripening and softening process. Many previous studies have demonstrated the effect of biological coatings on fruits, primarily by reducing enzymatic degradation and the rate of fruit deterioration [60,61].

Titrateable acidity (TA) of fruit juice is an indicator of the internal deterioration of fruits, which decreases and becomes acidic with an increase in storage time. However, the TA was significantly higher ($p < 0.05$) in the case of EPS-coated fruits than in uncoated fruits after 30 days of storage. The respective percentage declines in TA at 30 °C were 60.63%, 17.29%, and 31.26% for UC, EC, and PC, respectively. Similarly, at 4 °C storage conditions, TA was reduced. Although the rate of decline in TA at 4 °C storage was less than at 30 °C, the same reduction in TA was observed, i.e., EC < PC < UC. Hence, the halophilic EPS coating was deduced to be efficacious in preserving fruit quality and texture by delaying softening and rotting at both 4 °C and 30 °C, compared to uncoated and paraffin wax-coated fruits. Similarly, an edible coating composed of pullulan was developed through carboxymethylation and utilized for the preservation of blueberries. The outcome showed that pullulan coating was effective in delaying the ripening and extending the shelf life of coated blueberry fruits [60]. Another example of extensively utilized microbial EPS is Xanthan gum produced *Xanthomonas* bacteria. Recent discoveries by Mohammadi et al. (2024) [61] reported the impact of xanthan gum on Mexican lime fruits, which was similarly effective in reducing fruit weight loss and extending shelf life while preserving their color and quality. Moreover, the antioxidant activity and addition of essential oils from *Spirulina platensis* and pomegranate seeds helped maintain the freshness and color of the fruits [61]. Chitosan was also reported to have a preserving effect when used as a biological coating to prolong the shelf life of Agege sweet orange fruits. The findings revealed that the coating extended the shelf life by 8 weeks at 25 °C [62]. The findings and research on pullulan, chitosan, and cellulose-like biopolymer-based edible coatings have revealed that these biopolymers are potent in increasing the shelf life and post-harvest preservation of citrus fruits [63].

3.5.2. Determination of Shelf Life of Lemon Fruits

The shelf life of lemon fruits was determined for both storage conditions, yielding an obvious outcome of an extended shelf life at 4 °C compared to 30 °C, which was also reflected in the physiological analysis of the fruits. The shelf life of EPS-coated fruits was 26.6 ± 1.14 days and 18.0 ± 1.41 days at 4 °C and 30 °C, respectively, which was significantly higher than that of uncoated fruits, as demonstrated in Figure 9. EPS coating

revealed 62.19% and 109.30% of extension or rise in the shelf life of lemons in 4 °C and 30 °C storage conditions, respectively, compared to UC. The paraffin wax coating also revealed a shelf-life extension of 48.83% for lemons stored at 30 °C, which was lower (60.87%) than the EC. However, halophilic EPS coating formulation gave significantly higher ($p < 0.05$) shelf life than PC at 4 °C as well. Consequently, halophilic EPS coating can be conventionally used to prolong the shelf life of lemon fruits. Numerous studies have demonstrated that biological edible coatings can prolong the post-harvest shelf life of various fruits and food products. Chen et al. (2023) reported the shelf-life-prolonging action of curdlan or glucomannan edible coatings on cherry tomatoes [64]. Another investigation involved multiple polymeric coating materials, viz, xanthan gum (0.3%), carboxy methyl cellulose (1%), guar gum (0.75%), and gum arabic (10%) upon shelf life of ‘Misty’ blueberries stored at 1 ± 1 °C and 85–90% relative humidity. The findings revealed that the shelf life of blueberry fruits was extended up to 35 days without affecting their quality [65]. Analysis of pullulan EPS coating on cherry tomato fruits also revealed a similar extension of shelf life and maintenance of quality at low-temperature storage [66]. Previous findings from the literature have shown similar effects of different microbial exopolysaccharide-based edible coatings on various citrus fruits. EPS coating acts as a barrier that prevents moisture loss and gaseous exchange, which reduces weight loss and preserves firmness, along with slowing the ripening process and decay of fruits, thereby increasing shelf life [67]. Yet, the scope of betterment in terms of economic and more efficacious biological coating for food preservation presented halophilic EPS as a potent candidate. In the present study, EPS had an added advantage in the form of antimicrobial potential, which protects fruits from disease onset and resulting post-harvest losses.

