polymers

MDPI

Article

Antiseptic Potential of *Lactiplantibacillus plantarum*-Derived Postbiotic-Loaded Polymeric Gels: Comparative Evaluation of Chitosan and Carbopol Matrices

Pervin Soyer [1] and A. Alper Öztürk [2,*]

[1] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, Eskisehir 26470, Türkiye; pervinsoyer@anadolu.edu.tr
[2] Department of Pharmaceutical Technology, Faculty of Pharmacy, Anadolu University, Eskisehir 26470, Türkiye
* Correspondence: aaozturk@anadolu.edu.tr

Abstract

The development of multifunctional topical antiseptic formulations incorporating microbial-derived bioactive compounds represents a promising strategy for improving antimicrobial and antibiofilm performance. This study aimed to develop and comparatively evaluate chitosan- and Carbopol® 940-based antiseptic polymeric gels containing a *Lactiplantibacillus plantarum*-derived postbiotic. The postbiotic preparation was obtained through fermentation, cell removal, membrane filtration, and lyophilization and subsequently incorporated into polymeric gel formulations. The developed systems were characterized in terms of physicochemical and rheological properties, antimicrobial activity using agar well diffusion and minimum inhibitory concentration (MIC) assays, inhibitory effects on biofilm formation against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and antioxidant activity using the DPPH radical scavenging assay. Both formulations exhibited homogeneous appearances and shear-thinning behavior, supporting their potential suitability for topical application. The chitosan-based postbiotic formulation generally demonstrated superior antimicrobial performance, producing inhibition zones of up to 22.4 mm and achieving a two-fold reduction in MIC values compared with the non-formulated postbiotic. Concentration-dependent antibiofilm activity was observed, with the chitosan-based formulation achieving maximum biofilm inhibition of 62.5% against *S. aureus* and 59.3% against *P. aeruginosa*. Antioxidant evaluation further demonstrated enhanced radical scavenging activity following postbiotic incorporation, with the chitosan-based formulation reaching 72.0% DPPH inhibition and exhibiting the most favorable IC_{50} among the postbiotic-containing systems. The findings highlight the influence of polymeric matrix composition on the biological performance of postbiotic-containing polymeric gels and identify chitosan as a promising carrier for multifunctional topical antiseptic formulations. Further standardized antiseptic efficacy and safety studies are required to establish their practical applicability.

Keywords: *Lactiplantibacillus plantarum*; postbiotic; chitosan; Carbopol; antiseptic polymeric gel; antimicrobial activity; antibiofilm activity; antioxidant activity

Check for updates

Academic Editor: Xiaowen Shi

Received: 11 October 2026
Revised: 4 November 2026
Accepted: 5 November 2026
Published: 10 November 2026

Copyright: © 2026 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.

1. Introduction

Polymeric gels have emerged as versatile platforms for topical and biomedical applications due to their ability to incorporate bioactive compounds while providing desirable physicochemical properties, including spreadability, bioadhesion, local retention, and tunable rheological behavior. The polymeric matrix can influence the stability, diffusion, avail-

ability, and biological performance of incorporated substances. Consequently, polymers with intrinsic biological properties may contribute directly to formulation functionality rather than serving solely as inert carriers [1–3]. Postbiotics have attracted increasing interest as microbial-derived bioactive preparations that do not require the maintenance of viable microorganisms in the final formulation. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), postbiotics are preparations of inanimate microorganisms and/or their components that confer a health benefit on the host [4,5]. This definition emphasizes that postbiotics are complex preparations rather than individual purified metabolites and distinguishes them from probiotics, which require viable microorganisms. Their biological properties depend on the microbial strain, cultivation conditions, inactivation procedures, and downstream processing, which may influence the presence and activity of microbial components and associated metabolites [4,6–8].

Among lactic acid bacteria, *Lactiplantibacillus plantarum* represents a promising source of biologically active postbiotic preparations. Previous studies have demonstrated that *L. plantarum*-derived postbiotics exhibit antimicrobial and antioxidant activities, although their effectiveness varies according to the bacterial strain, cultivation medium, and preparation method. Chang et al. (2021) reported strain-dependent biological activities, while Khakpour et al. (2024) demonstrated the production of antimicrobial preparations using different culture media, including MRS broth, milk, and whey [9,10]. Further investigations have confirmed the multifunctional potential of these preparations [11,12]. Karaca et al. (2023) and Shaaban et al. (2025) reported antimicrobial and antibiofilm activities of *L. plantarum*-derived preparations against clinically relevant microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [13,14]. These findings, together with previous observations of biofilm inhibition [15], support the investigation of *L. plantarum* postbiotics as potential agents for controlling planktonic microbial growth and biofilm formation. The biological performance of postbiotic-containing formulations may be influenced by the polymeric carrier. Chitosan is a biodegradable, biocompatible, cationic polysaccharide with gel-forming, bioadhesive, antimicrobial, and antibiofilm properties. Its antimicrobial effects are commonly associated with electrostatic interactions between protonated amino groups and microbial surfaces [1,16]. Previous studies have demonstrated the feasibility of incorporating *L. plantarum*-derived postbiotics into chitosan-based systems while retaining biological functionality [15,16]. In contrast, Carbopol is a crosslinked poly(acrylic acid) polymer primarily used to improve the consistency, viscosity, and spreadability of semisolid pharmaceutical formulations [17–19]. Comparing these distinct polymeric matrices may therefore provide insight into formulation-dependent differences in postbiotic activity. In our previous study, Soyer and Öztürk (2026) developed chitosan- and Carbopol-based gel-serums incorporating *Lacticaseibacillus casei*-derived postbiotic and paraprobiotic fractions and demonstrated polymer-dependent differences in physicochemical, rheological, antimicrobial, antibiofilm, and antioxidant properties [20]. However, these findings cannot be directly generalized to *L. plantarum*, as postbiotic composition and biological activity depend on the producing microorganism and preparation conditions. Furthermore, differences in MIC values and inhibition zones may indicate formulation-associated effects but do not independently establish antimicrobial synergy [21–23]. The inclusion of corresponding blank formulations is therefore important for interpreting the contribution of the polymeric matrix. The potential application of postbiotic-containing polymeric gels in topical microbial control is supported by studies investigating lactobacilli-derived preparations in skin-related experimental models [24]. In addition to antimicrobial activity, inhibition of biofilm formation and antioxidant properties may contribute to the multifunctional potential of these formulations [6,9]. However, direct comparative investigations of chitosan- and Carbopol-based polymeric gels containing the same *L. plantarum*-derived

postbiotic remain limited, particularly when antimicrobial, antibiofilm, and antioxidant activities are evaluated alongside corresponding blank formulations [25]. Accordingly, this study aimed to develop and comparatively evaluate chitosan- and Carbopol-based polymeric gels containing an *L. plantarum*-derived postbiotic. Antimicrobial activity was assessed using agar well diffusion and minimum inhibitory concentration (MIC) assays, while inhibition of biofilm formation against *S. aureus* and *P. aeruginosa* was evaluated using concentration-dependent assays and $MBIC_{50}$ determination. Antioxidant activity was investigated using DPPH and ABTS radical scavenging assays [26]. The study aimed to determine how polymer composition influences the observed biological performance of postbiotic-containing polymeric gels and to explore their potential as multifunctional topical antiseptic formulations.

Although our previous research investigated *Lacticaseibacillus casei*-derived postbiotic and paraprobiotic preparations incorporated into polymeric gel formulations, the present study addresses a distinct scientific question by focusing on the formulation-dependent biological performance of *Lactiplantibacillus plantarum*-derived postbiotics. Given the strain-dependent composition and functional properties of postbiotic preparations, findings obtained using *L. casei* cannot necessarily be extrapolated to *L. plantarum*. Therefore, this study aimed to investigate whether incorporation of an *L. plantarum*-derived postbiotic into chitosan- and Carbopol-based formulations influences its antimicrobial, antibiofilm, and in vitro antioxidant activities. We hypothesized that the physicochemical characteristics and overall composition of the two formulations would result in different biological responses compared with the purified postbiotic. By integrating physicochemical, rheological, and biological evaluations, this study provides a formulation-specific assessment of *L. plantarum*-derived postbiotics as potential candidates for topical applications.

2. Materials and Methods

2.1. Materials

The *Lactiplantibacillus plantarum*-derived postbiotic was prepared as described in the following section. High-molecular-weight chitosan (Sigma-Aldrich, St. Louis, MO, USA; molecular weight: 310–375 kDa; degree of deacetylation: >75%; product no. 419419) and Carbopol® 940 (Zag Kimya, Istanbul, Türkiye) were used as gel-forming polymers. Citric acid monohydrate, polyethylene glycol 400 (PEG 400), urea, triethanolamine, and ammonium benzoate (Merck, Darmstadt, Germany), together with sorbitol, polysorbate 20, and sodium salicylate (Sigma-Aldrich, St. Louis, MO, USA), were used as formulation components. Ultrapure water was used for all aqueous preparations.The test microorganisms included *Bacillus subtilis* NRRL B-4378, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, and *Candida krusei* ATCC 6258. *S. aureus* and *P. aeruginosa* were additionally used for antibiofilm assays. De Man, Rogosa, and Sharpe (MRS) broth and agar (Merck, Darmstadt, Germany) were used for *L. plantarum* cultivation and viability assessment. Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB) (Merck, Darmstadt, Germany) were used for antibacterial assays. Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB), and RPMI 1640 medium (Merck, Darmstadt, Germany) were used, as appropriate, for antifungal testing.Ciprofloxacin and amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) served as antibacterial and antifungal reference agents, respectively. Crystal violet, phosphate-buffered saline (PBS), and acetic acid (Sigma-Aldrich, St. Louis, MO, USA) were used for biofilm quantification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) and methanol (Merck, Darmstadt, Germany) were used for antioxidant analysis, with gallic acid (Sigma-Aldrich, St. Louis, MO, USA) as the reference antioxidant. Unless otherwise specified, all reagents were of analytical grade.

2.2. Preparation of Lactiplantibacillus plantarum Postbiotic

The *Lactiplantibacillus plantarum* postbiotic was prepared using a modified cell-free supernatant (CFS) approach based on previously described postbiotic preparation procedures [6,20]. Briefly, *L. plantarum* was initially activated in MRS broth at 37 °C for 18–24 h under static conditions. The activated bacterial culture was standardized to approximately 1×10^8 CFU/mL and subsequently inoculated into fresh MRS broth at 2% (v/v). The culture was incubated at 37 °C for 48 h to promote the accumulation of extracellular microbial metabolites. Following fermentation, the bacterial culture was centrifuged at 10,000× g for 15 min at 4 °C to separate bacterial cells from the culture supernatant. The collected supernatant was subjected to a second centrifugation under the same conditions to further remove residual cellular material. The clarified supernatant was then passed through a sterile 0.22-µm membrane filter under aseptic conditions to obtain a cell-free postbiotic fraction. Cell-free supernatant preparation and verification of the absence of viable cells were performed with modifications from the procedure described by Almahbashi and Gunes Altuntas (2025) [6]. To confirm the absence of viable *L. plantarum* cells, 100 µL of the filtered preparation was spread onto MRS agar and incubated at 37 °C for 48 h. The absence of colony formation after incubation was considered confirmation that the preparation contained no detectable viable bacterial cells. A comparable culture-based viability control has been used for CFS-derived postbiotic preparations. The cell-free preparation was subsequently frozen at −80 °C and lyophilized to obtain a dry postbiotic powder. The lyophilized material was weighed and stored in sterile, light-protected containers at −20 °C until further use. Before formulation preparation and biological assays, the lyophilized postbiotic was reconstituted in sterile distilled water at the required concentrations. Concentrations used throughout the antimicrobial, antibiofilm, and antioxidant assays were expressed as µg/mL based on the dry mass of the lyophilized preparation. The resulting lyophilized preparation was considered a complex fermentation-derived postbiotic fraction rather than a chemically defined active ingredient. Accordingly, the preparation may contain soluble microbial metabolites together with residual constituents originating from the culture medium. The biological activities evaluated in this study therefore represent the overall effects of the recovered preparation rather than those of individually identified metabolites.

2.3. Confirmation of the Absence of Viable Cells

The absence of viable *Lactiplantibacillus plantarum* cells in the postbiotic preparation was verified using a culture-based viability assay, with modifications from previously described procedures for postbiotic preparation [6,20,27]. Following the inactivation and preparation process, 100 µL of the postbiotic preparation was aseptically spread onto de Man–Rogosa–Sharpe (MRS) agar plates. The plates were incubated at 37 °C for 48 h. Untreated viable *L. plantarum* culture was inoculated onto MRS agar under the same conditions and used as a positive growth control, whereas uninoculated MRS agar served as a sterility control. After incubation, the plates were visually examined for bacterial colony formation. The complete absence of colony growth was considered confirmation that no culturable viable *L. plantarum* cells remained in the postbiotic preparation. Only preparations showing no detectable growth were used for subsequent formulation and biological activity studies. Culture-based viability testing was performed using 100 µL of the filtered cell-free supernatant collected before lyophilization. The sample was assessed for detectable microbial growth under the specified culture conditions. The final lyophilized and reconstituted preparation was not separately subjected to viability testing.

2.4. Selection of Polymer Concentrations for Antiseptic Gel Formulation

Prior to preparation of the final postbiotic-containing formulations, high-molecular-weight (HMW) chitosan and Carbopol were each screened at concentrations of 0.5, 1.0, and 1.5% (*w*/*w*) to identify gel systems with suitable consistency and spreading characteristics for topical application. Chitosan was gradually dispersed in an aqueous citric acid solution under continuous magnetic stirring until complete hydration and a visually homogeneous gel were obtained. Carbopol was dispersed in purified water, allowed to hydrate, and subsequently neutralized to induce gel formation. The preliminary formulations were evaluated for visual homogeneity, gel consistency, ease of spreading, visible polymer aggregates, and phase separation. Polymer concentrations providing the most appropriate balance between gel consistency, homogeneity, and spreadability were selected for further studies. The selected concentration of each polymer was maintained in the corresponding blank and *Lactiplantibacillus plantarum* postbiotic-containing polymeric gels to assess the effect of postbiotic incorporation on physicochemical characteristics and biological activity.

