



Effect of *Corchorus olitorius* extract on physical properties of and antioxidant activities of chitosan/gelatin biocomposite films

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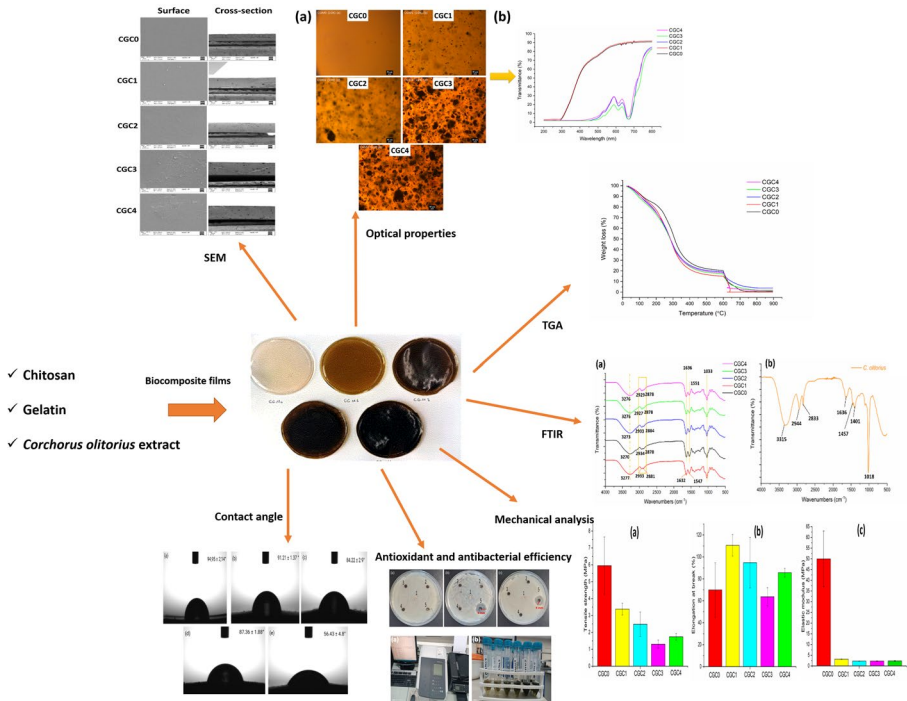
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Abstract

Wound healing is a complex physiological process that requires an optimal moist and antibacterial environment to promote tissue regeneration. In this study, chitosan/gelatin (CG) biocomposite films incorporated with different concentrations of *Corchorus olitorius* (*C. olitorius*) extract (2.5–10% v/v) were fabricated via the solvent casting method. The effects of extract loading on the structural, thermal, mechanical, optical, and biological characteristics of the films were systematically investigated. The tensile strength of the films decreased from 5.95 ± 1.70 MPa to 1.74 ± 0.20 MPa, while the elongation at break increased up to $110.5 \pm 9.8\%$. Contact angle measurements showed enhanced hydrophilicity with the highest extract content ($56.4^\circ \pm 4.8^\circ$). The incorporation of the extract also led to a notable reduction in transparency (from 7.18% to 2.57%) and a slight decrease in thermal stability. FT-IR spectra confirmed physical interactions, mainly hydrogen bonding, between the polymer matrix and the extract components. Furthermore, the 10% (v/v) *C. olitorius*-containing film exhibited significant antioxidant activity (80.5% DPPH scavenging) and antibacterial efficiency with inhibition zones of 9 mm against *E. coli* and *S. aureus*. These results indicate that the developed chitosan/gelatin/*C. olitorius* biocomposite films possess favorable physical and biological properties, suggesting their strong potential for wound dressing and biomedical applications.

Graphical abstract



Keywords Biocomposite film · Chitosan/gelatin · *Corchorus olitorius* extract · Antioxidant activity · Antibacterial efficiency

Introduction

Wound healing is a complex and dynamic biological process involving a series of cellular and molecular events, including hemostasis, inflammation, proliferation, and tissue remodeling, which collectively promote tissue repair and regeneration [1]. In recent years, biocomposite films have gained significant attention as wound dressing materials owing to their biocompatibility, biodegradability, and ability to maintain a moist environment conducive to healing [2–5]. Among the numerous natural biopolymers, gelatin and chitosan are particularly promising candidates for developing bioactive films and hydrogels due to their structural similarity to extracellular matrix components and favorable biological properties [6–8].

Beyond biomedical applications, bio-based and biodegradable polymeric films have been widely investigated for eco-friendly food packaging due to environmental concerns regarding synthetic plastics. Recent reviews have highlighted the development of sustainable packaging films derived from polysaccharides, proteins, and lipids, emphasizing their tunable physical, mechanical, and biological properties [9–11]. For example, Sharma et al. (2022) synthesized a gelatin/green tea-based self-healing

hydrogel designed for biomedical use. The incorporation of green tea extract, rich in polyphenolic compounds, not only enhanced the antioxidant and antibacterial performance of the gelatin matrix but also improved its mechanical flexibility and stretchability. The material exhibited remarkable self-healing capability due to dynamic hydrogen bonding and reversible physical crosslinking between gelatin chains and phytochemicals. Moreover, the hydrogel demonstrated biocompatibility and rapid recovery after deformation, indicating its strong potential for wound healing and soft tissue engineering applications. This study highlights how natural bioactive extracts can synergistically reinforce both the functional and structural performance of biopolymer networks [12]. Similarly, Casalini and Baschetti (2022) reported that cellulose/chitosan films containing rosemary essential oil exhibited significant antimicrobial activity against *E. coli* and *S. aureus*, while maintaining biodegradability and film integrity [13].

