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Propolis Incorporated Bovine Gelatin–Chitosan Polysaccharide-Based Films for Wound Dressing

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Abstract

The selection of appropriate materials is a critical step in designing an ideal wound dressing. One of the primary objectives of an ideal wound dressing is the prevention of bacterial infections. In this work bovine gelatine (BGE)–chitosan (CS) wound dressing films supplemented with propolis (PE) which is a well-known anti-bacterial agent were prepared by using solvent casting technique. The structures of the wound dressing films were characterized by FTIR and optical microscopy. Also, physical properties of the films such as thickness, mass, water vapor transmission rate (WVTR), water absorption capacity, water content and dressing pH were determined. The water content of films incorporating propolis was found to range from 18.8% to 23%. The water absorption capacity of these films varied between 137% and 646%, while their WVTR was observed to be between 1228.12 and 1593.75 g/m^2·day. Additionally, the pH values of the prepared films ranged from 5.86 to 7.34, aligning with the typical pH range of human skin. Furthermore, the antibacterial potential of the BGE-CS-PE films was preliminarily assessed using the agar disc diffusion method against Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*S. Typhi*, *E. coli*) microorganisms. The observed inhibition zones suggested the potential contribution of propolis incorporation to the antibacterial properties of the films. The combined physical properties and preliminary antibacterial observations of the prepared films indicate their potential for wound dressing applications.

Keywords: bovine gelatine-based film; chitosan; propolis; dressing; antibacterial activity

1. Introduction

Modern wound dressing theory suggests promoting dynamic equilibrium between exudate absorption and optimal surface moisture at the wound surface [1]. Numerous studies have been conducted to develop wound dressing materials that play a critical role in the healing process [2–8]. Recently, wound dressings based on biodegradable and renewable polymers have attracted growing interest. These natural polymers can better resemble the extracellular matrix and promote wound healing [9,10]. A wide range of natural polymers are known for their biocompatibility, biodegradability and are generally derived from natural tissues or artificial sources such as collagen, hyaluronic acid, chitosan, alginate and gelatin [11–15]. These materials are used alone or in combination depending on the nature and type of wound [16].

Chitosan is a cationic natural polysaccharide that has been widely investigated for wound dressing applications because of its intrinsic antimicrobial activity, biocompatibility, biodegradability, and film-forming ability [17–21]. Gelatin, obtained through the partial

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hydrolysis of collagen, is another widely used biopolymer owing to its excellent biocompatibility, biodegradability, and ability to maintain a moist wound environment, which is crucial for effective healing [22,23]. Furthermore, its glycine-rich composition contributes to tissue regeneration and supports new skin formation. Consequently, chitosan–gelatin composite films have attracted considerable attention as wound dressing materials because they combine the favorable biological properties of both polymers.

Recent studies have demonstrated the potential of chitosan/gelatin-based wound dressings with improved physicochemical, mechanical, and biological properties. For instance, composite biopolymer films with promising wound healing performance have been reported [24], while flexible chitosan-based hydrogel films exhibiting enhanced hemostatic properties have also been developed [25]. In addition, recent reviews have highlighted the rapid progress in chitosan-based wound dressings while emphasizing that further optimization is still needed to improve multifunctionality and overall wound healing performance [26–28]. These studies demonstrate the continued interest in chitosan–gelatin systems while also indicating opportunities for further improvement.

Despite the favorable properties of chitosan–gelatin composite films, certain limitations remain. Gelatin does not possess intrinsic antimicrobial activity, while the antimicrobial effectiveness of chitosan–gelatin films alone may not be sufficient to prevent wound infections, particularly in chronic wounds [29]. Wound infection is one of the major factors delaying tissue regeneration, causing pain, and increasing the risk of severe complications, including tissue loss and, in severe cases, life-threatening conditions. Therefore, improving the antimicrobial performance of biodegradable wound dressings remains an important research objective. Accordingly, numerous studies have investigated the incorporation of natural bioactive compounds into chitosan-based matrices to enhance their biological functionality [6,18,26,27].

Propolis, a resinous substance produced by bees, is collected by Apis mellifera from plant parts. The resin is mixed with mandibular gland secretions and beeswax with the purpose of sealing and protecting beehives against infective microorganisms. It has extensive plant sources and complex chemical compositions, including flavonoids, phenolic acids, terpenes, sugars, hydrocarbons and mineral elements, which enable it to perform a wide range of pharmacological activities including antibacterial, anti-inflammatory, antioxidant, immune stimulant, antitumor, and cytostatic activities. In addition to this range of beneficial effects, propolis also has great potential in promoting wound healing processes [30–36]. Propolis has also been widely incorporated into polysaccharide-based wound dressing systems, particularly in combination with chitosan, to enhance antimicrobial performance [34].