2.5. Preparation of Carbopol®- and Chitosan-Based Antiseptic Polymeric Gels

Carbopol® 940 and high-molecular-weight (HMW) chitosan were used as gel-forming polymers at a final concentration of 1.5% (*w*/*w*), as selected during the preliminary formulation studies. For preparation of the Carbopol®-based polymeric gels, Carbopol® 940 was gradually added to purified water under magnetic stirring and allowed to hydrate completely. Sorbitol, polyethylene glycol 400 (PEG 400), glycerol, sodium salicylate, ammonium benzoate, and polysorbate 20 were subsequently added in sequence, and mixing was continued to obtain a homogeneous base dispersion. The dispersion was then neutralized by controlled addition of triethanolamine (TEA) under stirring to promote gel formation. For preparation of the chitosan-based polymeric gels, citric acid monohydrate was first completely dissolved in purified water to provide an acidic medium for chitosan dissolution. HMW chitosan was gradually added to this solution under magnetic stirring to prevent agglomeration. Stirring was continued until a homogeneous appearance was obtained, after which the dispersion was allowed to equilibrate at room temperature to ensure complete polymer hydration. Sorbitol, PEG 400, glycerol, HPMC (0.25%, *w*/*w*), sodium salicylate, ammonium benzoate, and polysorbate 20 were subsequently incorporated under stirring to obtain a homogeneous gel base. The *Lactiplantibacillus plantarum*-derived postbiotic preparation was added to the respective gel bases at a final concentration of 5.0% (*w*/*w*) and mixed at low speed until a macroscopically homogeneous distribution was obtained. Corresponding blank polymeric gels were prepared without postbiotic. Purified water was used to bring each formulation to its final mass. Following preparation, formulation pH was checked, and all polymeric gels were visually inspected for homogeneity, consistency, visible aggregates, and phase separation. The formulations were transferred to tightly closed containers and stored until subsequent physicochemical characterization and biological activity studies [28–31]. Both postbiotic-loaded chitosan and Carbopol polymeric gels contained 5% (*w*/*w*) lyophilized *Lactiplantibacillus plantarum*-derived postbiotic. The concentrations of postbiotic-containing formulations used in the biological assays were expressed as postbiotic dry-mass equivalents (µg/mL), rather than total gel mass. Accordingly, the required formulation quantities were calculated based on the 5% (*w*/*w*) postbiotic loading. For example, a postbiotic concentration of 3125 µg/mL corresponded to a total gel concentration of 62,500 µg/mL. This normalization ensured that the purified postbiotic and postbiotic-loaded formulations were compared at equivalent nominal postbiotic concentrations.

2.6. Physicochemical and Rheological Characterization of Postbiotic-Containing Antiseptic Polymeric Gels

The physicochemical characteristics of the chitosan- and Carbopol®-based formulations were evaluated to assess differences between the polymeric matrices and changes associated with incorporation of the *Lactiplantibacillus plantarum*-derived postbiotic preparation. The blank and postbiotic-containing polymeric gels were characterized in terms of macroscopic appearance, pH, rheological behavior, and spreadability. These parameters are commonly used to evaluate semisolid topical formulations, as formulation composition may influence gel homogeneity, viscosity, flow behavior, and spreading characteristics. Unless otherwise stated, all quantitative measurements were performed in triplicate, and the results were expressed as mean ± standard deviation [28,32–35].

2.6.1. Determination of Formulation pH

The pH of the formulations was determined after dispersion in distilled water using a literature-based procedure [32,33,36]. An accurately weighed amount of each gel (1 g) was dispersed in distilled water (10 mL) under identical conditions for all formulations. Measurements were performed at room temperature using a digital pH meter calibrated with standard buffer solutions prior to analysis. After equilibration, the electrode was immersed in the dispersion, and the pH was recorded once the reading had stabilized. The electrode was rinsed with distilled water and gently blotted between measurements. Each formulation was analyzed in triplicate, and the results were expressed as mean ± standard deviation. The reported values represent the pH of the aqueous dispersions under the specified measurement conditions.

2.6.2. Flow Behavior and Rheological Properties

The rheological properties of the formulations were evaluated using a rotational rheometer at 25 ± 1 °C. Each gel was carefully loaded onto the measuring system while avoiding air bubbles and allowed to equilibrate before measurement. Flow measurements were performed over a predetermined shear-rate range, and the corresponding shear stress and apparent viscosity values were recorded. Flow behavior was assessed using shear stress–shear rate and apparent viscosity–shear rate curves. The experimental data were fitted to the Herschel–Bulkley model using the instrument software to obtain the consistency index, flow behavior index, and yield stress. The software-reported confidence of fit (CoF) was also recorded. A flow behavior index below unity was interpreted as shear-thinning behavior. Model-derived apparent viscosities at 50, 100, and 300 s^{-1} were calculated within the measured shear-rate range. The apparent viscosity ratio between 50 and 300 s^{-1} and the percentage decrease in apparent viscosity over this interval were calculated to quantify shear-dependent thinning. Rheological measurements were performed in triplicate, and the blank and postbiotic-containing polymeric gels were compared to assess formulation-associated differences in flow properties [28,33,37].

2.6.3. Organoleptic Properties and Physical Homogeneity

The macroscopic characteristics of the prepared formulations were evaluated by visual examination under standardized laboratory conditions. Each formulation was inspected for color, appearance, consistency, homogeneity, visible aggregates or particulate matter, and evidence of phase separation. The blank polymer matrices and their corresponding postbiotic-containing formulations were compared to identify visible changes associated with incorporation of the *L. plantarum* postbiotic. Macroscopic homogeneity was assessed by examining different portions of each formulation for uniform appearance and the absence of visible polymer agglomerates or particulate clusters. Syneresis and visible phase

separation were also recorded. These observations provided a preliminary assessment of the macroscopic uniformity of the prepared polymeric gels [32,33].

2.7. Antimicrobial Activity

2.7.1. Agar Well Diffusion Assay

The antimicrobial activities of the *Lactiplantibacillus plantarum* postbiotic and the polymeric formulations were initially evaluated using the agar well diffusion method [20,38]. The test microorganisms included the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the yeasts *Candida albicans* and *Candida krusei*. Fresh bacterial cultures were suspended in sterile 0.85% (*w*/*v*) NaCl and adjusted to a 0.5 McFarland turbidity standard, corresponding to approximately 1–2 × 10^8 CFU/mL. Yeast suspensions were separately prepared from fresh cultures and standardized to 0.5 McFarland before use. For the diffusion assay, 100 µL of each standardized microbial suspension was uniformly distributed over the surface of the corresponding agar medium. MHA was used for bacterial strains, whereas SDA was used for *Candida* spp. After inoculation, 6-mm-diameter wells were aseptically prepared in the agar using a sterile cork borer. The *L. plantarum* postbiotic, chitosan blank formula, Carbopol blank formula, chitosan + *L. plantarum* postbiotic formula, and Carbopol + *L. plantarum* postbiotic formula were tested at 12,500 µg/mL. An aliquot of 100 µL of each sample was transferred into the corresponding well. Ciprofloxacin was used as the antibacterial positive control, whereas amphotericin B was used as the antifungal positive control. After allowing the samples to diffuse into the agar for approximately 15–30 min, bacterial plates were incubated at 35–37 °C for 18–24 h and yeast plates were incubated under the corresponding incubation conditions for 24–48 h. Following incubation, the total diameter of the inhibition zone, excluding the 6-mm well diameter, was measured in millimeters. The results were expressed as mean ± standard deviation (SD). To quantify the change in diffusion-based antimicrobial activity following incorporation of the postbiotic into the polymeric formulations, inhibition-zone enhancement was calculated relative to the non-formulated postbiotic as follows (Equation (1)):

$$\text{Zone enhancement (\%)} = \frac{Z_f - Z_p}{Z_p} \times 100 \quad (1)$$

where Z_f is the mean inhibition-zone diameter of the postbiotic-containing formulation and Z_p is the mean inhibition-zone diameter of the non-formulated *L. plantarum* postbiotic. Zone enhancement was used as a comparative index of formulation-associated changes in agar diffusion activity and was not considered evidence of antimicrobial synergy.

2.7.2. Minimum Inhibitory Concentration (MIC)

The MICs of the *L. plantarum* postbiotic and polymeric formulations were determined using the broth microdilution method in sterile 96-well microplates according to the general principles of the Clinical and Laboratory Standards Institute (CLSI) reference methods for aerobic bacteria and yeasts [39]. MICs were determined using a broth microdilution method based on the general principles of CLSI M07 for bacteria and CLSI M27 for yeasts, with modifications according to the experimental conditions. The *L. plantarum* postbiotic, chitosan blank formula, Carbopol blank formula, chitosan + *L. plantarum* postbiotic formula, and Carbopol + *L. plantarum* postbiotic formula were evaluated separately. For bacterial MIC determination, fresh colonies were suspended in sterile 0.85% (*w*/*v*) NaCl and adjusted to 0.5 McFarland, corresponding to approximately 1–2 × 10^8 CFU/mL. The standardized suspension was subsequently diluted in MHB to obtain a working inoculum of approximately 1 × 10^6 CFU/mL. Following addition to the test wells at a 1:1 ratio, the

final bacterial density was approximately 5×10^5 CFU/mL. Two-fold serial dilutions of each test sample were prepared to obtain final concentrations of 12,500, 6250, 3125, 1562.5, and 781.25 µg/mL. Briefly, 100 µL of the 2× sample solution was combined with 100 µL of the bacterial working inoculum in each well, resulting in a final assay volume of 200 µL. Thus, the final sample concentrations ranged from 781.25 to 12,500 µg/mL and the final bacterial inoculum was approximately 5×10^5 CFU/mL. For *C. albicans* and *C. krusei*, fresh yeast suspensions were adjusted to 0.5 McFarland and subsequently diluted in the appropriate broth according to the CLSI M27 broth microdilution principle to obtain a final inoculum of approximately 0.5–2.5 × 10^3 CFU/mL in each well. As in the bacterial assay, 100 µL of the 2× sample dilution was mixed with 100 µL of the standardized yeast inoculum, giving a final volume of 200 µL per well and final sample concentrations of 781.25–12,500 µg/mL. Growth controls containing microbial inoculum without test sample and sterility controls containing uninoculated broth were included in each experiment. In addition, sample-specific blanks containing the corresponding concentration of the postbiotic or polymeric formulation without microbial inoculum were included to account for intrinsic turbidity associated with the formulations. Ciprofloxacin and amphotericin B were used as antibacterial and antifungal reference compounds, respectively. Bacterial microplates were incubated at 35–37 °C for 18–24 h, whereas yeast microplates were incubated at approximately 35 °C and evaluated after 24 h, with further incubation when required for adequate growth of the control. The MIC was defined as the lowest tested concentration that inhibited microbial growth relative to the corresponding growth control. When inhibition was not achieved within the tested concentration range, the MIC was reported as greater than the highest relevant concentration evaluated rather than extrapolated beyond the experimental range. To quantitatively compare the antimicrobial activity of the postbiotic-loaded formulations with that of the non-formulated postbiotic, MIC fold improvement was calculated as follows (Equation (2)):

$$\text{MIC fold improvement} = \frac{\text{MIC}_\text{p}}{\text{MIC}_\text{f}} \quad (2)$$

where MIC_p represents the MIC of the non-formulated *L. plantarum* postbiotic and MIC_f represents the MIC of the corresponding postbiotic-loaded polymeric formulation. A value greater than 1 indicated a reduction in MIC following incorporation into the polymeric matrix. Exact fold improvement was not calculated when an MIC was expressed only as greater than the highest concentration tested.

2.8. Antibiofilm Activity

The antibiofilm activities of the free *Lactiplantibacillus plantarum* postbiotic and the corresponding polymeric formulations were evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using a 96-well microplate crystal violet assay. The experiments were designed to evaluate the inhibition of biofilm formation. The *L. plantarum* postbiotic, chitosan blank formula, Carbopol blank formula, chitosan + *L. plantarum* postbiotic formula, and Carbopol + *L. plantarum* postbiotic formula were evaluated separately.

2.8.1. Biofilm Formation Inhibition Assay

The ability of the test samples to inhibit biofilm formation was determined using a crystal violet microplate assay [40,41]. It should be noted that the crystal violet assay quantifies attached biofilm biomass without distinguishing viable cells, non-viable cells, and extracellular matrix components. Therefore, the observed reductions should be interpreted as inhibition of biofilm formation or decreases in attached biomass rather than direct killing or eradication of biofilm-associated microorganisms. Complementary viability-based and imaging assays would be valuable for further characterizing the antibiofilm effects of these

formulations. Fresh cultures of *S. aureus* and *P. aeruginosa* were suspended in sterile 0.85% (w/v) NaCl solution and adjusted to a 0.5 McFarland turbidity standard, corresponding to approximately 1–2 × 10^8 CFU/mL for bacterial suspensions. The standardized suspensions were subsequently diluted in the appropriate growth medium to obtain a working bacterial suspension of approximately 2 × 10^6 CFU/mL. Two-fold serial dilutions of each test sample were prepared to obtain final concentrations of 12,500, 6250, 3125, 1562.5, and 781.25 µg/mL. Test solutions were prepared at twice the desired final concentration. Briefly, 100 µL of each 2× sample dilution was transferred into the corresponding wells of a sterile flat-bottom 96-well microplate. Subsequently, 100 µL of the bacterial working suspension was added to each well, resulting in a final assay volume of 200 µL, a final bacterial density of approximately 1 × 10^6 CFU/mL, and the desired final sample concentrations. Growth-control wells contained 100 µL of sterile growth medium and 100 µL of the bacterial working suspension but no test sample. Sterility controls contained 200 µL of sterile medium without bacteria. Because the postbiotic and polymer-containing formulations could contribute to background absorbance, sample-specific blank wells containing the corresponding concentration of each test sample and sterile medium without bacterial inoculum were included. The microplates were incubated statically at 37 °C for 24 h to allow bacterial attachment and biofilm development in the presence or absence of the test samples. Following incubation, the planktonic phase was carefully aspirated without disturbing the attached biofilm. Each well was gently washed twice with sterile phosphate-buffered saline (PBS) to remove non-adherent cells and residual test material. The attached biofilms were stained by adding 200 µL of 0.1% (w/v) crystal violet solution to each well and incubating the plates at room temperature for 15 min. The crystal violet solution was subsequently removed, and the wells were gently washed with PBS until excess unbound dye was eliminated. The plates were air-dried, after which the biofilm-bound crystal violet was solubilized by adding 200 µL of 30% (v/v) acetic acid to each well. The absorbance was measured at 570 nm using a microplate reader. For each test concentration, the absorbance attributable to the sample itself was corrected using the corresponding sample-specific blank. Biofilm inhibition was calculated relative to the untreated biofilm growth control according to the following equation (Equation (3)):

$$\text{Biofilm inhibition } (\%) = \frac{OD_{control} - OD_{treated}}{OD_{control}} \times 100 \quad (3)$$

where $OD_{control}$ represents the absorbance of the untreated bacterial biofilm and $OD_{treated}$ represents the blank-corrected absorbance of the biofilm formed in the presence of the corresponding test sample. The results were expressed as percentage biofilm inhibition at each tested concentration.