Incorporation of natural plant extracts into such matrices represents an efficient strategy to enhance their functional performance, imparting antioxidant, antimicrobial, and UV-shielding properties [14, 15]. Huda et al. (2025) developed ultrasonic-assisted gelatin-based biodegradable films containing turmeric extract (TE) to improve antioxidant and preservation performance. The optimized films showed 87% DPPH radical scavenging activity, reduced UV transmittance by ~ 25%, and enhanced flexibility (elongation at break 21 → 38%) while maintaining biodegradability. When applied to minced chicken, TE-incorporated films effectively inhibited microbial growth and delayed lipid oxidation, extending shelf life by about 4 days. These results confirm that natural antioxidants like turmeric can strengthen the functional and protective properties of gelatin-based biopolymer films [16]. These studies collectively show that the inclusion of polyphenolic compounds enhances radical scavenging capacity and delays oxidative degradation while maintaining biodegradability.

Gelatin, derived from collagen, is a natural polymer with excellent biocompatibility, hemostatic properties, and the ability to support cell adhesion and proliferation [17–19]. Its porous structure facilitates oxygen and nutrient exchange, crucial for wound treatment. Additionally, gelatin forms hydrogels that maintain a moist environment, preventing dehydration and infection [20]. Chitosan, obtained from crustacean exoskeletons, exhibits antimicrobial activity, promotes cell migration, and accelerates tissue regeneration [21, 22]. The combination of gelatin and chitosan in biocomposite films merges the beneficial attributes of both polymers, resulting in a synergistic material that provides structural integrity, antimicrobial activity, and a conducive environment for tissue repair [23].

Incorporating natural plant extracts can further enhance functionality. *Corchorus olitorius* (jute mallow) is rich in bioactive compounds such as chlorogenic acid, caffeoylquinic acid derivatives, gallic acid, and quercetin glycosides [24–29]. Previous studies by Abdel-Razek et al. (2021) demonstrated that DPPH radical scavenging activity of *Corchorus olitorius* leaf extracts prepared with different solvents (ethanol, methanol, water, petroleum ether, etc.) was measured as 17.5%, ~ 86.0%, and ~ 87.1% for the water, methanol, and ethanol extracts, respectively [25]. Further, İşeri et al. (2022) reported that *Corchorus olitorius* leaf and seed extracts exhibited strong antibacterial activity against food and plant pathogens, with MIC values of

8–128 $\mu\text{g/mL}$ and MBC values of 32–2048 $\mu\text{g/mL}$, and that methanol extracts rich in phenolics correlated with high DPPH radical scavenging activity [30]. Therefore, in this study, we developed gelatin/chitosan/*C. olitorius* extract biocomposite films and investigated their physicochemical, structural, thermal, mechanical, and biological properties. By establishing relationships between molecular interactions and macroscopic performance, this work aims to contribute to the growing body of research on biodegradable, bioactive films with potential applications in wound healing and eco-friendly packaging.

Materials and methods

Materials

Gelatin powder (~95% purity) was purchased from Zeta Matrix (İstanbul, Turkey). Chitosan powder was bought out (100% purity) from Smart Chemical Trd. and Cons. Lim. Comp. (İzmir, Turkey). Tannic acid was purchased from Zag Kimya (İstanbul, Turkey). Glycerol was purchased from a local market. *C. olitorius* leaves were purchased from a local herbalist. Methanol (~95% purity) and isopropyl alcohol ($\geq 99.5\%$) were obtained from Sigma-Aldrich (Chemie, Steinheim, Germany).

Extraction of *C. olitorius*

The bioactive components of *C. olitorius*, such as flavonoids and other phytochemicals, are naturally present in the plant and can be effectively obtained through solvent extraction. In this study, the dried leaves of *C. olitorius* were finely ground and subjected to methanolic extraction using a Soxhlet apparatus, following previously reported methods [31, 32]. This approach ensures efficient recovery of the plant's functional constituents, which can subsequently contribute to the antioxidant and bioactive properties of chitosan/gelatin biocomposite films.

Preparation of gelatin/chitosan biocomposite films

Chitosan was dissolved in a 2% (v/v) aqueous acetic acid solution at 50 °C and stirred continuously for 4 h to prepare a 4% (w/v) chitosan solution. Simultaneously, a 15% (w/v) gelatin solution was prepared in distilled water at 40 °C under continuous stirring for 2 h using a magnetic stirrer. The chitosan and gelatin solutions were then mixed in a 1:1 (v/v) ratio and stirred overnight to ensure complete homogenization. Glycerol was subsequently added as a plasticizer, and the resulting mixture was stirred for an additional hour at room temperature. Finally, specific amounts of citric acid and tannic acid, used as physical crosslinkers, were incorporated into the polymer mixture.