Based on these considerations, this study aimed to develop propolis-incorporated bovine gelatin/chitosan composite films and evaluate their suitability as potential wound dressing materials. The prepared films were characterized in terms of their physicochemical properties, swelling behavior, water vapor transmission rate, and antimicrobial activity. By combining the moisture-retaining capability of gelatin, the intrinsic antimicrobial properties of chitosan, and the bioactive functionality of propolis, this study seeks to provide further insight into the development of naturally derived multifunctional wound dressing materials.

2. Materials and Methods

2.1. Materials

High molecular weight chitosan (degree of deacetylation: 75%) was supplied by Nu-da Gıda Nebati Yağ ve Yem San. Ltd. (İstanbul, Türkiye). Bovine gelatin was obtained from Beşel Industrial Products Trade Inc. (İstanbul, Türkiye). Liquid propolis extract and Hypericum perforatum (St. John's wort) extract were provided by Aksuvital Natural Products Inc.

(İstanbul, Türkiye). Glycerol (technical grade) was used as a plasticizer. Glacial acetic acid (96%, Merck, Darmstadt, Germany) and Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) were used as received. Phosphate-buffered saline (PBS, pH 7.2) was used in the experiments. Nutrient Agar (Merck Microbiology, GranuCult, Germany) was used as the microbiological culture medium. Deionized water was used throughout all experiments.

2.2. Preparation of Dressing Films

Propolis extract (PE) was used as an active component. Propolis-incorporated wound dressing films and blank control samples were prepared using the solvent casting method. A typical experimental procedure is as follows: chitosan (CS) and bovine gelatin (BGE) aqueous solutions were prepared separately. CS (1% *w*/*v*) was dissolved in 100 mL of 1% (*w*/*v*) acetic acid solution and stirred at 400 rpm for 24 h. BGE (5% *w*/*v*) was dissolved in 100 mL of distilled water at 50 °C under continuous stirring for 4 h. Afterward, CS and BGE solutions were mixed, followed by the addition of glycerol (1 mL) and Tween 80 (1 mL) as plasticizers, together with propolis extract (PE, 5% *w*/*w* based on total polymer content). The mixture was further stirred under the same conditions for 2 h, followed by ultrasonication for 30 min and centrifugation at 5000 rpm for 30 min to ensure homogeneity. The resulting solutions were cast onto Petri dishes and dried at room temperature for 24 h. Subsequently, the films were vacuum-dried at 25 °C for 24 h. The dried films were carefully peeled off and stored in a desiccator until further analysis. In our previous study [18], which investigated the effect of plasticizers on gelatin-based wound dressing films, it was demonstrated that films exhibited brittleness and poor mechanical integrity when plasticizers were used individually, whereas improved properties were achieved when glycerol and Tween 80 were used in combination. Therefore, glycerol and Tween 80 were applied in combination in a 1:1 ratio to obtain an optimal film structure.

All prepared films were coded as shown in Table 1, and the schematic representation of the film preparation process is presented in Figure 1.

Table 1. Bovine gelatin–chitosan-based dressing film composition.

Code	K1J1	K1J5	K1J1P1	K1J1P5	K1J5P1	K1J5P5	K1J10P1	K1J10P5
CS:BGE	1:1	1:5	1:1	1:1	1:5	1:5	1:10	1:10
PE (%) *			1%	5%	1%	5%	1%	5%

* Weight ratio of total polymer.

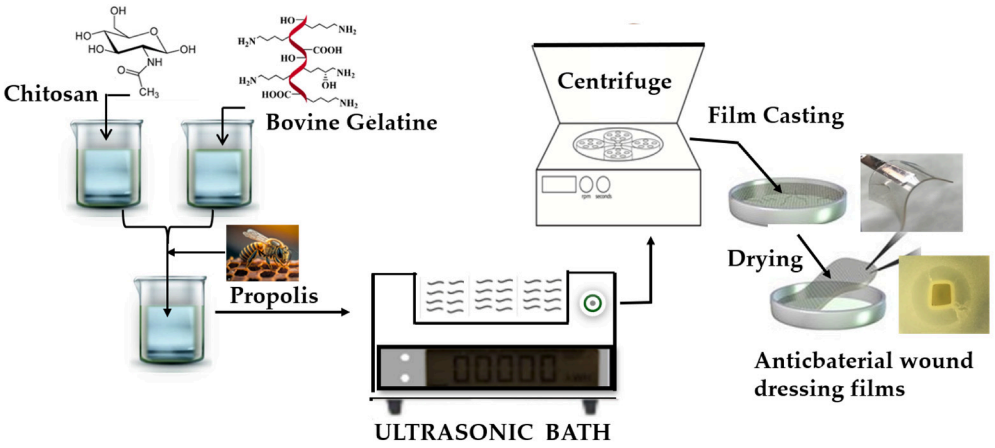


Figure 1. Preparation scheme for propolis incorporated dressing films.