2.8.2. Determination of $MBIC_{50}$

In addition to reporting concentration-dependent biofilm inhibition, the concentration required to inhibit biofilm formation by 50% ($MBIC_{50}$) was determined from the concentration–response data obtained in the biofilm formation inhibition assay. When 50% inhibition occurred between two consecutive tested concentrations, $MBIC_{50}$ was calculated by linear interpolation according to Equation (4):

$$MBIC_{50} = C_1 + \frac{50 - I_1}{I_2 - I_1}(C_2 - C_1) \quad (4)$$

where C_1 and C_2 represent the consecutive concentrations immediately below and above 50% biofilm inhibition, respectively, and I_1 and I_2 represent the corresponding percentage biofilm inhibition values. When 50% biofilm inhibition was not achieved at the highest concentration tested (12,500 µg/mL), the $MBIC_{50}$ was reported as >12,500 µg/mL. Values outside the experimentally evaluated concentration range were not extrapolated. Thus,

biofilm inhibition (%) represented the experimentally observed response at each individual concentration, whereas $MBIC_{50}$ provided a single quantitative parameter enabling comparison of the concentration-dependent antibiofilm activities of the free postbiotic and polymeric formulations. $MBIC_{50}$ values were evaluated by linear interpolation between the two experimentally tested concentrations immediately below and above 50% biofilm inhibition. These values were considered approximate results rather than precisely determined inhibitory concentrations, given the limited number of concentrations evaluated.

2.9. Determination of Antioxidant Activity by the DPPH Assay

The antioxidant activity of the *Lactiplantibacillus plantarum* postbiotic and the polymeric formulations was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with modifications to the method described by Brand-Williams et al. (1995) [42–45]. A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light until use. The chitosan blank formula, Carbopol blank formula, *L. plantarum* postbiotic, chitosan + *L. plantarum* postbiotic formula, and Carbopol + *L. plantarum* postbiotic formula were evaluated at final concentrations of 781.25, 1562.5, 3125, 6250, and 12,500 µg/mL. Gallic acid was used as the positive antioxidant control. For the assay, 100 µL of each sample prepared at twice the desired final concentration was mixed with 100 µL of 0.1 mM DPPH solution in a 96-well microplate, resulting in a final reaction volume of 200 µL. The reaction mixtures were incubated at room temperature for 30 min in the dark, after which absorbance was measured at 517 nm using a microplate reader. The DPPH control consisted of 100 µL of methanol and 100 µL of DPPH solution. To minimize potential interference from the intrinsic color or turbidity of the postbiotic and polymeric formulations, sample-specific blanks containing the corresponding sample without DPPH were included [38,46]. The absorbance of each sample blank was subtracted from the respective sample reading before calculation of radical scavenging activity. DPPH radical scavenging activity was calculated according to the following equation (Equation (5)) [47]:

$$\text{DPPH inhibition} = \frac{A_0 - A_s}{A_0} \times 100 \quad (5)$$

where A_0 represents the absorbance of the DPPH control and A_s represents the blank-corrected absorbance of the test sample.

The IC_{50} value, defined as the concentration required to achieve 50% DPPH radical inhibition, was determined from the concentration–response data by linear interpolation between the two consecutive concentrations immediately below and above 50% inhibition. IC_{50} values were not extrapolated beyond the experimentally evaluated concentration range. Accordingly, samples showing less than 50% inhibition at the highest concentration tested were reported as $IC_{50} > 12{,}500$ µg/mL, whereas samples exhibiting more than 50% inhibition at the lowest concentration were reported as $IC_{50} < 781.25$ µg/mL. The results were expressed as mean ± standard deviation (SD).

2.10. Determination of Antioxidant Activity by ABTS Assay

The antioxidant activity of *Lactiplantibacillus plantarum*-derived postbiotic and postbiotic-loaded chitosan and Carbopol polymeric gels was evaluated using the ABTS radical scavenging assay, following the method of Re et al. (1999) [48] with slight modifications. The ABTS radical cation (ABTS•+) was generated by mixing 2.5 mL of 7 mM ABTS solution with 44 µL of 140 mM potassium persulfate ($K_2S_2O_8$) and incubating overnight in the dark. The solution was subsequently diluted with deionized water to obtain an absorbance of 0.700–0.750 at 734 nm. Samples were diluted 1:10 and evaluated at postbiotic concentrations ranging from 781.25 to 12,500 µg/mL. Blank chitosan and Carbopol polymeric gels were also included as controls. Briefly, 100 µL of each sample was mixed with 1 mL of ABTS•+

working solution, and absorbance was measured at 734 nm after 2.5 min. ABTS radical scavenging activity was calculated as follows (Equation (6)) [48]:

$$\text{ABTS inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (6)$$

where $A_{control}$ and A_{sample} represent the control and blank-corrected sample absorbances, respectively. Trolox was used as the reference standard, and antioxidant capacity was expressed as mmol Trolox equivalents per gram of postbiotic (mmol TE/g) based on the calibration curve.

All concentrations used in the DPPH and ABTS assays were expressed on the basis of postbiotic dry mass (µg/mL). For postbiotic-loaded formulations, the tested concentrations were normalized to the actual postbiotic content rather than the total gel mass, ensuring equivalent postbiotic exposure across the purified postbiotic and postbiotic-containing formulations. Trolox equivalent antioxidant capacity (TEAC) was calculated using the Trolox calibration curve and expressed as mmol Trolox equivalents per gram of postbiotic dry mass (mmol TE/g postbiotic).

2.11. Statistical Analysis

Experimental results were expressed as mean ± standard deviation (SD). Differences among the purified *Lactiplantibacillus plantarum*-derived postbiotic, postbiotic-containing chitosan and Carbopol formulations, and their corresponding blank formulations were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, where applicable. For concentration-dependent antimicrobial, antibiofilm, and antioxidant assays, comparisons were performed separately at each tested concentration. Statistical significance was established at $p < 0.05$. Minimum inhibitory concentration (MIC) values were defined as the lowest tested concentrations preventing visible microbial growth. The minimum biofilm inhibitory concentration required to inhibit 50% of biofilm formation ($MBIC_{50}$) was calculated by linear interpolation between consecutive tested concentrations surrounding 50% inhibition, using the untreated biofilm control as the reference. Similarly, DPPH half-maximal inhibitory concentration (IC_{50}) values were calculated by linear interpolation from the concentration–response data. Values outside the investigated concentration range were reported as below or above the tested limits without extrapolation. DPPH and ABTS radical scavenging activities were expressed as percentage inhibition. Trolox equivalent antioxidant capacity (TEAC) was calculated from the ABTS inhibition values using the Trolox calibration equation and expressed as mmol Trolox equivalents per gram of postbiotic dry mass (mmol TE/g postbiotic). Concentrations of purified postbiotic and postbiotic-containing formulations were normalized to postbiotic dry-mass equivalents (µg/mL), rather than total formulation mass. The reported IC_{50} and $MBIC_{50}$ values were considered interpolation-based results, and no confidence intervals were assigned to these values.

3. Result and Discussion

The present study compares two complete formulation platforms rather than isolating the effect of the primary polymer. In addition to chitosan or Carbopol, the formulations differ in HPMC content, citric acid concentration, and the use of triethanolamine for neutralization. These compositional differences may contribute to the observed physicochemical and biological responses. Accordingly, the findings should be interpreted as formulation-dependent effects and cannot be attributed solely to chitosan versus Carbopol.

3.1. Selection of Polymer Concentrations

Preliminary screening of HMW chitosan and Carbopol® 940 at 0.5, 1.0, and 1.5% (*w*/*w*) was used to select polymer concentrations suitable for topical antiseptic gel development. Based on the qualitative assessment of consistency, macroscopic homogeneity, and ease of spreading, 1.5% (*w*/*w*) was selected for both polymers as providing the most suitable balance of these properties under the experimental conditions. This selection was specific to the investigated formulation systems and should not be regarded as a universally optimal polymer concentration. The selected concentration was maintained in the corresponding blank and *L. plantarum* postbiotic-containing polymeric gels for subsequent physicochemical and biological evaluation [30,31].

3.2. Development of Antiseptic Polymeric Gels

Carbopol® 940- and HMW chitosan-based polymeric gels containing 5.0% (*w*/*w*) *L. plantarum*-derived postbiotic were developed as candidate topical antiseptic formulations (Tables 1 and 2). The two polymers provided distinct formulation environments for evaluating the biological performance of the postbiotic preparation. Carbopol® 940 was selected for its gel-forming and rheology-modifying properties following neutralization with triethanolamine. In the chitosan-based formulations, citric acid facilitated polymer dissolution, while HPMC was included as a supplementary viscosity-modifying polymer. The use of chitosan together with HPMC and glycerol has previously been investigated in hydrogel development, supporting the technological rationale for this combination. Sorbitol and glycerol were included as humectants, while PEG 400 served as a hydrophilic formulation auxiliary with humectant and cosolvent functions. Polysorbate 20 was incorporated to facilitate wetting and uniform dispersion of the formulation components. Sodium salicylate and ammonium benzoate were included as part of the intended preservation system; however, their presence should also be considered when interpreting antimicrobial activity. Corresponding blank formulations enabled assessment of the contribution of the formulated vehicles. Because the chitosan blank and postbiotic-containing polymeric gels differed in citric acid concentration, differences between these formulations should be interpreted in conjunction with their measured pH rather than attributed exclusively to postbiotic incorporation [49–55].

Table 1. Composition of Carbopol®-based antiseptic formulations (% *w*/*w*).

Ingredient	PL-C-Blank	PL-C-PB
Carbopol® 940	1.50	1.50
Sorbitol	3.00	3.00
PEG 400	2.00	2.00
Glycerol	3.00	3.00
Sodium salicylate	0.50	0.50
Ammonium benzoate	0.30	0.30
Polysorbate 20	0.10	0.10
Triethanolamine	q.s.	q.s.
Postbiotic	–	5.00
Purified water	q.s. * to % 100.00	q.s. * to % 100.00

* q.s.: sufficient quantity to adjust the pH of the Carbopol-based formulation to a value comparable to that of the chitosan-based formulation.

Table 2. Composition of chitosan-based antiseptic formulations (% *w*/*w*).

Ingredient	PL-CS-Blank	PL-CS-PB
High-molecular-weight chitosan	1.50	1.50
Citric acid monohydrate	0.90	0.30
Sorbitol	1.00	1.00
PEG 400	2.00	2.00
Glycerol	3.00	3.00
Hydroxypropyl methylcellulose	0.25	0.25
Sodium salicylate	0.50	0.50
Ammonium benzoate	0.30	0.30
Polysorbate 20	0.10	0.10
Postbiotic	–	5.00
Purified water	q.s. * to % 100.00	q.s. * to % 100.00

* q.s.: sufficient quantity to adjust the pH of the chitosan-based formulation to a value comparable to that of the chitosan-based formulation.

3.3. Rheological Characterization of Antiseptic Gel Formulations

The postbiotic-containing Carbopol® and chitosan polymeric gels exhibited shear-thinning behavior, with flow behavior indices of 0.61 and 0.31, respectively (Table 3; Figure 1). The lower flow behavior index of PL-CS-PB indicated a more pronounced shear-dependent response. The software-reported CoF values were 100.0% for PL-C-PB and 99.3% for PL-CS-PB, indicating close agreement between the experimental data and the fitted curves within the evaluated range. Model-derived apparent viscosity decreased from 0.111 to 0.054 Pa·s for PL-C-PB and from 0.122 to 0.039 Pa·s for PL-CS-PB as the shear rate increased from 50 to 300 s^{-1}. These changes corresponded to viscosity reductions of 51.4% and 68.2%, respectively, and apparent viscosity ratios of 2.06 and 3.15 (Table 4). PL-CS-PB exhibited a slightly higher apparent viscosity at 50 s^{-1}, whereas PL-C-PB showed higher values at 100 and 300 s^{-1}. Therefore, the relative viscosity of the formulations depended on the applied shear rate. The different flow profiles may reflect the distinct structures and compositions of the polymeric matrices. Carbopol® 940 forms a hydrated, crosslinked network following neutralization, whereas HMW chitosan develops viscosity through hydration and interactions between polymer chains in an acidic medium. HPMC, present only in the chitosan formulation, may additionally influence chain interactions and resistance to flow. Under increasing shear, rearrangement and alignment of polymer chains may contribute to the more pronounced viscosity reduction observed for PL-CS-PB. However, these mechanisms were not directly investigated, and the differences cannot be attributed to polymer identity alone because the formulations also differed in their auxiliary components and acidification or neutralization conditions. For candidate topical antiseptic polymeric gels, shear-thinning behavior may facilitate distribution over the application surface by reducing resistance to flow during spreading. The stronger shear-dependent response of PL-CS-PB therefore represents a potentially useful application characteristic. Nevertheless, rheological findings should be interpreted alongside the measured spreadability and antimicrobial results, as they do not independently demonstrate surface coverage, residence time, or antiseptic efficacy [56–59]. The observed shear-thinning behavior indicates a decrease in apparent viscosity with increasing shear rate. However, this finding alone does not establish spreadability, uniform skin coverage, bioadhesion, or residence time. These properties were not quantitatively evaluated in this study and remain potential formulation advantages requiring further investigation.