A specific amount of *C. olitorius* extract (0, 2.5, 5, 7.5, and 10% v/v of the total polymer mixture) was added to the polymer solution and stirred for 2 h at 40 °C. Subsequently, 20 mL of the resulting mixture was poured into circular glass Petri dishes with a diameter of 12 cm and dried under ambient conditions overnight (Fig. 1). The

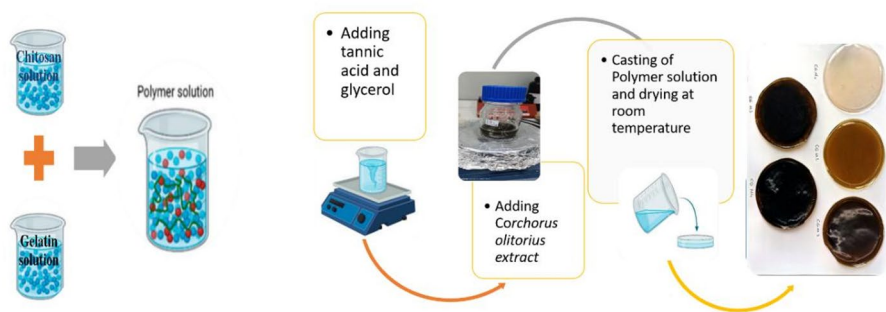


Fig. 1 Schematic procedure for preparation of biocomposite films

Table 1 Compositions of biocomposite films

Sample ID	Extract (% v/v)	Chitosan (% v/v)	Gelatin (% v/v)	Citric acid (% w/v)	Tannic acid (% w/v)	Glyc- erol (% v/v)
CGC0	-	4	15	0.5	0.1	3
CGC1	2.5	4	15	0.5	0.1	3
CGC2	5	4	15	0.5	0.1	3
CGC3	7.5	4	15	0.5	0.1	3
CGC4	10	4	15	0.5	0.1	3

obtained biocomposite films were designated as CGC0, CGC1, CGC2, CGC3, and CGC4, respectively, while chitosan/gelatin films without extract served as the control. The compositions of the prepared biocomposite films are summarized in Table 1.

Characterization methods

Optical properties

The macroscopic surface morphology of the resulting biocomposite films was examined using a light microscope (Leica BM 2500 M) at a magnification of 100× and a scale bar of 50 μm. Additionally, a Shimadzu UV-3600 spectrophotometer was employed to record the transmittance spectra of each polymer film in the UV–visible range. The transparency of the films was calculated according to Eq. 1.

$$Transparency = \frac{\log(\% T_{600})}{x} \quad (1)$$

where %T₆₀₀=the percent transmittance, and x=polymer film thickness (mm).

Scanning electron microscope (SEM)

The cross-sectional and surface microstructure of the samples were examined using a Carl Zeiss/Gemini 300 scanning electron microscope equipped with a Schottky FEG gun. For SEM analysis, the sample surfaces must be conductive to allow electron transfer. Therefore, the surfaces of the nanocomposite films were coated with a gold–palladium layer (15 nm) using a Leica ACE600 sputter coater prior to imaging.

Fourier transform infrared (FT-IR) spectroscopy

Chemical structural characterization of the obtained films was performed using a PerkinElmer Spectrum Two FT-IR spectrophotometer. The films were scanned in the wavenumber range of 4000–400 cm^{-1} using a diamond crystal.

Thermal properties

Thermal degradation behavior of the films was evaluated using a TA Instruments SDT 50 with an alumina pan. TGA measurements were conducted under a nitrogen atmosphere from 25 to 600 °C, followed by an oxygen atmosphere from 600 to 900 °C.

Contact angle

Wettability of the films was assessed using an Attension Theta Lite contact angle goniometer. Samples (2 cm × 2 cm) were measured by depositing a 3 μL droplet of deionized water in sessile drop mode. The contact angle was defined as the internal angle formed between the tangent drawn at the water–film interface and the water droplet.

Mechanical properties

Mechanical properties of the biocomposite films were determined using an AGS-X Series (1 kN, Shimadzu, Japan) universal testing machine according to ASTM D882-18: *Standard Test Method for Tensile Properties of Thin Plastic Sheeting*. Prior to testing, the samples were conditioned at 23 °C and 50% relative humidity for at least 40 h following ASTM D618 Procedure A. Films were cut into rectangular strips of 5 cm length and 1 cm width. Tensile tests were performed at a constant deformation rate of 10 mm/min. Tensile strength, Young's modulus, and elongation at break were measured five times for each sample, and the results are reported as mean $\pm$ standard deviation.

Film thickness

The film's thickness was measured using digital callipers (MACRONA, Turkey) at three random spots on each film. The film thickness was given as the arithmetic mean.