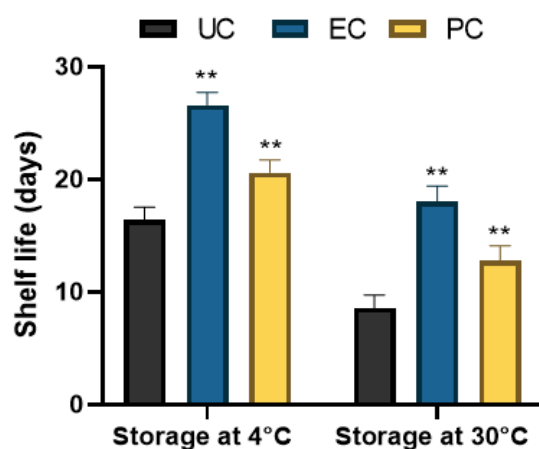


Figure 9. Assessment of EPS coating on shelf-life prolongation of lemon fruits [Results are represented as Mean $\pm$ SD and significant variations are denoted as ** for $p < 0.01$].

3.5.3. Effect of Coating on Biochemical Quality Parameters

Vitamin C Content

As a potent antioxidant, Vitamin C content is an essential indicator of fruit quality that declines significantly with prolonged storage. This was exemplified in the case of uncoated lemon fruits, where a 56.9% and 30% reduction in vitamin C was reported after 30 days of storage at 30 °C and 4 °C, respectively. Compared to them, EPS-coated fruits showed a 42.4% and 66.7% lesser decline in vitamin C at 30 °C and 4 °C, respectively. Moreover, it showed a better reduction in vitamin C loss than the positive control, paraffin coating (Figure 10a). Furthermore, preservation potential was found to be higher at 4 °C compared to 30 °C. Therefore, EPS was found to be efficacious in restoring the vitamin C content in coated lemons. For such preservation action, EPS coating was reported to reduce oxygen diffusion by acting as a barrier and preventing vitamin C loss due to oxidation [32,68].