2.3. Characterization

Structural characterization of dressing films was carried out using Perkin Elmer Spectrum 100 brand FTIR spectrometer (PerkinElmer Inc., Waltham, MA, USA), and all

measurements were performed in the wavelength range of 4000–600 cm^{-1} with a resolution of 4 cm^{-1}. Olympus BX53 optical microscope (Olympus Corporation, Hachioji, Tokyo, Japan) was used to analyze the surface morphologies of wound dressing films.

Wound dressing films were cut into 25 mm × 25 mm squares to determine thickness, mass and dressing pH. Their thicknesses were determined using a Fowler thickness gauge (±0.01 µm) at 8 different points on the films. Total mass loss was quantified by calculating the difference between the wet and dry weights.

pH measurements of the wound dressing films were carried out by immersing the samples in 0.9% NaCl solution and monitoring the pH at predetermined time intervals (24, 48, and 72 h) [6].

The water content of the films was calculated using Equation (1) [18,34] by first determining the initial weight (M_i) of the square-shaped samples (25 mm × 25 mm) cut from the films, followed by measuring their final weight (M_f) after being kept at 70 °C for 24 h.

$$\text{Water Content } \% = \frac{M_i - M_f}{M_f} \times 100 \quad (1)$$

The water absorption capacity of the films was calculated using Equation (2) [18]. Square-shaped film samples (25 mm × 25 mm) with known initial dry weights (M_d) were immersed in distillated water for 24 h. The wet weights (M_w) were recorded at 1 h intervals to monitor the swelling behavior. However, equilibrium swelling was reached within 4 h; therefore, the water absorption capacity at 24 h was reported as the final value for comparison

$$\text{Water Absorption Capasity } \% = \frac{M_w - M_d}{M_d} \times 100 \quad (2)$$

The water vapor transmission rates of the wound dressings were determined by modifying the ASTM E96-95 method [37]. Glass containers, filled with silica gel, were sealed with the prepared films and placed in a desiccator containing a saturated NaCl solution. Weight changes were monitored over a 24 h period. The weight change versus time data were plotted, and the slope of the resulting linear graph was divided by the film area to calculate the water vapor transmission rates [38,39].

The antibacterial activity of wound dressing films was studied using the agar disc diffusion method with Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* (*B. subtilis*, *S. aureus*) and Gram-negative bacteria *Escherichia coli* and *Salmonella Typhi* (*E. coli*, *S. Typhi*). Before testing the antibacterial activity, both Gram-negative and Gram-positive microorganisms were activated at 37 °C for 24 h. The films, cut into square shapes (25 mm × 25 mm), were placed in the center of Petri dishes containing agar media using sterile forceps. The Petri dishes containing bacteria and films were incubated, and the inhibition zones around the films were measured.

The antibacterial assay was performed as a preliminary qualitative evaluation, and representative inhibition zone images were recorded.

2.4. Statistical Analysis

All experiments, except for the preliminary antibacterial assay, were performed in triplicate ($n = 3$), and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using two-way analysis of variance (ANOVA) in Microsoft Excel. Differences were considered statistically significant at $p < 0.05$. The antibacterial assay was conducted as a single preliminary qualitative experiment ($n = 1$); therefore, no quantitative measurements or statistical analyses were performed, and representative inhibition zone images were presented.

3. Results and Discussion

Chitosan, as the polysaccharide component of the composite film matrix, may contribute to film formation through its functional groups, hydrophilic characteristics, and intermolecular interactions with gelatin. However, since the relative amount of chitosan changed simultaneously with gelatin content, the observed variations in film properties were interpreted considering the combined effects of gelatin composition and propolis incorporation.

3.1. Fourier Transform Infrared Spectroscopy (FTIR) and Morphology

To confirm the presence of CS, BGE, and propolis in the structure of the prepared wound dressing films, FTIR spectra of both the pure materials and the K1J5P1 film were compared (Figure 2). In the spectrum of pure CS and BGE film, bands characteristic of polysaccharide materials were observed. The bands observed at 1600 and 1550 cm^{-1} can be attributed to amide I (C-O stretching) and amide II (N-H bending), respectively [40]. The bands at 1000 and 1130 cm^{-1} can be assigned to symmetric stretching of C-O-C bond and C-O stretching vibrations, respectively [19,41]. FTIR spectrum of propolis showed peaks at 2960 and 1500 cm^{-1}, which correspond to –OH and NH stretching and bending vibration mode respectively. The addition of propolis extract to BGE-CS film solution caused changes in the structure of obtained films, as presented in Figure 2. BGE-CS films are related to the presence of propolis components, mainly phenolic compounds. The peaks at 2925 and 2855 cm^{-1} can be connected with stretching modes of C-H, arising from methyl groups [11]. The peak at 1680 cm^{-1} indicates the C-O bond, which is present in propolis constituents, such as lipid or phenols [11], and confirmed the presence of propolis in the film structure. Also, optical microscope images of K1J10 and K1J10S5 wound dressing films shown in Figure 2 indicate that PE is homogeneously distributed in the chitosan and bovine gelatin-based matrix.