Antibacterial efficiency

The antibacterial activity of the biocomposite films was evaluated using standard strains of *Escherichia coli* (ATCC® 25922) and *Staphylococcus aureus* (ATCC® 25923). Lyophilized bacterial strains were cultured on Tryptone Soy Agar (Merck Millipore™ 105458) and incubated for 24 h at 37 °C under aerobic conditions. Bacterial suspensions were prepared in isotonic saline and adjusted to a turbidity of 0.5 McFarland (1×10^8 CFU/mL). The antimicrobial efficacy of the films was assessed qualitatively using the disk diffusion method.

Antioxidant activity

The short-term antioxidant efficiency of biocomposite films was evaluated using a free radical scavenging experiment with 2,2-diphenyl-1-picryl hydrazyl (DPPH). In this study, the analysis and extraction of samples were performed using from literature [33]. The absorbance at 517 nm was measured using a spectrophotometer (Fig. 2). The DPPH radical scavenging activity was determined using the following Eq. 2:

$$\% \text{ Radical scavenging activity} = \frac{A_{\text{reference sample}} - A_{\text{sample}}}{A_{\text{sample}}} \times 100 \quad (2)$$

in which A is the amount of water absorbed at 517 nm.

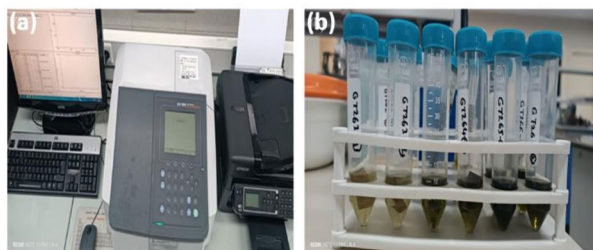
Results and discussion

Optical properties

Recently, the adverse effects of ultraviolet (UV) radiation on living organisms have attracted considerable attention. Over time, humans are exposed to various environmental stressors, including UV radiation, pollution, and lifestyle factors, which can accelerate skin aging, trigger inflammation, and even increase the risk of skin cancer [34–36].

It has been reported that gelatin-based films exhibit higher UV-blocking capacity compared to some synthetic films [37], whereas chitosan films generally possess poor UV barrier properties. In one study, the UV light transmittance of chitosan–gelatin composite films was very low at 200 nm and increased with higher chitosan content

Fig. 2 The photograph of a spectrophotometer (a), and preparation of biocomposite film samples for antioxidant activity (b)



at 280 nm [38]. Conversely, increasing gelatin content led to decreased light transmission at 280 nm [39].

Overall, chitosan–gelatin composite films demonstrate excellent UV-blocking ability, particularly when gelatin is present at optimal concentrations. As the macromolecular chains of chitosan and gelatin approach each other, the internal structure becomes more organized, reducing light transmission and rendering the films more opaque [40]. Furthermore, incorporating functional materials into chitosan–gelatin composites has been shown to enhance their UV barrier properties [40, 41].

Plant extracts, rich in potent antioxidants such as flavonoids, play a significant role in protecting against UV-induced damage and have considerable scientific and therapeutic relevance. Incorporating such extracts into polymer films can improve UV protection by imparting UV-absorbing properties to the films [42]. For instance, chitosan–gelatin films containing hawthorn (*Crataegus pinnatifida*) fruit extract exhibited enhanced UV-blocking performance, attributed to the polyphenols in the extract and the UV-absorbing capacity of the film matrix [43].

In the present study, the UV–Vis barrier properties of the produced films were assessed by measuring light transmittance. Pure chitosan–gelatin films without extract displayed the highest transparency, whereas the addition of *C. olitorius* extract led to a concentration-dependent decrease in transparency, consistent with observable color changes (Table 2). This reduction is likely due to light scattering caused by the presence of extract particles within the polymer matrix. Moreover, the dark brown/black color of the *C. olitorius* extract obtained via Soxhlet extraction highlights its potential as a UV-absorbing agent in polymer films.

In the 200–800 nm, it was observed that chitosan/gelatin films have a transmittance value of 90% from 800 nm to about 280 nm, and the permeability decreases with increasing extract concentration (~82%) (Fig. 3). Nevertheless, no significant variation in transmittance values of the between the CGC1 films with the lowest extract concentrations.

Morphology by scanning electron microscopy (SEM)

SEM is a valuable technique for assessing the homogeneity of components in active films. Microstructural analysis provides detailed information on the surface and cross-sectional morphology, which is crucial for understanding the relationship between the microstructure and the macroscopic properties of the films (Fig. 4). Both pure chitosan/gelatin films and those containing extracts exhibited a continuous structure without apparent phase separation. However, this homogeneous structure was disrupted in films with higher amounts of *C. olitorius* extract. Specifically, excessive extract led to the formation of agglomerates, particularly in CGC3 and CGC4

Table 2 Transparency values of the biocomposite films

Sample ID	Extract (% v/v)	Transparency (%)
CGC0	-	7.18±0.3
CGC1	2.5	4.74±0.2
CGC2	5	3.59±0.18
CGC3	7.5	2.42±0.12
CGC4	10	2.57±0.12