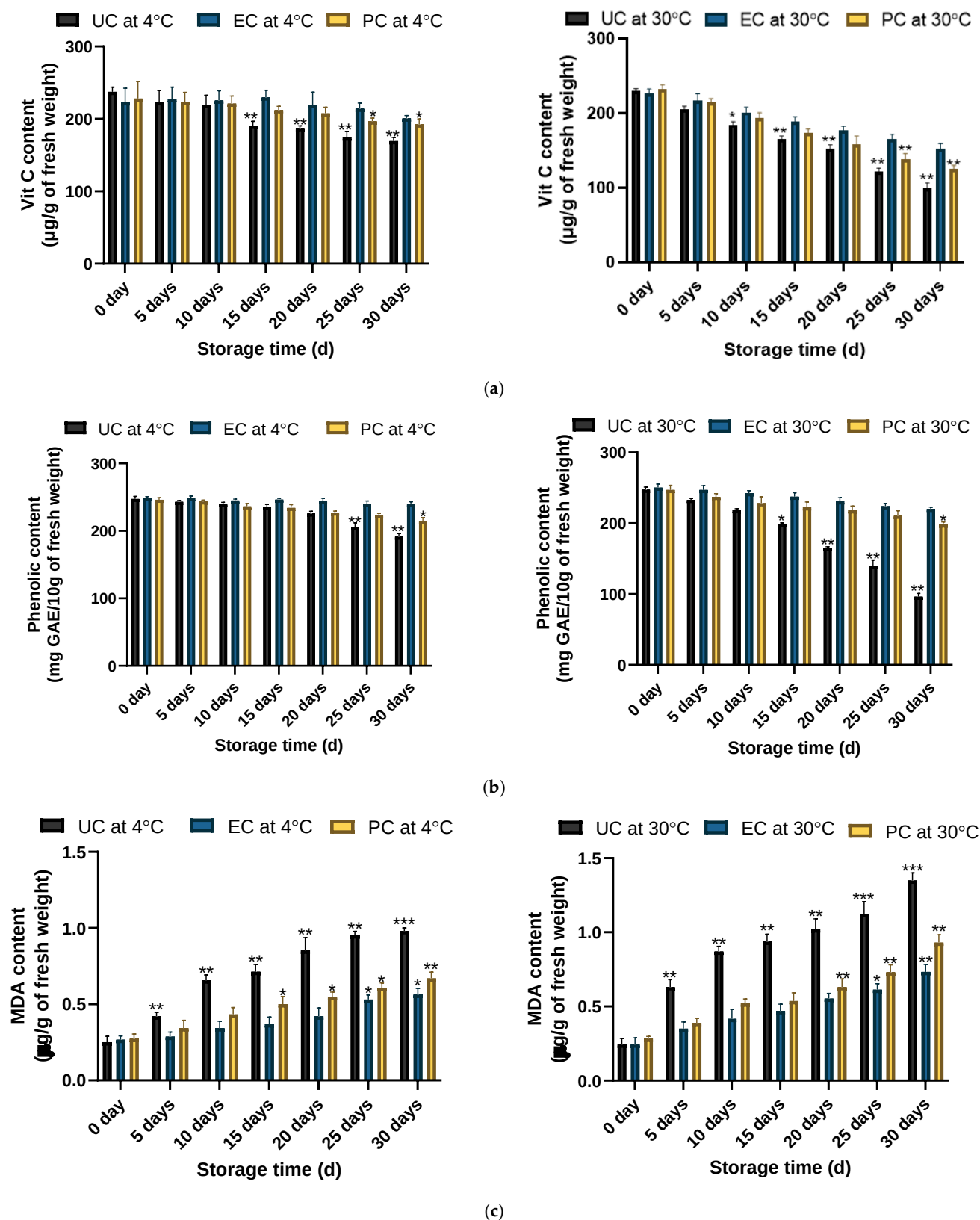


Figure 10. Effect of EPS coating on biochemical quality parameters (a) vitamin C content; (b) phenolic compound content, and (c) MDA content of lemon fruits [Results are represented as Mean $\pm$ SD and significant variations are denoted as * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$].

Phenolic Compounds Content

Phenolic content is associated with the high quality of fruits, as they contribute to overall texture, firmness, flavour profile, and antioxidant properties. Fruits with higher phenolic content show high antioxidant potential. Prolonged storage mainly reduces the phenolic content of fruits. In uncoated lemons, a non-significant decline in phenolic content was reported up to 10 days at 30 °C storage and 20 days at 4 °C storage, after which phenolic content declined at a higher rate up to 30 days. The EPS coating demonstrated the preservation of phenolic content throughout the storage period under both conditions. At 4 °C storage, uncoated fruits experienced a 22.64% decrease in phenolic content, whereas EPS-coated lemons showed only a 3.57% reduction. A more significant decrease in phenolic compounds was reported in lemons stored at 30 °C, with a 12.00% decrease in EPS-coated lemons compared to uncoated lemons, representing a 61.13% reduction (Figure 10b). A similar outcome was obtained in coated strawberry fruits. Zebua et al. (2025) [33] reported the preserving effect of a biological coating on strawberry fruits, with a significantly smaller decrease in phenolic content compared to uncoated fruits. Some fruits show a reduction in phenolic content during prolonged storage. In contrast, some fruits undergo the ripening process during storage and are reported to have an increase in phenolic content as a result of ripening [69].