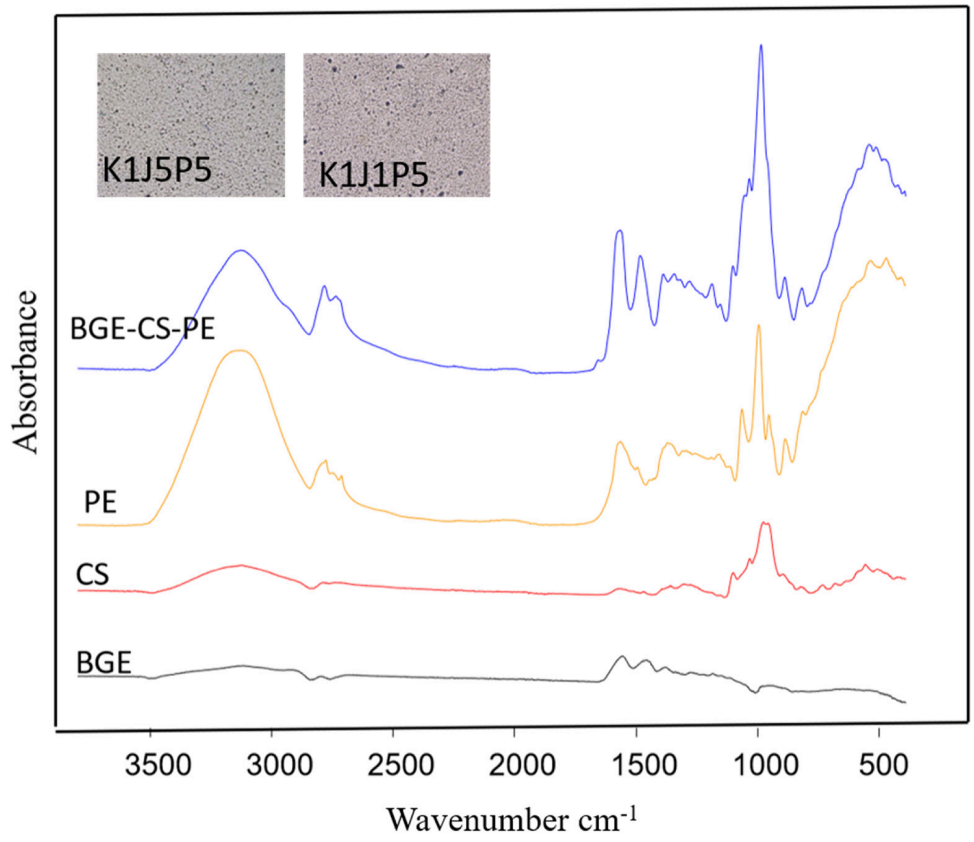


Figure 2. FTIR spectrums and optical microscope images.

3.2. Thickness, Mass Measurement, Dressing pH

Film thickness and mass varied from 36 ± 9 to 98 ± 5 µm and from 53.2 ± 4.44 to 110.7 ± 12.85 mg, respectively, for samples prepared with different casting diameters of 8.8, 9.1, and 9.8 cm. However, it was determined that the change in the thickness of each film was between 0.5% and 1%, and the change in its mass was between 1% and 2%. These low variations show that the films can be prepared homogeneously. The results indicate that the total amount of substance removed varies between 94 and 97 and that the total amount of solid matter used in the structure of chitosan-bovine gelatin films is between 3 and 5% (wt.); it is an expected result that the total amount of removed substance is between 94 and 97%.

It is important that the pH values of wound dressing films are compatible with skin pH, which has been reported to range from 5.25 to 7.9 in the literature [6]. The pH values of the prepared wound dressing films are shown in Figure 3. The pH of all films ranged from 5.86 ± 0.01 to 7.34 ± 0.04, indicating compatibility with skin pH.