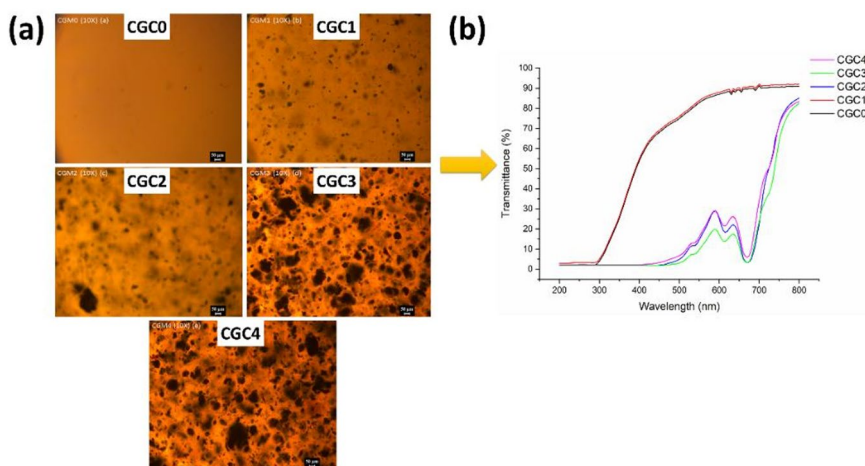


Fig. 3 Optical images of (a) and (b) transmittance spectra of biocomposite films

samples. Similar agglomeration phenomena have been reported in SEM images of films containing modified *Acca sellowiana* extract [44] and in fish gelatin films with incorporated additives [42].

FT-IR analysis

FT-IR analysis was performed to identify the functional groups and investigate the interactions between the extract and the polymer matrix in pure chitosan/gelatin films (CGC0) and *C. olitorius* extract-containing films (CGC1–CGC4). The spectra of all films are presented in Fig. 5a.

Gelatin, a typical polyampholyte, contains charged carboxyl groups ($-\text{COOH}$) that can form ionic interactions with protonated amine groups ($-\text{NH}_3^+$) on chitosan chains under acidic conditions [45]. In addition, the polar groups in gelatin, such as $-\text{COOH}$, $-\text{NH}_2$, and $-\text{OH}$, can establish hydrogen bonds with $-\text{OH}$ and $-\text{NH}_2$ groups on chitosan chains [46, 47].

The characteristic peaks of pure chitosan/gelatin films (CGC0) include a broad $-\text{OH}$ stretching band at 3277 cm^{-1} , asymmetric and symmetric $-\text{CH}$ stretching peaks at 2933 and 2881 cm^{-1} , respectively, a sharp amide I peak at 1632 cm^{-1} ($-\text{C}=\text{O}$ stretching), an amide II peak at 1547 cm^{-1} ($-\text{NH}$ bending), $-\text{CH}_2$ bending at 1451 cm^{-1} , $-\text{CH}_3$ bending at 1389 cm^{-1} , $-\text{CH}$ bending at 1240 cm^{-1} , and bands at 1153 and 1033 cm^{-1} associated with saccharide unit vibrations and $-\text{CO}$ asymmetric stretching in chitosan [48–50].

Upon incorporation of the highest concentration of *C. olitorius* extract (10%), shifts were observed in several characteristic peaks: $-\text{CH}$ stretching shifted from 2933 to 2929 cm^{-1} , amide I from 1632 to 1636 cm^{-1} , amide II from 1547 to 1552 cm^{-1} , $-\text{CH}_2$ bending from 1451 to 1457 cm^{-1} , and $-\text{CH}_3$ bending from 1389 to 1397 cm^{-1} . The intensity of the amide I band also decreased. Such peak shifts indicate interactions between the extract and polymer matrix, consistent with previous reports of extract–polymer interactions in composite films [51].

Fig. 4 The surface morphology and cross-sectional images of all films (Magnification: 1 kX and 50 X, scale bar: 10 μ m, 100 μ m, respectively)