MDA Content

MDA content is an indicator of lipid peroxidation, whose levels increase during storage. Uncoated fruits were reported to have increased MDA content from the 5th day onwards, up to 30 days in both 30 °C and 4 °C storage conditions. The EPS coating decreased MDA synthesis and lipid peroxidation up to 25 days in both storage conditions, viz., 60.6% lower MDA compared to uncoated control and 22.3% lower MDA compared to paraffin-coated controls stored at 4 °C (Figure 10c). Similarly, the least MDA increase was observed in EPS-coated lemons during 30 °C storage. These findings align with previous research, which shows a significant reduction in MDA accumulation in fortified chitosan-coated Xinyu Tangerine citrus fruits [34].

Based on previous findings, exopolysaccharide-like polymer-based coatings serve as a barrier that reduces respiration rate, prevents the deterioration of phenolic compounds, flavonoids, antioxidant pigments, and vitamins, thereby helping to preserve the quality of fruits and vegetables [70].

3.5.4. Effect of EPS Coating on Citrus Canker Disease by Assessing % Disease Incidence

All groups of fruits were treated with an inoculum of *X. citri* and evaluated for canker development after 10 to 15 days of incubation or storage at both 4 °C and 30 °C. This treatment showed a similar low disease incidence at 4 °C compared to 30 °C. The EPS coating provided 89% and 70.4% protection against the onset of citrus canker at 4 °C and 30 °C, respectively. EPS-coated lemons showed 68.64% and 67.03% lower disease incidence than UC- and PC-treated fruits at 30 °C. In contrast, the paraffin wax coating cannot significantly inhibit disease incidence compared to the uncoated fruits at 30 °C. At 4 °C, PC-coated fruits exhibited a 74.29% higher disease incidence compared to EC (Figure 11). Consequently, the presented halophilic EPS showed more promising effects than paraffin wax and potentially helped to reduce the incidence of citrus canker disease in lemon fruits. Based on the literature review, a similar assessment of the percentage of disease incidence mitigation by microbial EPS after forced inoculation of phytopathogen *X. citri* was not reported to the best of our knowledge. The anti-phytopathogenic potential of *Bacillus* sp. EPS against fungal pathogens has been previously reported, demonstrating a protective effect and a reduction in postharvest losses in cherry tomatoes [29]. The halophilic EPS

coating had the potential to resist the onset of phytopathogenic infection and, hence, is a promising candidate to be used in the food industry. It exerts such effects by acting as a barrier that possesses bioactive reactive groups, which inhibit the interaction and growth of phytopathogens over the surface of coated fruits. However, the exact mechanism driving this antagonistic response to pathogens is yet to be fully understood.