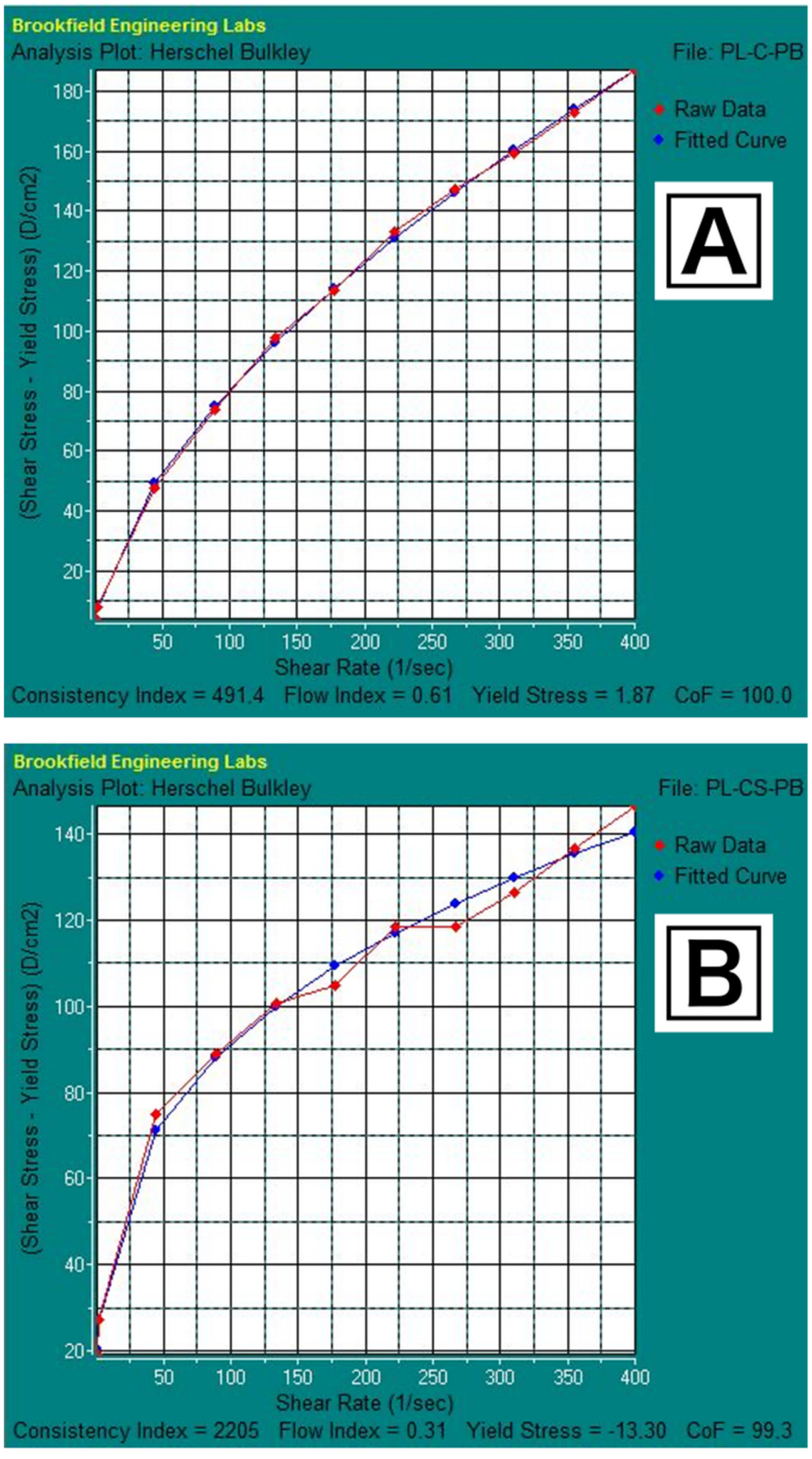


Figure 1. Herschel–Bulkley fits of the flow data for the postbiotic-containing (**A**) Carbopol® 940 gel (PL-C-PB) and (**B**) high-molecular-weight chitosan gel (PL-CS-PB). Red symbols represent experimental data, and blue symbols represent fitted values. The ordinate shows shear stress minus the fitted yield-stress parameter, as displayed by the instrument software. Software-reported confidence of fit (CoF) values were 100.0% and 99.3% for panels A and B, respectively; these values do not independently establish model appropriateness or the physical validity of the fitted parameters. The negative fitted yield-stress parameter in panel B represents a model-fitting limitation rather than a physical yield threshold. The fits are therefore interpreted descriptively within the investigated shear-rate range.

Table 3. Herschel–Bulkley rheological parameters of antiseptic polymeric gels.

Code	Consistency Index (K)	Flow Behavior Index (*n*)	Apparent Viscosity * at 50 s^{-1} (Pa·s)	Apparent Viscosity * at 100 s^{-1} (Pa·s)	Apparent Viscosity * at 300 s^{-1} (Pa·s)	CoF (%) *
PL-C-PB	0.491	0.61	0.111	0.083	0.054	100.0
PL-CS-PB	2.205	0.31	0.122	0.079	0.039	99.3

* Apparent viscosities are approximate values calculated using the complete Herschel–Bulkley fits. CoF: software-reported confidence of fit.

Table 4. Summary of shear-dependent flow characteristics of the postbiotic-containing antiseptic polymeric gels.

Code	Flow Behavior	Apparent Viscosity Ratio (η_{50}/η_{300}) *	Viscosity Decrease from 50 to 300 s^{-1} (%)	Rheological Characteristics
PL-C-PB	Shear-thinning	2.06	51.4	Less pronounced shear-dependent thinning; higher apparent viscosity at 100 and 300 s^{-1}
PL-CS-PB	Shear-thinning	3.15	68.2	More pronounced shear-dependent thinning; slightly higher apparent viscosity at 50 s^{-1}

* η_{50} and η_{300} denote model-derived apparent viscosities at shear rates of 50 and 300 s^{-1}, respectively. Viscosity ratios and percentage decreases were calculated using the complete Herschel–Bulkley fits.

3.4. pH of Antiseptic Gel Formulations

The pH was found to vary between 4.0 and 7.0 range, optimum for the skin treatment [60,61]. The postbiotic-containing Carbopol® and chitosan formulations exhibited acidic pH values of 4.24 ± 0.30 and 4.19 ± 0.10, respectively (Table 5). The similar values indicated a comparable acidic environment in both gel dispersions, with PL-CS-PB showing a slightly lower mean pH. The acidic profile is consistent with the preparation conditions and the potential contribution of fermentation-derived organic acids in the *L. plantarum* postbiotic preparation. Citric acid facilitated chitosan dissolution, while controlled neutralization with triethanolamine enabled formation of the Carbopol® gel. The resulting pH therefore reflects the combined influence of the microbial preparation and the formulation components. An acidic environment is relevant to topical formulation development and may also contribute to the antimicrobial performance of these candidate antiseptic polymeric gels. Previous studies have associated the activity of *L. plantarum*-derived preparations with organic acids and other microbial constituents. Accordingly, the observed pH values provide a coherent physicochemical basis for the postbiotic antiseptic gel concept and should be considered alongside the antimicrobial and formulation performance results [62–64]. These values represent the pH of the diluted dispersions and do not independently establish skin compatibility. Suitability for topical application depends on the application site, exposure duration, and complete formulation composition and requires confirmation through skin compatibility studies.

Table 5. pH values of the developed Carbopol®- and chitosan-based antiseptic gel formulations (mean ± SD, n = 3).

Formulation	pH
PL-C-PB	4.24 ± 0.3
PL-CS-PB	4.19 ± 0.1

3.5. Macroscopic Characteristics of Antiseptic Gel Formulations

Both postbiotic-containing formulations exhibited a light brown color, macroscopically homogeneous appearance, and gel consistency, with no visible phase separation or precipitation at the time of evaluation (Table 6). The light brown coloration was consistent with incorporation of the *L. plantarum*-derived postbiotic preparation. The uniform appearance and absence of visible separation or precipitation support the feasibility of incorporating the preparation into both Carbopol®- and chitosan-based matrices [65,66]. These macroscopic characteristics provide an initial technological basis for further evaluation of the formulations as candidate topical antiseptic polymeric gels.

Table 6. Macroscopic characteristics of antiseptic gel formulations *.

Code	Color	Homogeneity	Phase Separation	Precipitation	Consistency
PL-C-PB	light brown	+	None	None	gel
PL-CS-PB	light brown	+	None	None	gel

* The observed macroscopic homogeneity and absence of visible phase separation describe the formulations only at the time of evaluation and should not be interpreted as evidence of storage stability. No systematic stability study under defined storage conditions was performed. Changes in appearance, pH, rheological properties, and antimicrobial activity over time require evaluation before practical product suitability can be established.

3.6. Antimicrobial Activity

The antimicrobial activities of the purified *Lactiplantibacillus plantarum* postbiotic and the chitosan- and Carbopol-based formulations were evaluated against Gram-positive and Gram-negative bacteria and *Candida* spp. using agar diffusion and broth microdilution assays [67,68]. As shown in Figure 2, clear differences were observed among the blank polymer formulations, purified postbiotic, and postbiotic-loaded formulations. Overall, incorporation of the postbiotic into the polymeric matrices increased diffusion-based antimicrobial activity, with the postbiotic-containing chitosan formula showing the highest inhibition-zone diameters against most of the tested microorganisms.

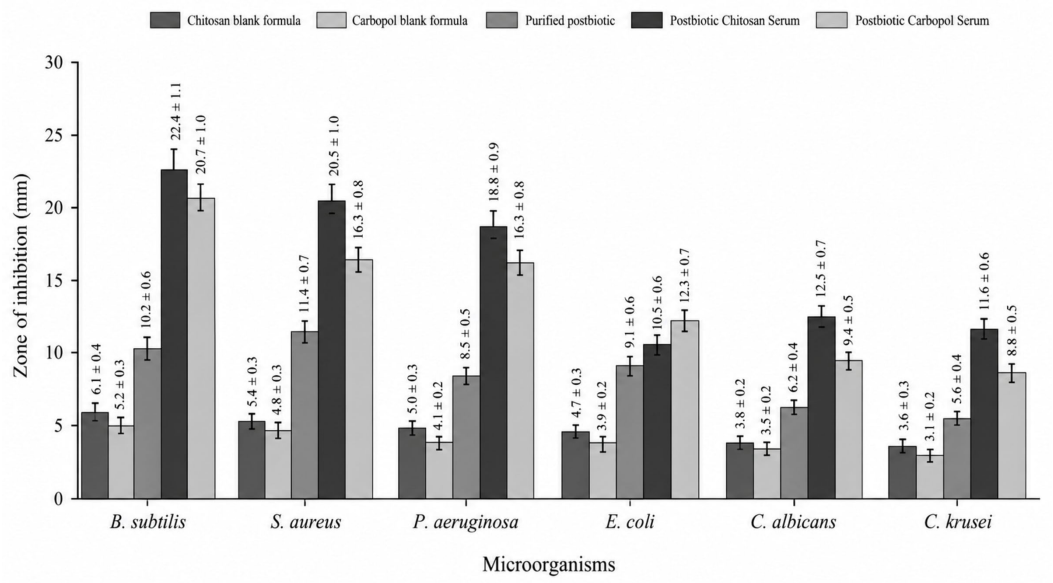


Figure 2. Antimicrobial activity of the chitosan blank formula, Carbopol blank formula, purified *Lactiplantibacillus plantarum* postbiotic, postbiotic-containing chitosan formula, and postbiotic-containing Carbopol formula against the tested microorganisms. Results are expressed as inhibition-zone diameter (mm; mean ± SD).

3.6.1. Agar Diffusion Activity

The *Lactiplantibacillus plantarum*-derived postbiotic exhibited antimicrobial activity against all six tested microorganisms, with net inhibition zones ranging from 5.6 ± 0.4 mm against *Candida krusei* to 11.4 ± 0.7 mm against *Staphylococcus aureus* (Figures 2 and 3). The chitosan and Carbopol® blank formulations exhibited lower inhibitory activity, producing net inhibition zones of 3.6–6.1 mm and 3.1–5.2 mm, respectively. Incorporation of the postbiotic into either polymeric matrix increased the inhibition zones against all tested microorganisms. The chitosan-based postbiotic gel showed the greatest activity against *Bacillus subtilis* (22.4 ± 1.1 mm), S. aureus (20.5 ± 1.0 mm), and *Pseudomonas aeruginosa* (18.8 ± 0.9 mm), whereas the corresponding Carbopol® formulation produced inhibition zones of 20.7 ± 1.0, 16.3 ± 0.8, and 16.3 ± 0.8 mm, respectively. The chitosan-based formulation also produced larger inhibition zones against both Candida species. However, the Carbopol®-based formulation exhibited greater activity against *Escherichia coli* (12.3 ± 0.7 mm) than the chitosan-based formulation (10.5 ± 0.6 mm). All inhibition-zone values represent net diameters after subtraction of the 6 mm agar well diameter.

The antimicrobial activity of the postbiotic preparation is consistent with previous findings demonstrating the inhibitory potential of *L. plantarum*-derived metabolites. Chang et al. (2021) reported antimicrobial activity in postbiotic preparations obtained from different *L. plantarum* strains and characterized their organic acid composition, highlighting the possible contribution of fermentation-derived metabolites to microbial inhibition [9]. Similarly, Butrungrod et al. (2023) demonstrated that postbiotic metabolites from *L. plantarum* PD18 exhibited activity against periodontal pathogens and affected biofilm-associated virulence characteristics [15]. These findings support the biological plausibility of the antimicrobial effects observed in the present study. Nevertheless, the specific compounds responsible for the activity of the current postbiotic preparation were not chemically identified, and their individual contributions cannot be established from agar diffusion results alone. The greater inhibition-zone diameters obtained with the chitosan-based postbiotic gel may be associated with the antimicrobial characteristics of chitosan in addition to the activity of the incorporated postbiotic. Chitosan is a cationic polysaccharide whose protonated amino groups can interact with negatively charged microbial surfaces, potentially affecting cell envelope integrity and permeability. Its antimicrobial properties are influenced by several factors, including molecular weight, degree of deacetylation, concentration, and environmental pH [69]. Furthermore, Thirupathi et al. (2023) highlighted the potential of chitosan-based hydrogels as antimicrobial and antibiofilm delivery systems [1]. In the present study, the chitosan blank formulation itself exhibited measurable inhibitory activity, suggesting that the polymeric formulation may contribute to the overall antimicrobial response. However, the greater inhibition zones of the postbiotic-containing gel do not independently establish a synergistic interaction between chitosan and the postbiotic. The difference between the chitosan- and Carbopol®-based formulations was not uniform across microorganisms. Notably, *E. coli* showed a larger inhibition zone with the Carbopol®-based postbiotic gel, despite the generally greater activity of the chitosan-based system. Agar diffusion assays are influenced by the diffusion of antimicrobial constituents through the agar matrix, meaning that inhibition-zone diameter does not necessarily reflect intrinsic antimicrobial potency alone [70]. Consequently, differences in polymer composition, release behavior, and diffusion characteristics may have contributed to the observed microorganism-dependent responses. Because release kinetics and diffusion coefficients were not measured, these possibilities require further investigation. The results demonstrate that incorporation of the *L. plantarum*-derived postbiotic into polymeric gels increased agar diffusion-based antimicrobial activity, with the chitosan formulation exhibiting the most consistent enhancement across the tested microorganisms. These findings support further investigation of

postbiotic-containing polymeric gels for potential topical antiseptic applications. However, the results should be interpreted together with broth microdilution and antibiofilm findings, since agar diffusion activity alone does not establish antiseptic efficacy under practical application conditions.