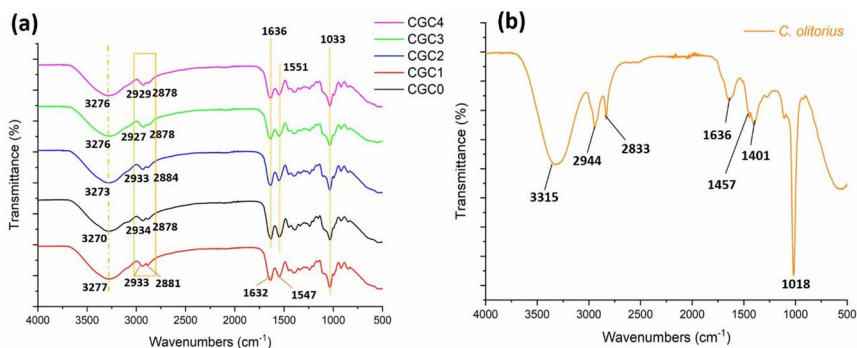
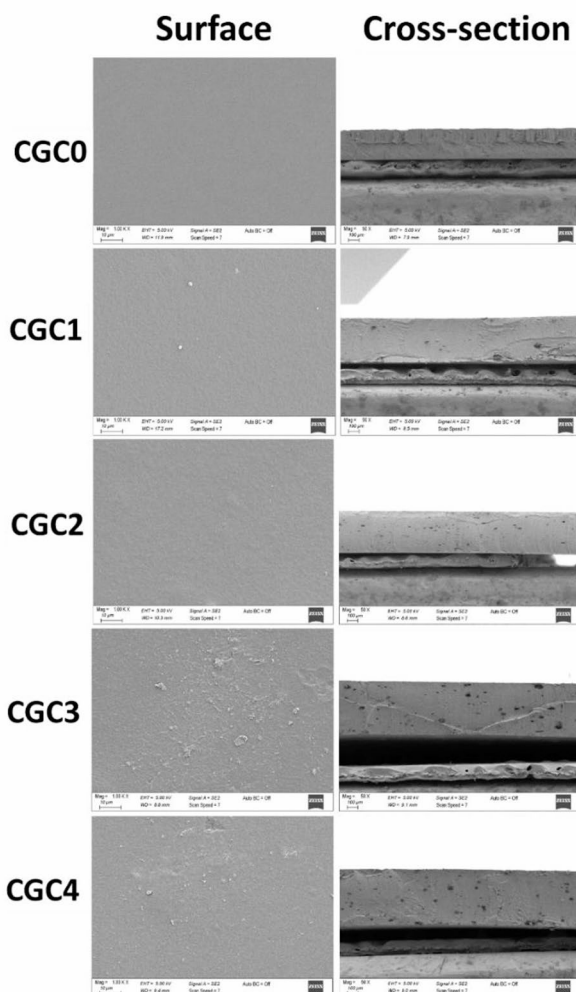


Fig. 5 FT-IR spectra of (a) all film samples, and (b) *C. olitorius* extract

Overall, the FT-IR spectra of the films were similar to the control CGC0 film, reflecting the relatively low concentrations of the extract. Detailed analysis suggests that the interactions among chitosan, gelatin, and *C. olitorius* extract are primarily physical, involving hydrogen bonding. The shifts in amide I, amide II, and $-\text{CH}_2$ bending bands suggest hydrogen bond formation between phenolic hydroxyl groups in the extract (e.g., chlorogenic acid, caffeic acid, gallic acid derivatives) and amino or hydroxyl groups on the polymer chains. Broadening of the $-\text{OH}$ stretching band ($3270\text{--}3310\text{ cm}^{-1}$) further supports the formation of hydrogen-bonded networks within the matrix. These interactions improved miscibility between chitosan and gelatin, enhanced film hydrophilicity, and promoted uniform dispersion of the extract at low concentrations, although higher extract loadings led to partial aggregation due to saturation of binding sites. Overall, the results demonstrate that *C. olitorius* extract acts as both a hydrogen-bond donor and acceptor, reinforcing interpolymeric associations and influencing the physicochemical behavior of the biocomposite films.

In the FT-IR spectrum of *C. olitorius* extract (Fig. 5b), a prominent peak at 3314 cm^{-1} corresponds to $-\text{OH}$ and $-\text{C}=\text{O}$ stretching vibrations of compounds such as 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid, ascorbic acid, α -tocopherol, and quercetin-3-galactoside [52, 53]. A strong band at 1050 cm^{-1} is attributed to $\text{C}-\text{O}$ vibrations of proteins present in the leaves [54, 55].

Thermogravimetric analysis (TGA)

The TGA thermograms of all prepared bio-based chitosan/gelatin film samples are presented in Fig. 6. According to the thermograms, pure chitosan/gelatin films (CGC0) exhibited three distinct degradation steps. In the first step, a 15.9% weight loss occurring between 100 and 180°C is attributed to the evaporation of water and residual acetic acid. The second step, corresponding to 61% weight loss between 200 and 500°C , is associated with the decomposition of saccharide rings in chitosan and the cleavage of protein chains and peptide bonds in gelatin [56, 57]. The final degra-

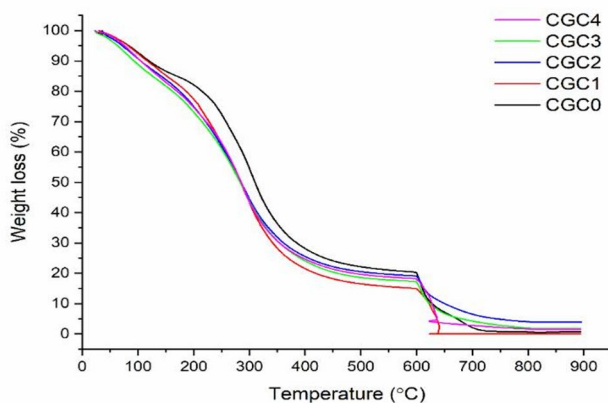
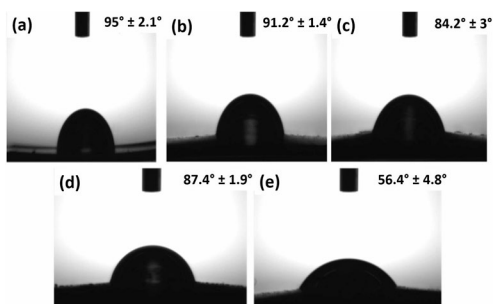