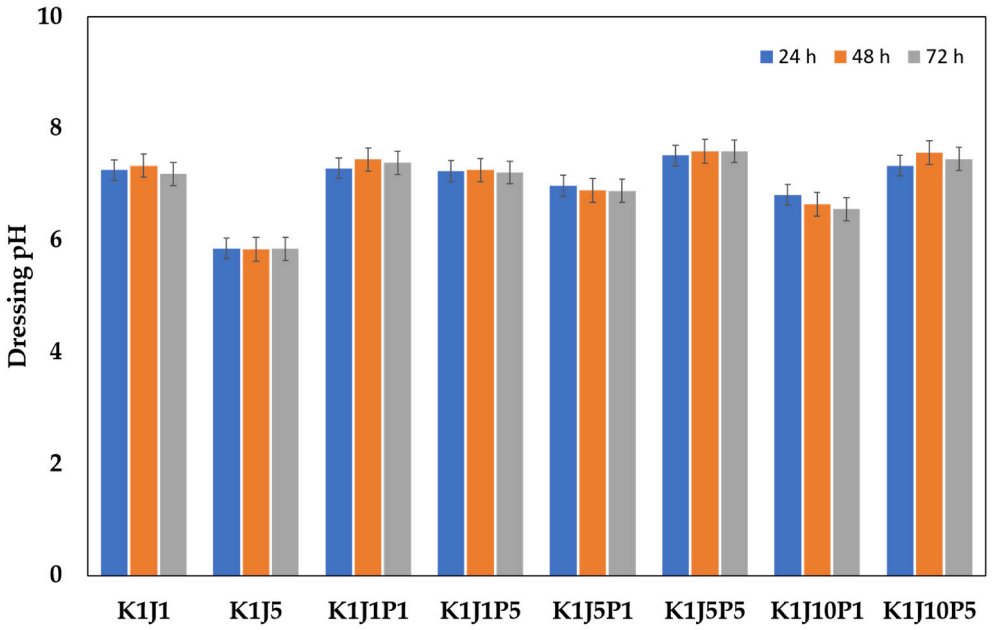


Figure 3. Dressing pH of films.

3.3. Water Content and Water Absorption Capacity

The ability of wound dressing films to maintain a moist environment at the wound site is a crucial characteristic for the healing process [40]. The water content of all prepared films ranged from 7.2% to 23.0% (Figure 4).

Two-way ANOVA revealed that both gelatin content and propolis incorporation significantly affected the water content of the films ($p < 0.05$). The effect of propolis was highly significant ($F = 927.92$, $p = 6.96 \times 10^{-19}$), while gelatin content also had a significant effect ($F = 206.15$, $p = 3.92 \times 10^{-13}$). Furthermore, a significant interaction between the two factors was observed ($F = 89.05$, $p = 1.32 \times 10^{-11}$), indicating that the effect of propolis on water content depended on the gelatin content of the films.

As shown in Figure 4, increasing the gelatin concentration generally decreased the water content of the films. This behavior may be attributed to the formation of a denser and more compact polymer network at higher gelatin concentrations, which restricts the retention of free water within the matrix after drying. Similar relationships between increased network compactness and changes in water-related properties have been reported for gelatin–chitosan composite films [42,43]. In contrast, the incorporation of propolis

significantly increased the water content compared with the corresponding control films, although the magnitude of this increase depended on both the gelatin and propolis concentrations. This behavior may be associated with interactions between phenolic constituents of propolis and the gelatin–chitosan matrix, which can modify the microstructure of the polymer network and promote water retention. The effect of propolis on the water-related properties of polymeric films appears to be highly dependent on the composition of the polymer matrix. In pure gelatin films, propolis has been reported to decrease water content because of its relatively hydrophobic nature [44]. Therefore, the significant interaction observed between gelatin and propolis indicates that the moisture-retention capacity of the films is governed by their combined effect on the composite network rather than by either component alone. These findings demonstrate that the water content of the films can be tailored by adjusting the gelatin and propolis contents, providing a means to optimize the hydration characteristics of wound dressing materials.

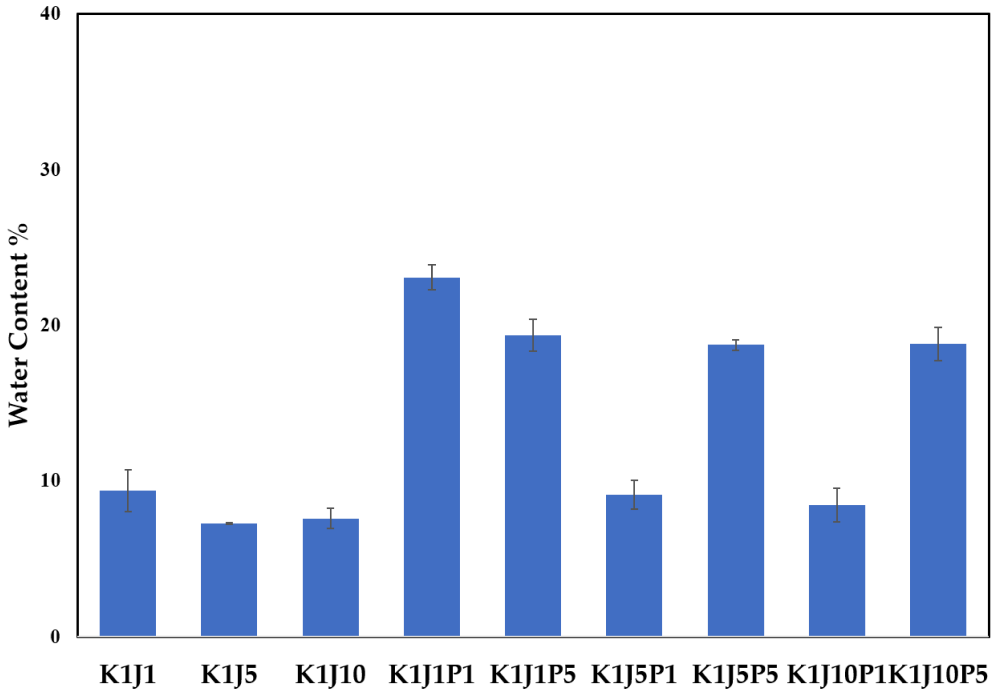


Figure 4. Water content of wound dressing films.