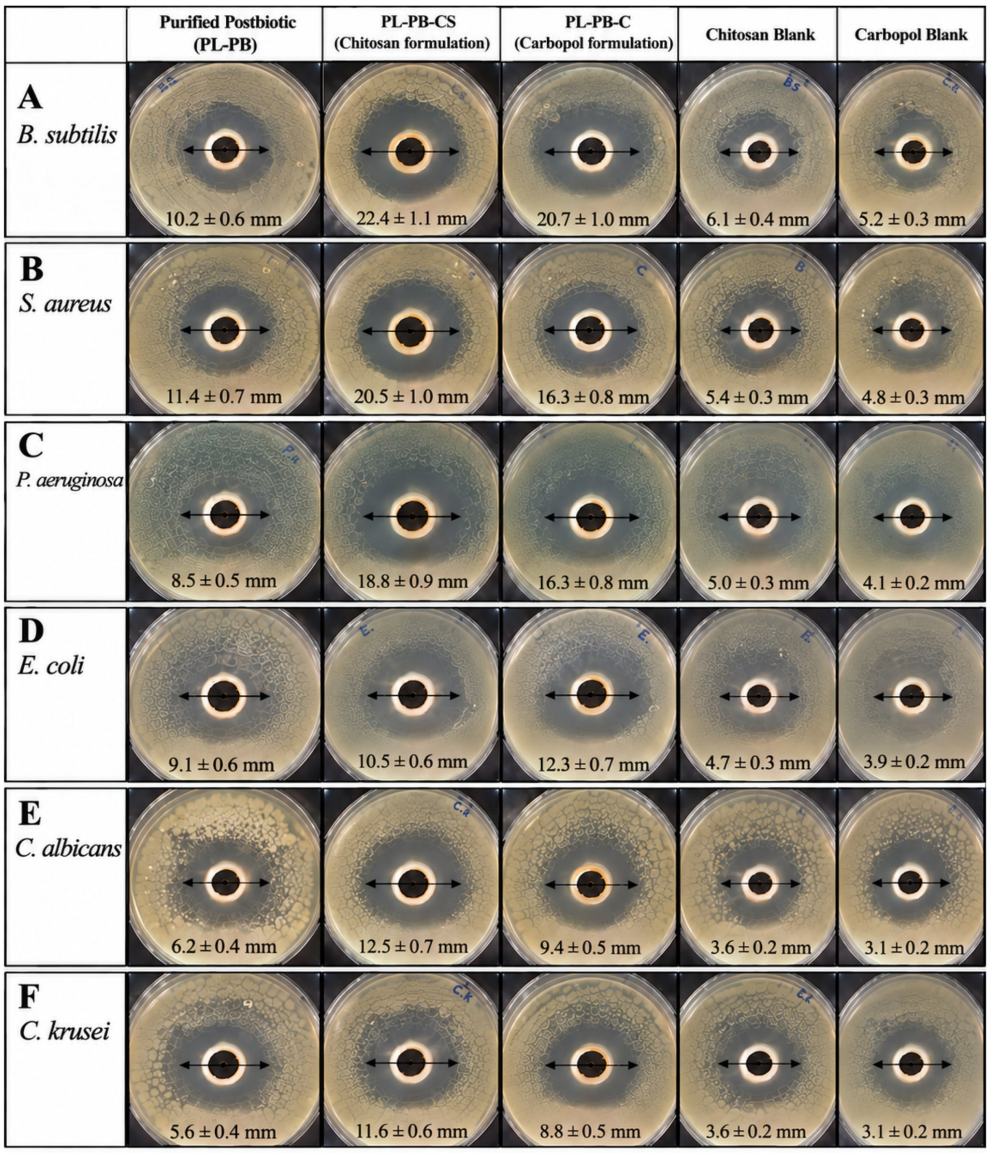


Figure 3. Representativeagar well diffusion assay images illustrating the antimicrobial activity of purified *Lactiplantibacillus plantarum*-derived postbiotic (PL-PB), chitosan-based postbiotic formulation (PL-PB-CS), Carbopol-based postbiotic formulation (PL-PB-C), and their corresponding blank formulations against (**A**) *Bacillus subtilis*, (**B**) *Staphylococcus aureus*, (**C**) *Pseudomonas aeruginosa*, (**D**) *Escherichia coli*, (**E**) *Candida albicans*, and (**F**) *Candida krusei*. Inhibition-zone diameters are expressed as mean ± standard deviation (SD) in millimeters (mm). Black double-headed arrows indicate the inhibition zones. Well-diameter-corrected inhibition zone (mm), calculated by subtracting the 6 mm well diameter from the total inhibition-zone diameter.

It should be noted that inhibition-zone diameters are influenced not only by antimicrobial activity but also by the diffusion of active constituents through agar and their release from the polymeric formulations. Therefore, larger inhibition zones do not necessarily indicate greater intrinsic antimicrobial potency. These findings should be interpreted alongside the MIC results, considering the potential contributions of the postbiotic preparation, polymer matrices, and other formulation components to the observed antimicrobial effects.

3.6.2. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) results demonstrated distinct antimicrobial responses depending on the polymeric matrix and target microorganism. The *Lactiplantibacillus plantarum*-derived postbiotic exhibited MIC values of 3125 µg/mL against all four bacterial strains and 6250 µg/mL against both Candida species. Incorporation into the chitosan-based gel reduced these values to 1562.5 and 3125 µg/mL, respectively, corresponding to a two-fold improvement in MIC against all six microorganisms. In contrast, the Carbopol®-based postbiotic formulation exhibited bacterial MIC values of 3125 µg/mL, identical to those of the postbiotic preparation alone, while the MICs against both Candida species were reported as >3125 µg/mL. The blank polymer formulations exhibited substantially higher MIC values, indicating limited inhibitory activity within the evaluated concentration ranges. As expected, ciprofloxacin and amphotericin B showed considerably lower MIC values than the postbiotic-containing formulations (Table 7).

Table 7. Minimum inhibitory concentrations (MICs, µg/mL) of the blank polymer formulations, purified *Lactiplantibacillus plantarum* postbiotic, postbiotic-containing chitosan and Carbopol formulations, and reference antimicrobial agents against the tested microorganisms.

Sample	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*C. krusei*
Chitosan + *L. plantarum* postbiotic formula	1562.5	1562.5	1562.5	1562.5	3125	3125
Carbopol + *L. plantarum* postbiotic formula	3125	3125	3125	3125	>3125	>3125
Chitosan blank formula	>6250	>6250	>6250	>6250	>6250	>6250
Carbopol blank formula	>12,500	>12,500	>12,500	>12,500	>12,500	>12,500
L. plantarum postbiotic	3125	3125	3125	3125	6250	6250
Ciprofloxacin	≥1	≤2	≥1	≥0.5	–	–
Amphotericin B	–	–	–	–	≥1	≥1

The MIC findings were generally consistent with the agar diffusion results, although the magnitude of enhancement differed between the two methods. Relative to the postbiotic preparation alone, the chitosan-based formulation increased net inhibition-zone diameters by 15.4–121.2%, with the greatest enhancement observed against *Pseudomonas aeruginosa* (121.2%) and *Bacillus subtilis* (119.6%) (Table 8). The Carbopol®-based formulation also increased inhibition-zone diameters by 35.2–102.9%, despite showing no improvement in bacterial MIC values. Notably, the Carbopol® formulation produced a larger inhibition zone against *Escherichia coli*, whereas the chitosan formulation exhibited a lower MIC against this microorganism. These findings indicate that diffusion-based inhibition and broth microdilution susceptibility were not directly proportional across the investigated formulations.

Table 8. MIC fold improvement and inhibition-zone enhancement of the postbiotic-containing chitosan and Carbopol formulations relative to the purified *Lactiplantibacillus plantarum* postbiotic.

Microorganism	Chitosan Formula MIC Fold Improvement [a]	Chitosan Formula Zone Enhancement [b] (%)	Carbopol Formula MIC Fold Improvement [a]	Carbopol Formula Zone Enhancement [b] (%)
B. subtilis	2.00×	119.6	1.00×	102.9
S. aureus	2.00×	79.8	1.00×	43.0
P. aeruginosa	2.00×	121.2	1.00×	91.8
E. coli	2.00×	15.4	1.00×	35.2
C. albicans	2.00×	101.6	NC [c]	51.6
C. krusei	2.00×	107.1	NC [c]	57.1

[a] MIC fold improvement was calculated as MIC of the purified postbiotic/MIC of the corresponding postbiotic-containing formulation. [b] Zone enhancement (%) was calculated relative to the inhibition-zone diameter of the purified postbiotic. [c] NC, not calculated because an exact MIC value was not obtained within the indicated concentration range.

The superior MIC performance of the chitosan-based formulation may be associated with the intrinsic antimicrobial properties of chitosan and its contribution to the overall activity of the postbiotic-containing matrix. Kong et al. (2010) reported that chitosan-mediated antimicrobial activity is influenced by polymer characteristics, environmental conditions, and microbial susceptibility [69]. In addition, Thirupathi et al. (2023) highlighted the potential of chitosan-based hydrogels for antimicrobial applications [1]. The present findings are also consistent with Chang et al. (2021), who demonstrated inhibitory activity of *L. plantarum*-derived postbiotic preparations and identified associations between organic acid composition and antimicrobial performance [9]. Nevertheless, the specific contribution of individual postbiotic metabolites and their interactions with the polymer matrix were not determined in the present study. The discrepancy between inhibition-zone enhancement and MIC improvement, particularly for the Carbopol® formulation, emphasizes the importance of interpreting antimicrobial activity using complementary assays. Balouiri et al. (2016) described the methodological differences between diffusion-based and dilution-based susceptibility tests, including the influence of antimicrobial diffusion on inhibition-zone measurements [70]. Accordingly, the larger inhibition zones observed with certain formulations may reflect differences in compound mobility and release from the polymeric matrices rather than proportional changes in intrinsic antimicrobial potency. These potential mechanisms require further investigation through release and physicochemical characterization. The combined results indicate that the chitosan-based postbiotic formulation provided the most consistent antimicrobial enhancement, achieving a two-fold reduction in MIC against all six microorganisms alongside increased inhibition-zone diameters. Although the Carbopol® formulation improved diffusion-based inhibition, its bacterial MIC values remained unchanged relative to the postbiotic preparation alone. These findings support the potential of chitosan as a functional polymeric carrier for developing *L. plantarum*-derived postbiotic polymeric gels with antimicrobial properties. However, the substantially lower MIC values of the reference antimicrobial agents indicate that the formulations should be considered investigational systems rather than alternatives with demonstrated equivalent antiseptic efficacy. The chitosan-based postbiotic formulation exhibited a two-fold lower observed MIC than the unformulated postbiotic preparation under the tested conditions. However, this difference should not be interpreted as evidence of synergistic antimicrobial activity, as the observed response may reflect the combined contributions of chitosan, other formulation components, and formulation-dependent physicochemical effects. Therefore, the lower MIC represents a difference in the activity of the complete formulation rather than an increase in the intrinsic antimicrobial potency of the postbiotic. The antimicrobial responses were not uniform across the tested microorganisms or assay methods. Notably, the Carbopol-based postbiotic formulation produced a larger inhibition zone against *E. coli*, whereas the chitosan-based formulation exhibited a lower observed MIC against the same microorganism. This discrepancy indicates that agar diffusion and broth microdilution results are not necessarily directly proportional, potentially reflecting differences in formulation composition, diffusion, and release characteristics. Therefore, the findings do not support the universal superiority of either polymeric formulation.

3.7. Antibiofilm Activity

The *Lactiplantibacillus plantarum*-derived postbiotic and its polymeric formulations exhibited concentration-dependent inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm formation (Figure 4A,B). The antibiofilm experiments were designed to evaluate inhibition during biofilm formation rather than the eradication of pre-established biofilms. Accordingly, the observed reductions in crystal violet-stained biomass indicate an inhibitory effect on biofilm development under the tested conditions. These findings do not

establish the ability of the formulations to eradicate mature biofilms, which would require separate experiments involving preformed biofilms. The postbiotic-containing formulations consistently demonstrated greater antibiofilm activity than the purified postbiotic and their corresponding blank matrices. At the highest concentration tested (12,500 µg/mL), the chitosan-based postbiotic formulation achieved biofilm inhibition of 62.5 ± 3.1% against *S. aureus* and 59.3 ± 3.0% against *P. aeruginosa*, compared with 54.8 ± 2.9% and 53.1 ± 2.8%, respectively, for the Carbopol®-based formulation. The purified postbiotic produced inhibition values of 46.2 ± 2.7% and 44.0 ± 2.6%, whereas both blank formulations exhibited lower activity (Figure 4). These findings indicate that incorporation into polymeric matrices was associated with enhanced inhibition of biofilm formation, particularly in the chitosan-based system.