Fig. 6 TGA thermograms of the biocomposite films

Table 3 Thermal properties of all films

Sample ID	Extract (v/v %)	T _{d10} (°C)	T _{d50} (°C)	T _{d90} (°C)	Char content at 900 °C (%)
CGC0	0	119.6	309.8	627.6	0.74
CGC1	2.5	116.3	283.3	621.8	0.00
CGC2	5	104.6	284.7	650.5	3.92
CGC3	7.5	92.6	283.2	623.4	1.86
CGC4	10	104.6	285.1	626.7	1.5

Fig. 7 The contact angle of chitosan/gelatin films with different amounts of *C. olitorius* extract. (a) CGC0, (b) CGC1, (c) CGC2, (d) CGC3, and (e) CGC4, respectively

dation step, observed above 600 °C, is related to the pyrolytic decomposition of the chitosan/gelatin matrix in an O₂ atmosphere [58].

All chitosan/gelatin films containing *C. olitorius* extract exhibited similar thermal degradation behavior. This phenomenon can be attributed to the formation of secondary interactions, primarily hydrogen bonds, between the polymer chains and the extract, which help stabilize the polymer network [59]. Nevertheless, the decomposition temperature of the films slightly decreased with increasing extract concentration (Table 3). Such a reduction is considered negligible and has been observed in similar studies; for instance, the onset temperature (T_{onset}) of films decreased with higher concentrations of *Cynara cardunculus* leaf extract [60].

Contact angle

A humid microenvironment is beneficial for wound healing, as it helps reduce dehydration, promotes angiogenesis and collagen formation, and facilitates the removal of necrotic tissue and fibrin [3]. Contact angle measurements are commonly used to assess surface hydrophobicity and wettability, reflecting the relationship between the film's microstructure and its chemical properties [61].

The wettability of polymeric materials used as wound dressings is directly related to cellular behaviors such as adhesion and differentiation. Materials are classified as hydrophilic, partially hydrophilic, or hydrophobic based on contact angle values of 0–30°, 30–90°, and > 90°, respectively [62].

Pure chitosan/gelatin films have exhibited hydrophobic properties. In this study, the contact angle value obtained for chitosan/gelatin films was 95° ± 2.1°, 91.2° ± 1.4° (Fig. 7). It was compatible with the literature values (92.57° and 96.2°) [63]. It has been found that as the amount of extract increases, the polymer films become hydrophilized and their wettability increases in correlative. Generally, the contact

angle values of pure chitosan/gelatin film decrease with increasing addition of *C. olitorius* extract compared to other films. This reduction in contact angle can be attributed to the formation of hydrogen bonds between the hydrophilic groups of the extract (mainly phenolic –OH) and the polar groups of chitosan and gelatin, as evidenced by the FTIR results. The stronger hydrogen-bonded network likely increased the surface energy and improved water spreading on the film surface. Thus, the contact angle data are consistent with the FTIR findings, confirming the enhanced interfacial interactions among the components.

Mechanical property determination

Table 4 presents the mechanical properties of the biocomposite films, including average tensile strength, elongation at break (EAB), and Young's modulus values for each extract concentration. Mechanical performance is a critical parameter for polymer films intended for wound dressing applications, as such films are expected to exhibit adequate flexibility and sufficient tensile strength. Therefore, the mechanical properties of chitosan/gelatin biocomposite films were evaluated as a function of *C. olitorius* extract content.

The tensile strength of the pure chitosan/gelatin film (CGC0) was measured at 5.95 ± 1.70 MPa, which is higher than previously reported values for chitosan/gelatin films [64]. Incorporation of 2.5% extract reduced the tensile strength to 3.38 ± 0.35 MPa. Mechanical properties are influenced not only by the amount of additives but also by the type of plasticizer used. Al-Hassan and Norziah [65] reported that the tensile strength of sago starch/fish gelatin films decreased with variations in plasticizer type and gelatin content. The reduction in tensile strength is likely due to limited compatibility between the polymer chains and extract molecules. As the extract concentration increased from 2.5% to 10%, the tensile strength further decreased to 1.74 ± 0.20 MPa (Fig. 8), which may be attributed to agglomeration of extract particles at higher concentrations, resulting in weaker interactions with the polymer matrix.

The Young's modulus of the pure chitosan/gelatin film was 49.96 ± 13.02 MPa, while all extract-incorporated films exhibited significantly lower values, decreasing to 2.35 ± 0.20 MPa. Similar findings were reported by Bitencourt [66], where incorporation of ethanolic *Curcuma* extract into a gelatin matrix reduced Young's modulus due to interactions between phenolic compounds in the extract and gelatin peptides.