Ideal wound dressings are expected to absorb exudate from the wound surface [45]. The calculated water absorption capacities of the prepared films are presented in Figure 5. It was observed that the water absorption capacity of all films reached equilibrium after 4 h, and the films maintained their structural integrity for 24 h. The water absorption capacity of the K1J1 film is 646%, but with the addition of PE, this capacity decreases by 44% to 362% for the K1J1P1 film and by 78% to 137% for the K1J1P5 film. The water absorption capacity of the K1J5P1 film is 365%. Two-way ANOVA revealed that both gelatin content and propolis incorporation significantly affected the water absorption capacity of the films ($p < 0.05$). The effect of propolis was highly significant ($F = 87.19$, $p = 5.49 \times 10^{-10}$), while gelatin content also showed a statistically significant effect ($F = 7.78$, $p = 0.0037$). Furthermore, a significant interaction between the two factors was observed ($F = 15.07$, $p = 1.42 \times 10^{-5}$), indicating that the swelling behavior of the films depends on the combined effect of gelatin network structure and propolis incorporation.

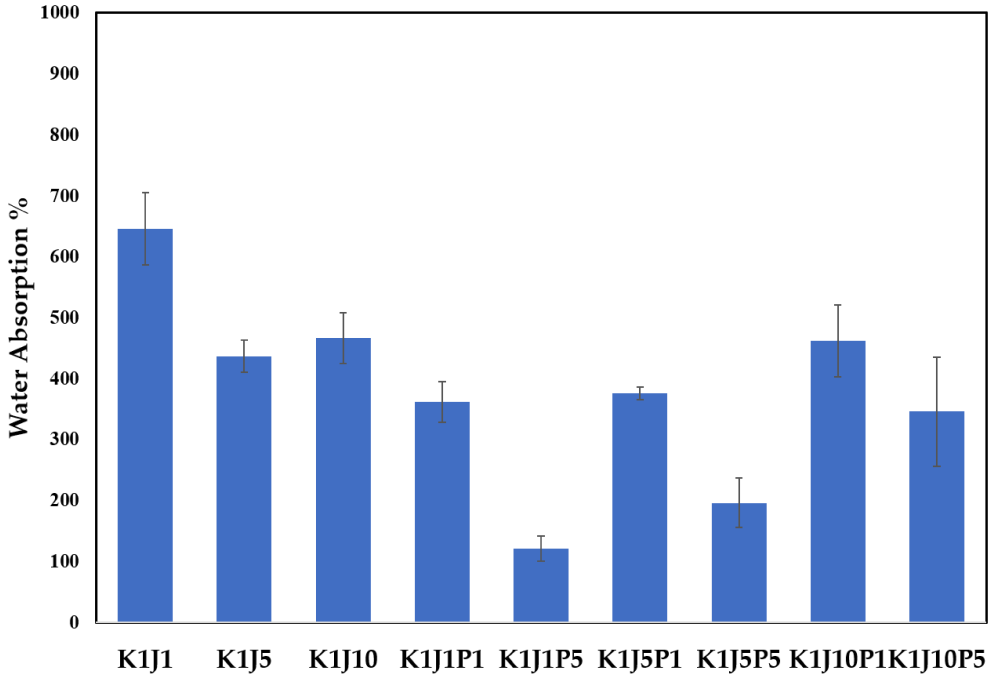


Figure 5. Water absorption capacity of wound dressing films.

It was observed that the incorporation of PE into the wound dressing films was associated with a slight decrease in water content and a more pronounced reduction in water absorption capacity. This behavior may be attributed to the interactions between the polyphenolic compounds present in propolis and the chitosan matrix. Previous studies have reported that interactions between phenolic compounds and chitosan reduce the availability of hydrophilic groups for hydrogen bonding with water, thereby decreasing the swelling capacity of the resulting films [39,46]. Consistent with these findings, the two-way ANOVA demonstrated that both gelatin content and propolis incorporation, as well as their interaction, significantly influenced the water absorption capacity of the films. Furthermore, the water absorption capacities of chitosan-based wound dressing films have been reported to range from 100% to 900% [21,47]. The PE-incorporated films prepared in the present study exhibited water absorption capacities between 137% and 647%, which fall within the range considered suitable for wound dressing applications.