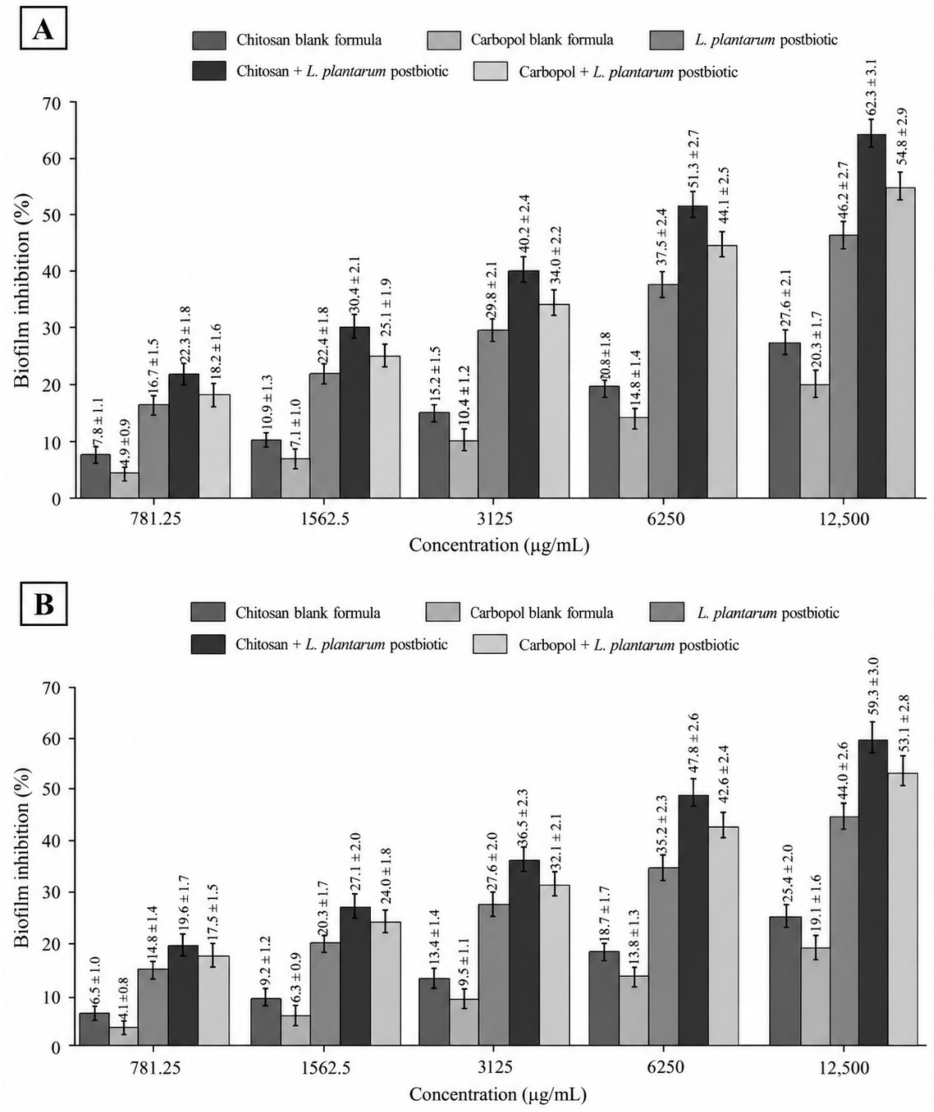


Figure 4. Concentration-dependent inhibition of biofilm formation by the chitosan blank formula, Carbopol blank formula, purified *Lactiplantibacillus plantarum* postbiotic, chitosan + *L. plantarum* postbiotic formula, and Carbopol + *L. plantarum* postbiotic formula against (**A**) *Staphylococcus aureus* and (**B**) *Pseudomonas aeruginosa*. Biofilm inhibition was determined using the crystal violet assay and expressed as percentage inhibition relative to the untreated biofilm control. Values are presented as mean ± SD.

The concentration–response profiles were further reflected in the $MBIC_{50}$ values (Table 9). The chitosan-based postbiotic formulation exhibited $MBIC_{50}$ values of 5884 µg/mL against *S. aureus* and 7446 µg/mL against *P. aeruginosa*, compared with 9696 and 10,655 µg/mL,

respectively, for the Carbopol®-based formulation. Neither the purified postbiotic nor the blank polymer formulations reached 50% inhibition within the tested concentration range, resulting in $MBIC_{50}$ values >12,500 µg/mL. These results indicate that the chitosan-based formulation achieved the 50% biofilm inhibition threshold at lower concentrations than the Carbopol®-based formulation.

Table 9. $MBIC_{50}$ values of the chitosan and Carbopol blank formulations, purified *Lactiplantibacillus plantarum* postbiotic, and postbiotic-containing polymeric formulations.

Sample	*S. aureus* $MBIC_{50}$ (µg/mL)*	*P. aeruginosa* $MBIC_{50}$ (µg/mL)*
Chitosan blank formula	>12,500	>12,500
Carbopol blank formula	>12,500	>12,500
L. plantarum postbiotic	>12,500	>12,500
Chitosan + *L. plantarum* postbiotic	5884	7446
Carbopol + *L. plantarum* postbiotic	9696	10,655

* $MBIC_{50}$, concentration required to inhibit biofilm formation by 50%. Values expressed as ">" indicate that the corresponding endpoint was not reached at the highest concentration evaluated.

The observed antibiofilm activity is consistent with previous investigations demonstrating the potential of *L. plantarum*-derived preparations to interfere with biofilm-associated processes. Butrungrod et al. (2023) reported that postbiotic metabolites obtained from *L. plantarum* PD18 exhibited inhibitory effects against periodontal pathogens and biofilm-related virulence characteristics [15]. Although the microbial species and experimental conditions differed from those used in the present study, these findings support the potential contribution of fermentation-derived microbial metabolites to antibiofilm activity. Nevertheless, the chemical composition of the present preparation was not characterized sufficiently to identify the compounds responsible for the observed effects. The greater activity of the chitosan-based formulation may also be associated with the physicochemical and antimicrobial properties of chitosan. Kong et al. (2010) described the interactions of chitosan with microbial cell surfaces, while Thirupathi et al. (2023) discussed the antimicrobial and antibiofilm applications of chitosan-based hydrogels [1,69]. These properties may contribute to the inhibitory performance of chitosan-containing systems. However, the observed enhancement cannot be attributed exclusively to chitosan–postbiotic interactions, because differences in polymer composition, formulation-associated ingredients, and microbial exposure may also influence biofilm inhibition. Furthermore, crystal violet staining primarily quantifies attached biofilm biomass rather than directly measuring bacterial viability, and therefore, the results should be interpreted as inhibition of biofilm formation rather than confirmed killing of biofilm-associated cells. Although the results showed slightly greater inhibition of *S. aureus* biofilm formation than *P. aeruginosa* biofilm formation, both microorganisms exhibited similar concentration-dependent responses. The comparatively lower susceptibility of *P. aeruginosa* may be related to the protective properties of its biofilm matrix and its capacity to tolerate antimicrobial exposure. Flemming et al. (2016) emphasized the importance of the extracellular polymeric matrix in biofilm structure and protection, while Hall and Mah (2017) described multiple mechanisms contributing to antimicrobial tolerance in bacterial biofilms [71,72]. However, these mechanisms were not directly investigated in the present study. The findings demonstrate that postbiotic incorporation into chitosan- and Carbopol®-based formulations increased inhibition of biofilm formation, with the chitosan-based formulation exhibiting the most favorable concentration–response profile and lowest $MBIC_{50}$ values against both microorganisms. These results support further investigation of *L. plantarum*-derived postbiotic polymeric gels for potential topical applications where inhibition of microbial biofilm formation is desirable.

3.8. Antioxidant Activity: Comparative Evaluation of DPPH and ABTS Radical Scavenging Assays

The antioxidant activities of the purified *Lactiplantibacillus plantarum*-derived postbiotic and its chitosan- and Carbopol-based formulations were evaluated using DPPH and ABTS radical scavenging assays over a concentration range of 781.25–12,500 µg/mL, expressed as postbiotic dry mass. These complementary assays were employed to assess the antioxidant responses of the formulations under different reaction conditions, considering the potential influence of solvent compatibility and polymer-related interference [38,73].

The DPPH assay demonstrated concentration-dependent radical scavenging activity (Figure 5A). At 12,500 µg/mL, the postbiotic-containing chitosan formulation exhibited the highest inhibition (72.0 ± 3.0%), followed by the Carbopol formulation (65.0 ± 2.9%) and purified postbiotic (50.4 ± 3.0%). The corresponding blank chitosan and Carbopol formulations showed inhibition values of 34.2 ± 2.2% and 27.1 ± 2.0%, respectively, while gallic acid exhibited 82.1 ± 1.0% inhibition. The apparent IC_{50} values were 2983, 3606, and 4830 µg/mL for the chitosan formulation, purified postbiotic, and Carbopol formulation, respectively (Table 10). Neither blank formulation reached 50% inhibition within the tested range (IC_{50} >12,500 µg/mL). These findings suggest that formulation composition influenced the observed antioxidant response, although the activity of the blank formulations indicates that the effect cannot be attributed exclusively to the postbiotic. The ABTS assay supported the concentration-dependent antioxidant response observed with DPPH (Figure 5B). At 12,500 µg/mL, ABTS inhibition reached 83.4% for the postbiotic-containing chitosan formulation, 78.1% for the Carbopol formulation, and 76.2% for the purified postbiotic. The blank chitosan and Carbopol formulations exhibited inhibition values of 34.5% and 25.6%, respectively. The corresponding TEAC values at 781.25 µg/mL were 0.0900, 0.0749, and 0.0720 mmol TE/g postbiotic for the chitosan formulation, Carbopol formulation, and purified postbiotic, respectively. At 12,500 µg/mL, these values decreased to 0.0178, 0.0164, and 0.0159 mmol TE/g postbiotic (Table 11). Although ABTS inhibition increased with concentration, the decline in mass-normalized TEAC values may reflect nonlinear assay responses and limitations associated with the Trolox calibration range rather than reduced intrinsic antioxidant capacity.

Table 10. DPPH IC_{50} values of gallic acid, blank polymer formulations, purified *Lactiplantibacillus plantarum* postbiotic, and postbiotic-containing chitosan and Carbopol formulations.

Formulation	DPPH IC_{50} (µg/mL)
L. plantarum postbiotic	3606
Chitosan + *L. plantarum* postbiotic	2983
Carbopol + *L. plantarum* postbiotic	4830
Gallic acid	<781.25
Chitosan blank serum	>12,500
Carbopol blank serum	>12,500

Table 11. Trolox equivalent antioxidant capacity (TEAC) of *L. plantarum*-derived postbiotic and polymeric gel formulations (mmol TE/g).

Concentration (µg/mL)	Purified Postbiotic	Postbiotic + Chitosan	Postbiotic + Carbopol
781.25	0.0720	0.0900	0.0749
1562.5	0.0583	0.0696	0.0602
3125	0.0440	0.0489	0.0434
6250	0.0264	0.0304	0.0271
12,500	0.0159	0.0178	0.0164

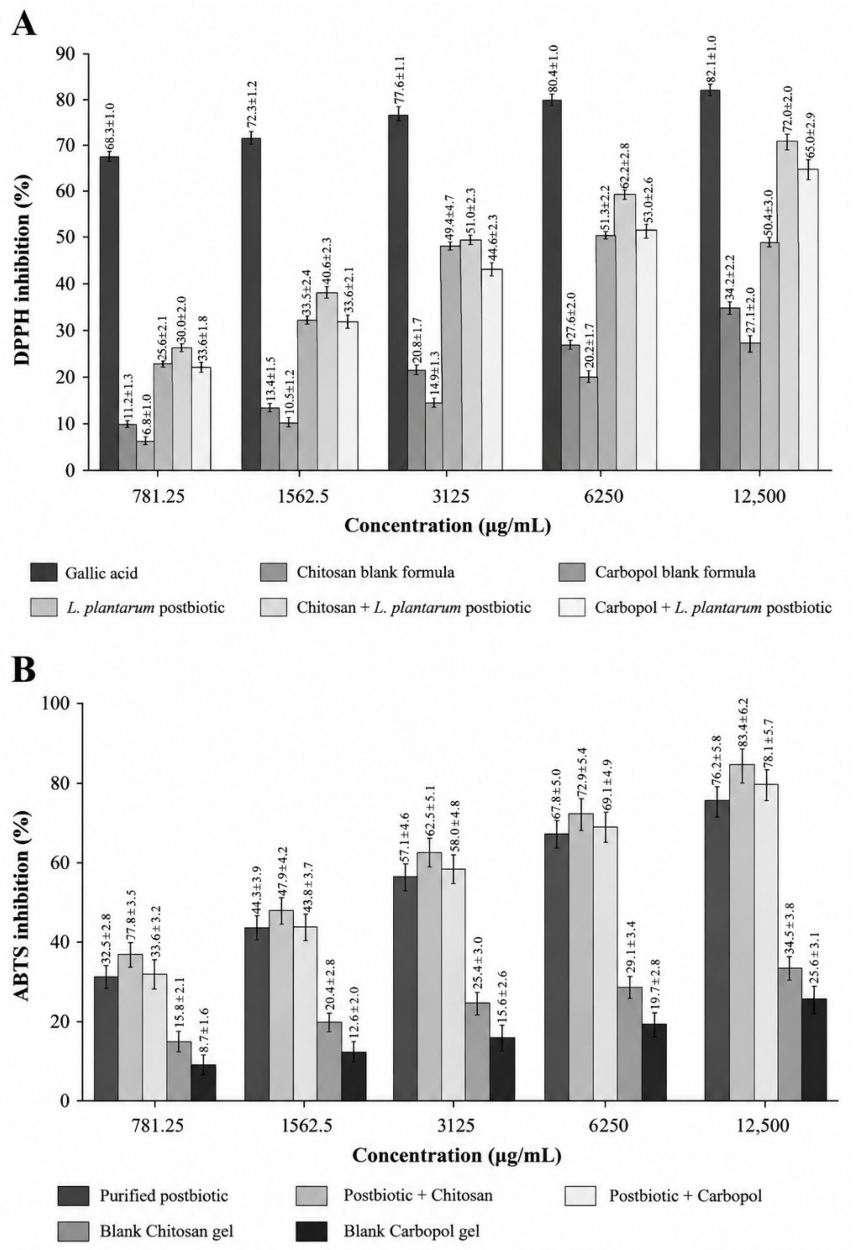


Figure 5. Concentration-dependent in vitro radical scavenging activity of purified *Lactiplantibacillus plantarum*-derived postbiotic, postbiotic-containing chitosan and Carbopol formulations, and their corresponding blank formulations. (**A**) DPPH radical scavenging activity, including gallic acid as the positive control. (**B**) ABTS radical cation scavenging activity. Values are expressed as mean ± SD.