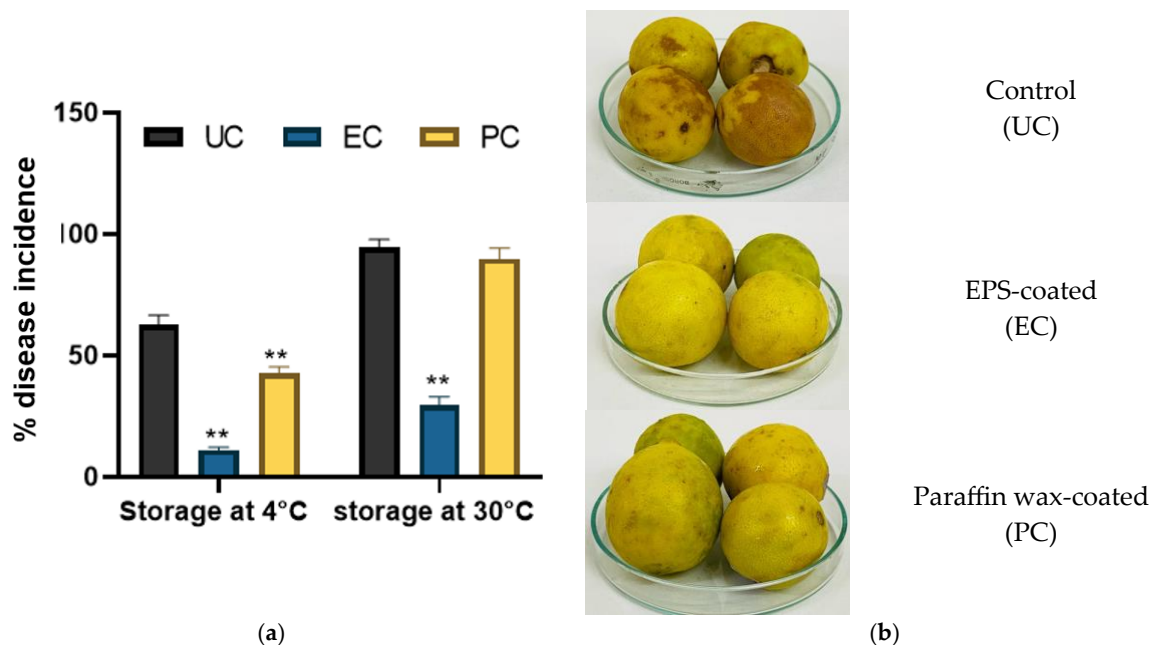


Figure 11. Effect of EPS coating on % disease incidence of *Xanthomonas citri* in lemon fruits stored at 4 °C and 30 °C temperatures (a) % disease incidence, and (b) Fruits after 15 days incubation at 30 °C [Results are represented as Mean $\pm$ SD and significant variations are denoted as ** for $p < 0.01$].

4. Conclusions

The presented study concluded that the screened isolate was a potent producer of exopolysaccharide, which had good stability and growth-inhibitory effects on *Xanthomonas citri*. The produced EPS was characterized as a heteropolymer with bioactive properties, such as antimicrobial and pseudoplastic nature, with no cytotoxicity, making it a suitable and safe candidate for edible coating formation. The halophilic EPS-coated lemons demonstrated a shelf-life extension by reducing the % weight loss by 61.9%, firmness loss by 75.77%, and titratable acidity reduction by 71.48% compared to uncoated lemons at 30 °C. EPS-coated (EC) lemons showed a shelf life of 26.6 ± 1.14 days at 4 °C and 18.0 ± 1.41 days at 30 °C, which increased by 62.19% and 109.30%, respectively, under these storage conditions compared to uncoated lemons. Additionally, the EPS coating preserved vitamin C and phenolic compounds and prevented lipid peroxidation in the coated fruits, thereby maintaining their quality. The % disease incidence reduction on lemon fruits was determined by force inoculation of *X. citri* in all three treatments, which revealed a 68.64% and 67.03% decrease in disease incidence compared to uncoated and paraffin wax-coated lemons incubated at 30 °C. The breakthrough of the presented research was the growth-inhibitory bioactive action of EPS in protecting lemon fruits during prolonged storage. Therefore, halophilic EPS has been identified as a promising candidate for current and future use as an edible coating material in postharvest applications to extend the shelf life of citrus fruits in the food industry.

Author Contributions: Methodology, C.U.; Software, T.U.; Validation, H.P.; Formal analysis, C.U.; Investigation, C.U., H.P. and T.U.; Resources, I.P., T.U. and H.P.; Writing—original draft, C.U., H.P. and T.U.; Writing—review and editing, T.U. and H.P.; Supervision, H.P.; Funding acquisition, T.U. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank P P Savani University, Surat, for supporting the research. The authors would also like to acknowledge Charotar University of Science & Technology (CHARUSAT), Changa, Anand, India, for funding the publication under the CHARUSAT Employee Development and Research Support Scheme.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting this study are included within the article.

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

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