3.4. Water Vapor Transmission Rate (WVTR)

High water vapor permeability of wound dressing films is a desirable feature because it prevents bacterial growth. The water vapor permeability rates of K1J1 and K1J5 films were calculated as 984.38 ± 48.61 g/m^2·day, 721.88 ± 24.80 g/m^2·day, respectively. The water vapor permeability of K1J1P1 and K1J5P5 films was calculated as 1228.12 ± 53.39 g/m^2·day and 1593.75 ± 47.83 g/m^2·day, respectively.

Two-way ANOVA revealed that propolis incorporation significantly affected the water vapor transmission rate (WVTR) of the films ($F = 520.00$, $p = 1.45 \times 10^{-8}$), whereas gelatin content alone did not have a statistically significant effect ($F = 4.35$, $p = 0.0705$). Nevertheless, a significant interaction between gelatin content and propolis incorporation was observed ($F = 190.16$, $p = 7.38 \times 10^{-7}$), indicating that the effect of propolis on the WVTR depended on the gelatin content of the films. These findings suggest that although gelatin content alone did not significantly influence WVTR, the combined effect of gelatin and propolis played an important role in regulating the moisture permeability of the developed films.

The water vapor permeability rate for normal skin is reported to be 204 g/m^2·day [47]. However, in cases of injury, this value can range from 200 to 5138 g/m^2·day [47,48]. Con-

sidering the water vapor permeability rates of the propolis-supplemented films prepared in this study, it is evident that they are suitable for use as wound dressing films.

3.5. Antibacterial Activity

One of the most important features of wound dressing films is their antibacterial effect, which helps protect the wound from microorganisms that may be present in the wound area [49]. Chitosan, a natural polysaccharide, has been widely reported to exhibit antibacterial activity, which is attributed to electrostatic interactions between its protonated amino groups and negatively charged bacterial cell surfaces, resulting in membrane disruption and inhibition of bacterial growth [50]. Although gelatin provides favorable characteristics for wound dressing applications, including biocompatibility and film-forming ability, its intrinsic antibacterial activity is limited; therefore, it is commonly combined with antimicrobial polymers or bioactive agents to improve infection control properties [51]. The antibacterial behavior of the developed chitosan–bovine gelatin films may be associated with the combined contribution of the polymer matrix components and propolis incorporation. Propolis contains bioactive compounds, particularly phenolic constituents, which have been associated with antibacterial activity against various microorganisms. Previous studies have demonstrated that incorporation of propolis into chitosan-based films and wound dressing systems can enhance their antibacterial potential [52,53].

The antibacterial effects of the K1J5P5 and K1J10P5 films against four different microorganisms were evaluated, considering their water vapor permeability, water absorption capacity, and water content properties. Table 2 and Figure 6 present the inhibition diameters of the films against microorganisms. The tested films exhibited antibacterial activity against the evaluated microorganisms, while larger inhibition zones were observed for the propolis-containing films compared with the control sample.

Table 2. Inhibition zone of wound dressing films.

	Gram (+)		**Gram (−)**	
	S. aureus	***B. cereus***	***E. coli***	***S. Tyhpi***
	Inhibition Diameter (mm)		**Inhibition Diameter (mm)**	
K1J5 (Control)	19	30	19	--
K1J5P5	38	45	32	25
K1J10P5	41	40	30	23

While the control film K1J5 did not show any inhibition against *S. Typhi*, the PE-incorporated films K1J5P5 and K1J10P5 demonstrated antibacterial activity with inhibition diameters of 25 mm and 23 mm, respectively. For the control film, the highest antibacterial activity was observed against the Gram-positive bacterium *B. cereus*, with an inhibition diameter of 30 mm. Both propolis-containing films exhibited enhanced antibacterial activity, increasing the inhibition diameters to 45 mm and 40 mm, respectively.

The control film K1J5 showed inhibition diameters of 19 mm against both *S. aureus* and *E. coli*, whereas the PE-incorporated films exhibited larger inhibition zones of 38 and 41 mm for *S. aureus* and 32 and 30 mm for *E. coli*, respectively.

These findings suggest that propolis incorporation may contribute to the antibacterial potential of the chitosan–gelatin films. The higher inhibition zones observed for propolis-containing films are in agreement with previous reports on propolis-containing chitosan-based materials, supporting the potential role of propolis as an antibacterial component in polymeric wound dressing systems [52,53].

The present study has several limitations that should be acknowledged. First, mechanical properties such as tensile strength, elongation at break, and Young's modulus were not

evaluated. Second, neither in vitro cytotoxicity nor in vivo wound healing performance was investigated, which are important for a comprehensive assessment of biomedical applicability. Future studies should therefore focus on mechanical characterization, biocompatibility evaluation, and in vivo testing to further validate the potential of the developed gelatin–chitosan–propolis films for wound dressing applications.