The differences between the two assays may be associated with their distinct physicochemical characteristics. DPPH is generally evaluated in alcohol-containing media, where interactions with aqueous polymeric formulations may influence polymer dispersion, turbidity, and radical accessibility [9,42,74]. In contrast, the ABTS radical cation is compatible with aqueous environments, providing a complementary approach for evaluating complex formulations [73]. Nevertheless, polymer aggregation, diffusion limitations, and potential interference from excipients such as PEG 400 and polysorbate 20 may affect the measured responses. Sample-specific blanks reduce background absorbance interference but do not necessarily eliminate all matrix-related effects. The higher apparent antioxidant activity of the chitosan-based formulation may reflect contributions from both the postbiotic constituents and the complete formulation. Previous studies have demonstrated that chitosan and its derivatives can exhibit radical scavenging properties depending on their structural

characteristics [75]. However, because the formulations differ in composition, the observed differences cannot be attributed solely to the principal polymer or interpreted as evidence of synergy. Furthermore, DPPH and ABTS assays measure chemical radical scavenging and do not establish cellular antioxidant protection or activation of endogenous pathways such as Nrf2, SOD, catalase, or glutathione peroxidase. Both assays indicated that the postbiotic-containing chitosan formulation exhibited the highest apparent in vitro radical scavenging activity among the investigated postbiotic preparations. However, the results should be interpreted in the context of formulation-dependent matrix effects and analytical limitations. Further studies involving validated interference controls and cell-based oxidative stress models are required to establish the biological relevance of these findings.

The safety of the formulations for topical application has not been established. Cytotoxicity in relevant skin cells, irritation, sensitization, and skin-barrier compatibility were not evaluated in this study. These assessments are particularly important given the complex postbiotic preparation, preservatives, sodium salicylate, and acidic formulation environment. Accordingly, the formulations should be regarded as potential topical antiseptic candidates requiring further safety evaluation rather than products with demonstrated suitability for skin application.

4. Conclusions

This study evaluated Lactiplantibacillus plantarum-derived postbiotic-loaded chitosan- and Carbopol-based polymeric gels as potential topical antiseptic candidates. Both formulations exhibited shear-thinning behavior and in vitro antimicrobial, biofilm-inhibitory, and radical scavenging activities. The chitosan-based formulation generally showed more favorable biological responses, although antimicrobial performance varied with the microorganism and assay. These findings represent a comparison of two complete formulation platforms; differences cannot be attributed solely to the primary polymers, and antimicrobial synergy was not established. Biofilm inhibition also does not demonstrate eradication of established biofilms. These preliminary findings support further investigation but do not establish practical antiseptic efficacy, storage stability, or safety for skin application. Further work should address postbiotic composition and batch consistency, standardized antiseptic efficacy, formulation stability, and skin safety, including cytotoxicity, irritation, sensitization, and barrier compatibility. Quantitative assessment of spreadability, bioadhesion, and local retention is also needed to establish the proposed topical advantages.

Author Contributions: Conceptualization, P.S. and A.A.Ö.; methodology, P.S. and A.A.Ö.; investigation, P.S. and A.A.Ö.; data curation, P.S.; formal analysis, P.S.; visualization, P.S.; validation, P.S. and A.A.Ö.; resources, P.S. and A.A.Ö.; supervision, A.A.Ö.; project administration, A.A.Ö.; funding acquisition, A.A.Ö.; writing—original draft preparation, P.S.; writing—review and editing, P.S. and A.A.Ö. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Scientific Research Projects Commission of Anadolu University under Project No. 3735.

Institutional Review Board Statement: Not applicable. This study did not involve human participants or experimental animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank the Scientific Research Projects Commission of Anadolu University (Project No. 3735) for its financial support throughout this study.

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

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
CFS	Cell-Free Supernatant
CFU	Colony-Forming Units
CV	Crystal Violet
DPPH	2,2-Diphenyl-1-picrylhydrazyl
IC_{50}	Half-Maximal Inhibitory Concentration
$MBIC_{50}$	Minimum Biofilm Inhibitory Concentration Required for 50% Biofilm Inhibition
MHA	Mueller–Hinton Agar
MHB	Mueller–Hinton Broth
MIC	Minimum Inhibitory Concentration
MMW	Medium Molecular Weight
MRS	de Man–Rogosa–Sharpe
NRRL	Agricultural Research Service Culture Collection
OD	Optical Density
PB	Postbiotic
PBS	Phosphate-Buffered Saline
PEG	Polyethylene Glycol
SD	Standard Deviation
v/*v*	Volume per Volume

References

1. Thirupathi, K.; Raorane, C.J.; Ramkumar, V.; Ulagesan, S.; Santhamoorthy, M.; Raj, V.; Krishnakumar, G.S.; Phan, T.T.V.; Kim, S.C. Update on Chitosan-Based Hydrogels: Preparation, Characterization, and Its Antimicrobial and Antibiofilm Applications. *Gels* **2022**, *9*, 35. [CrossRef] [PubMed]
2. Wang, Y.; Wang, Z.; Lu, W.; Hu, Y. Review on Chitosan-Based Antibacterial Hydrogels: Preparation, Mechanisms, and Applications. *Int. J. Biol. Macromol.* **2024**, *255*, 128080. [CrossRef] [PubMed]
3. Draelos, Z.K. Cosmetics: An Overview. *Curr. Probl. Dermatol.* **1995**, *7*, 45–64. [CrossRef]
4. Salminen, S.; Collado, M.C.; Endo, A.; Hill, C.; Lebeer, S.; Quigley, E.M.M.; Sanders, M.E.; Shamir, R.; Swann, J.R.; Szajewska, H.; et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Postbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2021**, *18*, 649–667. [CrossRef] [PubMed]
5. Oelschlaeger, T.A. Mechanisms of Probiotic Actions—A Review. *Int. J. Med. Microbiol.* **2010**, *300*, 57–62. [CrossRef] [PubMed]
6. Almahbashi, A.; Gunes Altuntas, E. From Preparation to Bioactivity: A Comparative Study on Preparation Methods and Characterization of Postbiotics. *Food Sci. Nutr.* **2025**, *13*, e70294. [CrossRef]
7. Soyer, P.; Tekgöz, M.; Tunalı, Y. Determination of Potential Probiotic Properties of Lactic Acid Bacteria Isolated from Colostrum Milk. *Eur. J. Life Sci.* **2023**, *2*, 31–38. [CrossRef]
8. Soyer, P.; Öztürk, A.A. Comparative Evaluation of Extrusion and Emulsification Techniques for Alginate-Based Microencapsulation of Bifidobacterium Longum. *Fabad J. Pharm. Sci.* **2026**, *51*, 853–864. [CrossRef]
9. Chang, H.M.; Foo, H.L.; Loh, T.C.; Lim, E.T.C.; Abdul Mutalib, N.E. Comparative Studies of Inhibitory and Antioxidant Activities, and Organic Acids Compositions of Postbiotics Produced by Probiotic Lactiplantibacillus Plantarum Strains Isolated From Malaysian Foods. *Front. Vet. Sci.* **2021**, *7*, 602280. [CrossRef] [PubMed]
10. Khakpour, M.; Mohsenzadeh, M.; Salari, A. Feasibility of Lactiplantibacillus Plantarum Postbiotics Production in Challenging Media by Different Techniques. *AMB Express* **2024**, *14*, 47. [CrossRef] [PubMed]
11. Khorshidi, S.; Mehdizadeh, T.; Tajik, H.; Hamishekar, H.; Reale, A. Characterization of Lactiplantibacillus Plantarum Subsp. Plantarum and Bifidobacterium Animalis Spp. Lactis BB-12 Postbiotics: In Vivo and in Vitro Experiments against Foodborne Pathogens. *LWT* **2025**, *224*, 117823. [CrossRef]
12. Islam, F.; Azmat, F.; Imran, A.; Zippi, M.; Hong, W.; Tariq, F.; Shehzadi, U.; Fatima, A.; Safdar, M.; Ahmed, H.; et al. Role of Postbiotics in Food and Health: A Comprehensive Review. *CyTA-J. Food* **2024**, *22*, 2386412. [CrossRef]
13. Karaca, B.; Gursoy, M.; Kiran, F.; Loimaranta, V.; Söderling, E.; Gursoy, U.K. Postbiotics of the Lactiplantibacillus Plantarum EIR/IF-1 Strain Show Antimicrobial Activity against Oral Microorganisms with pH Adaptation Capability. *Microbiol. Res.* **2023**, *14*, 1442–1456. [CrossRef]

14. Shaaban, M.T.; Khalil, F.O.; Hamdy, E.; Ahmed, A.A.M. Antimicrobial and Antibiofilm Activities of Culture Filtrates from Lactiplantibacillus Plantarum Isolated from Traditional Dairy Products in Menoufia, Egypt. *BMC Microbiol*. **2025**, *25*, 745. [CrossRef] [PubMed]
15. Butrungrod, W.; Chaiyasut, C.; Makhamrueang, N.; Peerajan, S.; Chaiyana, W.; Sirilun, S. Postbiotic Metabolite of Lactiplantibacillus Plantarum PD18 against Periodontal Pathogens and Their Virulence Markers in Biofilm Formation. *Pharmaceutics* **2023**, *15*, 1419. [CrossRef] [PubMed]
16. Afrasiabi, S. Chitosan-Based Structures for Targeting Bacterial Biofilm-Associated Genes. *Carbohydr. Polym. Technol. Appl*. **2026**, *13*, 101077. [CrossRef]
17. Pérez-Marcos, B.; Gutiérrez, C.; Gómez-Amoza, J.; Martínez-Pacheco, R.; Souto, C.; Concheiro, A. Usefulness of Certain Varieties of Carbomer in the Formulation of Hydrophilic Furosemide Matrices. *Int. J. Pharm*. **1991**, *67*, 113–121. [CrossRef]
18. Abu-Rumman, A.; Abu-Huwaij, R.; Hamed, R. Development and in Vitro Appraisal of Soluplus® and/or Carbopol® 971 Buccoadhesive Patches Releasing Atorvastatin. *J. Adhes*. **2022**, *98*, 915–933. [CrossRef]
19. Abu-Huwaij, R.; Obaidat, R.M.; Sweidan, K.; Al-Hiari, Y. Formulation and in Vitro Evaluation of Xanthan Gum or Carbopol 934-Based Mucoadhesive Patches, Loaded with Nicotine. *AAPS PharmSciTech* **2011**, *12*, 21–27. [CrossRef] [PubMed]
20. Soyer, P.; Öztürk, A.A. Development of Pharmabiotic Gel-Serums with Lacticaseibacillus Casei Postbiotics and Paraprobiotics Using Chitosan and Carbopol. *Gels* **2026**, *12*, 838. [CrossRef] [PubMed]
21. Spirescu, V.A.; Chircov, C.; Grumezescu, A.M.; Andronescu, E. Polymeric Nanoparticles for Antimicrobial Therapies: An up-to-Date Overview. *Polymers* **2021**, *13*, 724. [CrossRef] [PubMed]
22. Chen, Y.; Huang, Y.; Jin, Q. Polymeric Nanoplatforms for the Delivery of Antibacterial Agents. *Macromol. Chem. Phys*. **2022**, *223*, 2100440. [CrossRef]
23. Birk, S.E.; Boisen, A.; Nielsen, L.H. Polymeric Nano- and Microparticulate Drug Delivery Systems for Treatment of Biofilms. *Adv. Drug Deliv. Rev*. **2021**, *174*, 30–52. [CrossRef] [PubMed]
24. Dinić, M.; Burgess, J.L.; Lukić, J.; Catanuto, P.; Radojević, D.; Marjanović, J.; Verpile, R.; Thaller, S.R.; Gonzalez, T.; Golić, N.; et al. Postbiotic Lactobacilli Induce Cutaneous Antimicrobial Response and Restore the Barrier to Inhibit the Intracellular Invasion of Staphylococcus Aureus in Vitro and Ex Vivo. *FASEB J*. **2024**, *38*, e23801. [CrossRef] [PubMed]
25. Öğüt, K.; Soyer, P. Evaluation of Antimicrobial and Biofilm Inhibitory Activities of Flower, Leaf, Seed, and Pulp Extracts of Mahonia Aquifolium (Pursh) Nutt. *Fabad J. Pharm. Sci*. **2026**, *51*, 755–766. [CrossRef]
26. Soyer, P.; Öztürk, A.A. Development and Functional Evaluation of a Pharmabiotic Food Supplement Containing Microencapsulated Lactiplantibacillus Pentosus for Oral Microbiome Health. *Food Sci. Nutr*. **2026**, *14*, e72243. [CrossRef] [PubMed]
27. Soyer, P.; Ayhan, M.; İrdem, E.; Dikmen, M.; Cantürk, Z. Evaluation of Saccharomyces Cerevisiae Yeast Cell Wall as a Microbial-Derived Prebiotic Substrate for Enhancing the Functional Properties of Lactic Acid Bacteria. *Int. J. Food Sci. Technol*. **2026**, *61*, vvag191. [CrossRef]
28. Islam, M.T.; Rodríguez-Hornedo, N.; Ciotti, S.; Ackermann, C. Rheological Characterization of Topical Carbomer Gels Neutralized to Different pH. *Pharm. Res*. **2004**, *21*, 1192–1199. [CrossRef] [PubMed]
29. Cid, Y.P.; Pedrazzi, V.; De Sousa, V.P.; Pierre, M.B.R. In Vitro Characterization of Chitosan Gels for Buccal Delivery of Celecoxib: Influence of a Penetration Enhancer. *AAPS PharmSciTech* **2011**, *13*, 101. [CrossRef] [PubMed]
30. Öztürk, A.A.; Güven, U.M.; Yenilmez, E. Flurbiprofen Loaded Gel Based Topical Delivery System: Formulation and in Vitro Characterization with New Developed Uplc Method. *ACTA Pharm. Sci*. **2018**, *56*, 81. [CrossRef]
31. Ismailovi, N.; Kıyan, H.T.; Öztürk, A.A. A Novel Phytotherapy Application: Preparation, Characterization, Antioxidant Activities and Determination of Anti-Inflammatory Effects by In Vivo HET-CAM Assay of Chitosan-Based DDSs Containing Endemic Helichrysum Pamphylicum P.H. Davis & Kupicha Methanolic Extract. *Curr. Drug Deliv*. **2024**, *21*, 901–916. [CrossRef] [PubMed]
32. Dantas, M.G.B.; Reis, S.A.G.B.; Damasceno, C.M.D.; Rolim, L.A.; Rolim-Neto, P.J.; Carvalho, F.O.; Quintans-Junior, L.J.; Da Silva Almeida, J.R.G. Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene Borneol. *Sci. World J*. **2016**, *2016*, 7394685. [CrossRef] [PubMed]
33. Shiehzadeh, F.; Mohebi, D.; Chavoshian, O.; Daneshmand, S. Formulation, Characterization, and Optimization of a Topical Gel Containing Tranexamic Acid to Prevent Superficial Bleeding: In Vivo and In Vitro Evaluations. *Turk. J. Pharm. Sci*. **2023**, *20*, 261–269. [CrossRef] [PubMed]
34. Uma Shankar, M.S.; Vishnu, V.; Sharmila, S.; Kumar, A. Formulation and evaluation of cefixime trihydrate topical gel for wound infections. *Asian J. Pharm. Clin. Res*. **2018**, *11*, 369–373. [CrossRef]
35. Öztürk, A.A.; Uzun, Y.; Saltan, F.Z. Multiparametric Analysis of Functional Group–Performance Relationships in Tannic Acid-Based Hydrogels from a Pharmaceutical Technology Standpoint. *J. Drug Deliv. Sci. Technol*. **2025**, *113*, 107352. [CrossRef]
36. Jabrayıl, S.M.; İsmayılova Rajab, N.; Jahangirova Rafik, İ.; Jafarova Bayram, S.; Alper Öztürk, A. Phyto-Functionalized Niosomal Gel for Acne Therapy: Dual Delivery of Clindamycin and Botanical Actives via a Sustained Topical Platform—in Vitro, Ex Vivo, in Silico and in Vivo Evaluation. *J. Drug Deliv. Sci. Technol*. **2026**, *121*, 108313. [CrossRef]