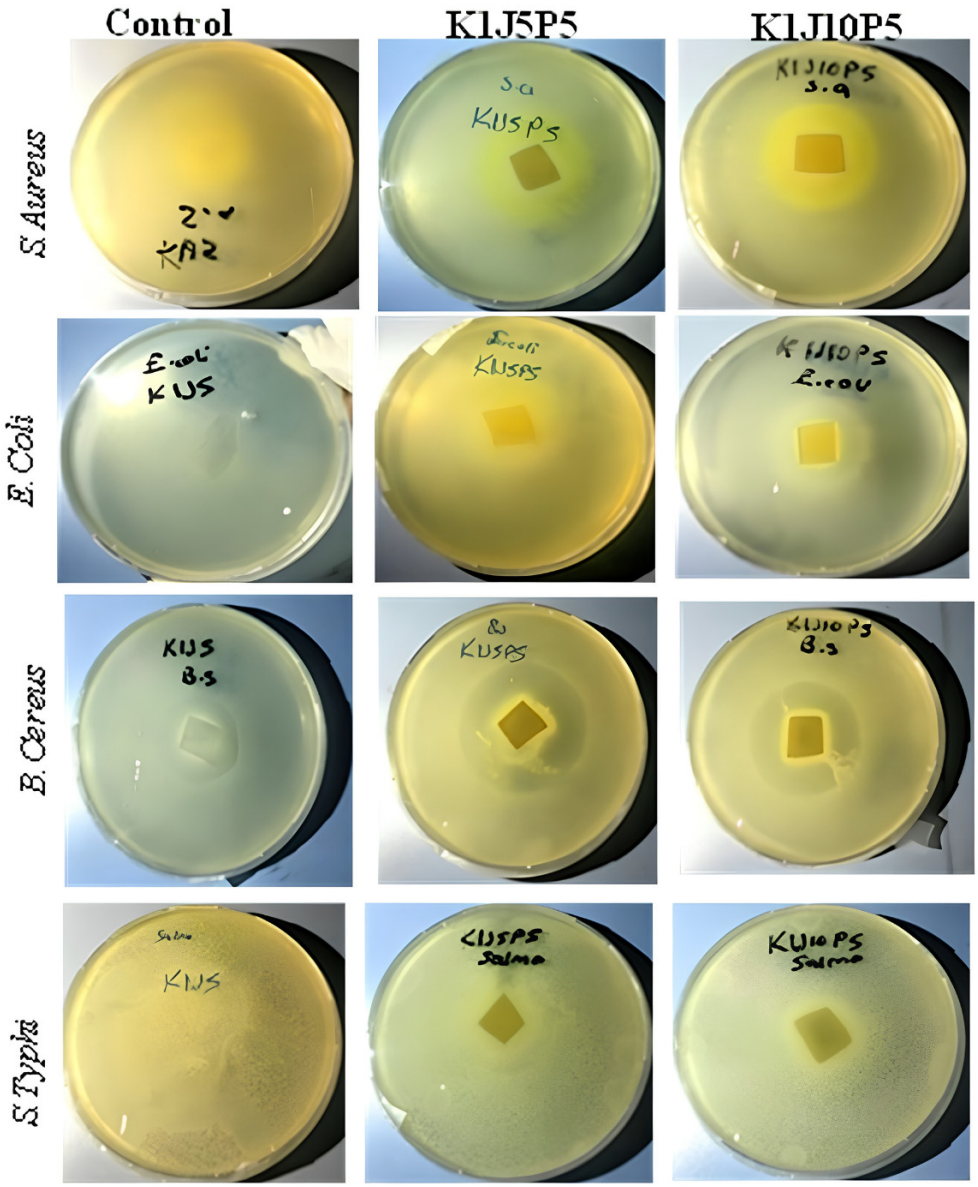


Figure 6. Antibacterial activity of films against to microorganisms and inhibition zones.

4. Conclusions

Chitosan and bovine gelatin-based, propolis-incorporated films intended for wound dressing applications were successfully prepared, and their structural and morphological properties were characterized using FTIR and optical microscopy. The water content of the propolis-incorporated films ranged from 18.8 to 23%, while the water absorption capacity varied between 137 and 646%, and the water vapor permeability rate ranged from 1228.12 to 1593.75 g/m^2·day. The pH values of the prepared films were within the range of 5.86–7.34, indicating compatibility with physiological skin pH. In addition, the propolis-incorporated films showed preliminary antibacterial activity against both Gram-negative and Gram-positive bacteria.

Taken together, the results suggest that the BGE-CS-PE films have potential as wound dressing materials. These films represent promising candidates for future wound dressing applications due to their favorable water absorption capacity, water vapor permeability, and antibacterial properties.

Mechanical properties (tensile strength, elongation at break, and Young's modulus) were not evaluated in this study and should be considered in future work to fully assess the applicability of these films as wound dressing materials.

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

Abbreviations

The following abbreviations are used in this manuscript:

BGE	Bovine Gelatine
CS	Chitosan
PE	Propolis
WVTR	water vapor transmission rate

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