37. Mammadov, B.S.; Mehraliyeva, S.J.; Sevinc Özakar, R.; Özakar, E.; Bayır, Y.; Öztürk, A.A.; Gasimov, E.; Rzayev, F. Engineering Pectin- and Sodium Alginate-Based Nanoemulgels for Topical Delivery of *Hibiscus sabdariffa* Flower Extract. *RSC Adv.* **2026**, *16*, 57258–57273. [CrossRef] [PubMed]
38. Solak, B.; Soyer, P.; Kaya-Tilki, E.; Öztürk, A.A. Evaluation of Antimicrobial Properties and Antioxidant Activity of Rosmarinic Acid Loaded Resomer RG 502 H Based Nanoparticles against Tert-Butyl Hydroperoxide-Induced Oxidative Stress: A Detailed Formulation, Characterization and Efficacy Determination Study. *J. Drug Deliv. Sci. Technol.* **2025**, *106*, 106721. [CrossRef]
39. Öztürk, A.A.; Aygül, A.; Şenel, B. Influence of Glyceryl Behenate, Tripalmitin and Stearic Acid on the Properties of Clarithromycin Incorporated Solid Lipid Nanoparticles (SLNs): Formulation, Characterization, Antibacterial Activity and Cytotoxicity. *J. Drug Deliv. Sci. Technol.* **2019**, *54*, 101240. [CrossRef]
40. Stepanović, S.; Vuković, D.; Hola, V.; Di Bonaventura, G.; Djukić, S.; Ćirković, I.; Ruzicka, F. Quantification of Biofilm in Microtiter Plates: Overview of Testing Conditions and Practical Recommendations for Assessment of Biofilm Production by Staphylococci. *APMIS* **2007**, *115*, 891–899. [CrossRef] [PubMed]
41. O'Toole, G.A. Microtiter Dish Biofilm Formation Assay. *J. Vis. Exp.* **2011**, *47*, 2437. [CrossRef] [PubMed]
42. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
43. Mammadov, A.; Çimentepe, M.; Öztürk Çimentepe, Ö.; Demirci Kayiran, S.; Güven Bolgen, U.M.; Çevikelli, T.; Öztürk, A.A.; Dörtbudak, M.B.; Yiğin, A.; Yildirim, M. Antibacterial, Anti-Inflammatory, and Wound-Healing Effects of Micromeria Fruticosa Essential Oil-Loaded Nanoemulsion in MRSA-Infected Diabetic Wounds: In Vitro and In Vivo Evaluation. *Pharmaceutics* **2026**, *18*, 1252. [CrossRef]
44. Arınmış, K.N.; Kıyan, H.T.; Öztürk, A.A. Preparation, Characterization, Antioxidant Activities, and Determination of Anti-Alzheimer Effects of PLGA-Based DDSs Containing Ferulic Acid. *ACS Omega* **2024**, *9*, 11321–11338. [CrossRef] [PubMed]
45. Öztürk, A.A.; Martin Banderas, L.; Cayero Otero, M.D.; Yenilmez, E.; Yazan, Y. New Approach to Hypertension Treatment: Carvediol-Loaded PLGA Nanoparticles, Preparation, in Vitro Characterization and Gastrointestinal Stability. *Lat. Am. J. Pharm.* **2018**, *37*, 1730–1741.
46. Hoşer, H.C.; Soyer, P.; Tilki, E.K.; Arısoy, S.; Alper Öztürk, A. CQA and DoE Guided Development of Brimonidine Tartrate Loaded Polymeric Nanoparticles for Sustained Ocular Delivery and in Vitro Neuroprotective Evaluation in Glaucoma. *J. Drug Deliv. Sci. Technol.* **2026**, *123*, 108568. [CrossRef]
47. Öztürk, A.A.; Başaran, E.; Şenel, B.; Demirel, M.; Sarıca, Ş. Synthesis, Characterization, Antioxidant Activity of Quercetin, Rutin and Quercetin-Rutin Incorporated β-Cyclodextrin Inclusion Complexes and Determination of Their Activity in NIH-3T3, MDA-MB-231 and A549 Cell Lines. *J. Mol. Struct.* **2023**, *1282*, 135169. [CrossRef]
48. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
49. Wang, T.; Chen, L.; Shen, T.; Wu, D. Preparation and Properties of a Novel Thermo-Sensitive Hydrogel Based on Chitosan/Hydroxypropyl Methylcellulose/Glycerol. *Int. J. Biol. Macromol.* **2016**, *93*, 775–782. [CrossRef] [PubMed]
50. Alhakamy, N.A.; Kotta, S.; Ali, J.; Alam, M.S.; Hosny, K.M.; Shaik, R.A.; Eid, B.G.; Riadi, Y.; Asfour, H.Z.; Ashy, N.; et al. Formulation Development, Statistical Optimization, In Vitro and In Vivo Evaluation of Etoricoxib-Loaded Eucalyptus Oil-Based Nanoemulgel for Topical Delivery. *Appl. Sci.* **2021**, *11*, 7294. [CrossRef]
51. Nair, A.B.; Shah, J.; Aljaeid, B.M.; Al-Dhubiab, B.E.; Jacob, S. Gellan Gum-Based Hydrogel for the Transdermal Delivery of Nebivolol: Optimization and Evaluation. *Polymers* **2019**, *11*, 1699. [CrossRef] [PubMed]
52. Czajkowska-Kośnik, A.; Perkowska, P.; Winnicka, K. Trehalose in an Emulsion-Based Carrier: Effects on Skin Hydration and Transepidermal Water Loss. *Cosmetics* **2026**, *13*, 196. [CrossRef]
53. Bialik-Wąs, K.; Pluta, K.; Malina, D.; Barczewski, M.; Malarz, K.; Mrozek-Wilczkiewicz, A. The Effect of Glycerin Content in Sodium Alginate/Poly(Vinyl Alcohol)-Based Hydrogels for Wound Dressing Application. *Int. J. Mol. Sci.* **2021**, *22*, 12022. [CrossRef] [PubMed]
54. Ozon, E.A.; Anastasescu, M.; Musuc, A.M.; Burloiu, A.M.; Socoteanu, R.P.; Atkinson, I.; Mitran, R.-A.; Culita, D.C.; Lupuliasa, D.; Mihai, D.P.; et al. Formulation and Characterization of Carbopol-Based Porphyrin Gels for Targeted Dermato-Oncological Therapy: Physicochemical and Pharmacotechnical Insights. *Int. J. Mol. Sci.* **2025**, *26*, 3641. [CrossRef] [PubMed]
55. Stancu, A.I.; Oprea, E.; Dițu, L.M.; Ficai, A.; Ilie, C.-I.; Badea, I.A.; Buleandra, M.; Brîncoveanu, O.; Ghica, M.V.; Avram, I.; et al. Development, Optimization, and Evaluation of New Gel Formulations with Cyclodextrin Complexes and Volatile Oils with Antimicrobial Activity. *Gels* **2024**, *10*, 645. [CrossRef] [PubMed]
56. Mohebbi, F.; Sellier, M. Identification of Rheological Parameters of Herschel–Bulkley Fluids from Free Surface Data. *Int. J. Thermofluids* **2022**, *16*, 100253. [CrossRef]
57. Ashena, R.; Badrouchi, F.; Elmgerbi, A.; Mishani, S.; Sotoudeh, F.; Nekoeian, S. Stepwise Mathematical Derivation of the Herschel–Bulkley Laminar Fluid Flow Equations—In Pipes. *J. Pet. Explor. Prod. Technol.* **2023**, *13*, 625–643. [CrossRef]

58. Ostróżka-Cieślik, A.; Strasser, C.; Dolińska, B. Insulin-Loaded Chitosan–Cellulose-Derivative Hydrogels: In Vitro Permeation of Hormone through Strat-M® Membrane and Rheological and Textural Analysis. *Polymers* **2024**, *16*, 2619. [CrossRef] [PubMed]
59. Varges, P.R.; Costa, C.M.; Fonseca, B.S.; Naccache, M.F.; Mendes, P.D.S. Rheological Characterization of Carbopol® Dispersions in Water and in Water/Glycerol Solutions. *Fluids* **2019**, *4*, 3. [CrossRef]
60. Lukić, M.; Pantelić, I.; Savić, S.D. Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products. *Cosmetics* **2021**, *8*, 69. [CrossRef]
61. Çevikelli, T.; Güven, U.M.; Öztürk, A.A. Metronidazole Loaded Novel Microemulsion Formulation for Topical Delivery and Characterization With Validated New UPLC Method. *Fabad J. Pharm. Sci.* **2024**, *49*, 111–128. [CrossRef]
62. Çevikelli, T.; Güven Bölgen, U.M.; Öztürk, A.A.; Kandır, S.; Balcı Özyurt, A.; Akyüz-Çetin, S.; Çelenk, A.; Eroğlu Özkan, E.; Demirci Kayıran, S. Lavandula Angustifolia and Pinus Brutia Loaded Nanoemulsion Enhances In Vivo Burn Wound Healing: Histopathological and Cytokine-Based Mechanistic Evidence. *J. Drug Deliv. Sci. Technol.* **2026**, *122*, 108372. [CrossRef]
63. Selmi, H.; Rocchetti, M.T.; Capozzi, V.; Semedo-Lemsaddek, T.; Fiocco, D.; Spano, G.; Abidi, F. Lactiplantibacillus Plantarum from Unexplored Tunisian Ecological Niches: Antimicrobial Potential, Probiotic and Food Applications. *Microorganisms* **2023**, *11*, 2679. [CrossRef] [PubMed]
64. Kim, S.W.; Kang, S.I.; Shin, D.H.; Oh, S.Y.; Lee, C.W.; Yang, Y.; Son, Y.K.; Yang, H.-S.; Lee, B.-H.; An, H.-J.; et al. Potential of Cell-Free Supernatant from Lactobacillus Plantarum NIBR97, Including Novel Bacteriocins, as a Natural Alternative to Chemical Disinfectants. *Pharmaceuticals* **2020**, *13*, 266. [CrossRef] [PubMed]
65. Taléns-Visconti, R.; Belarbi, Y.; Díez-Sales, O.; Julián-Ortiz, J.V.d.; Vila-Busó, O.; Nácher, A. A New Hyaluronic Emulgel of Hesperetin for Topical Application—An In Vitro Evaluation. *J. Funct. Biomater.* **2024**, *15*, 89. [CrossRef] [PubMed]
66. Stancu, A.I.; Dițu, L.M.; Oprea, E.; Ficai, A.; Badea, I.A.; Buleandră, M.; Brîncoveanu, O.; Mirea, A.G.; Voicu, S.N.; Musuc, A.M.; et al. New Antimicrobial Gels Based on Clove Essential Oil–Cyclodextrin Complex and Plant Extracts for Topical Use. *Gels* **2025**, *11*, 653. [CrossRef] [PubMed]
67. Öztürk, A.A.; Aygül, A. Design of Cefaclor Monohydrate Containing Nanoparticles with Extended Antibacterial Effect by Nano-Spray Dryer: A Nanoenglobing Study. *J. Res. Pharm.* **2020**, *24*, 100–111. [CrossRef]
68. Öztürk, A.A.; Namlı, İ.; Aygül, A. Cefaclor Monohydrate-Loaded Colon-Targeted Nanoparticles for Use in COVID-19 Dependent Coinfections and Intestinal Symptoms: Formulation, Characterization, Release Kinetics, and Antimicrobial Activity. *Assay Drug Dev. Technol.* **2021**, *19*, 156–175. [CrossRef] [PubMed]
69. Kong, M.; Chen, X.G.; Xing, K.; Park, H.J. Antimicrobial Properties of Chitosan and Mode of Action: A State of the Art Review. *Int. J. Food Microbiol.* **2010**, *144*, 51–63. [CrossRef] [PubMed]
70. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in Vitro Evaluating Antimicrobial Activity: A Review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef] [PubMed]
71. Flemming, H.C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S.A.; Kjelleberg, S. Biofilms: An Emergent Form of Bacterial Life. *Nat. Rev. Microbiol.* **2016**, *14*, 563–575. [CrossRef] [PubMed]
72. Hall, C.W.; Mah, T.F. Molecular Mechanisms of Biofilm-Based Antibiotic Resistance and Tolerance in Pathogenic Bacteria. *FEMS Microbiol. Rev.* **2017**, *41*, 276–301. [CrossRef] [PubMed]
73. 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. [CrossRef] [PubMed]
74. Prior, R.L.; Wu, X.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [CrossRef] [PubMed]
75. Ngo, D.H.; Kim, S.K. Antioxidant Effects of Chitin, Chitosan, and Their Derivatives. *Adv. Food Nutr. Res.* **2014**, *73*, 15–31. [CrossRef] [PubMed]

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.