The addition of the extract also affected the elongation at break. Except for CGC3, the films became more flexible compared to the pure chitosan/gelatin film, with CGC1 (lowest extract concentration) exhibiting the best polymer–extract interaction.

Table 4 Mechanical properties of all biocomposite films

Sample ID	Thickness	Tensile Strength (MPa)	Young Modulus (MPa)	Elongation at Break (%)
CGC0	0.32	5.95 ± 1.70	49.96 ± 13.02	69.91 ± 24.80
CGC1	0.44	3.38 ± 0.35	3.15 ± 0.11	110.53 ± 9.83
CGC2	0.49	2.48 ± 0.73	2.26 ± 0.10	94.78 ± 23.17
CGC3	0.56	1.30 ± 0.4	2.26 ± 0.13	63.66 ± 8.35
CGC4	0.52	1.74 ± 0.2	2.35 ± 0.20	85.62 ± 3.96

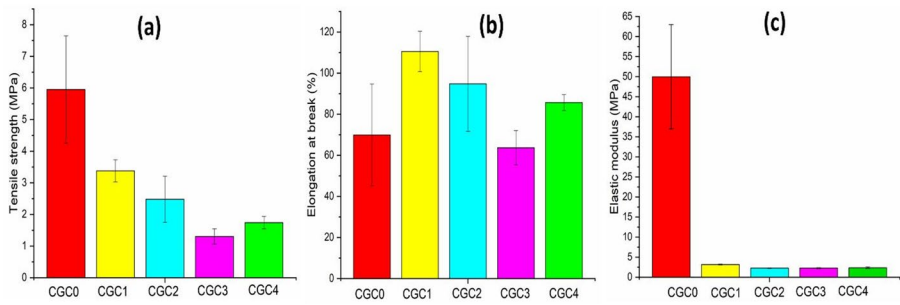


Fig. 8 (a) Tensile strength and (b) Elongation at break, and (c) Elastic modulus graphs of the films

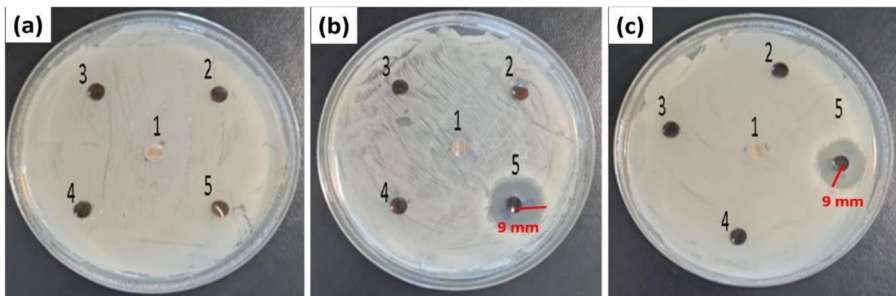


Fig. 9 Antibacterial efficiencies of the films against (a) *E. coli*, (b) *S. aureus*, and (c) *P. aeruginosa* pathogens

Similar trends have been observed in other systems; for example, the incorporation of guarana ethanolic extracts significantly increased EAB in gelatin-based films [67].

Antibacterial efficiency

Bacterial infections have become a significant public health concern due to the emergence of drug-resistant strains, highlighting the need for novel antimicrobial therapies. Antibacterial activity is particularly critical in wound treatment, as wounds exposed to physical and environmental stressors are more susceptible to pathogenic infection, leading to delayed healing. In this study, the disk diffusion method was employed to evaluate the antibacterial activity of pure chitosan/gelatin films (CGC0) and biocomposite films containing *C. olitorius* extract (CGC1–CGC4) against *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative), and *Staphylococcus aureus* (Gram-positive).

As shown in Fig. 9, none of the films, except CGC4, exhibited antibacterial activity against *E. coli* and *S. aureus*. This lack of activity may be attributed to the low extract concentration or restricted extract availability due to cross-linking of the polymer matrix with tannic acid. In the literature, Kurtuluş et al. (2024) reported that nanofibrillated cellulose incorporated into chitosan/gelatin bioaerogels exhibited strong antibacterial activity against both *S. aureus* and *E. coli* [19]. Additionally, the

Table 5 Antioxidant properties of films

Sample ID	DPPH activity (% scavenging activity)
CGC0	-
CGC1	24.3±0.8
CGC2	42.5±0.3
CGC3	65.3±0.1
CGC4	80.5±0.1
<i>C. olitorius</i> extract	89.5±0.2

hydrophobic nature or poor solubility of certain antibacterial agents in hydrophilic environments can limit their efficacy. In line with contact angle results, CGC4 exhibited the highest hydrophilicity, which likely contributed to its superior antibacterial performance.

C. olitorius possesses antibacterial activity due to the presence of phytochemicals such as triterpenes, alkaloids, coumarins, flavonoids, and anthraquinones [68, 69]. Previous studies have demonstrated the antibacterial activity of *C. olitorius* against *E. coli*, *S. aureus*, and *Bacillus pumilus* [70, 71], while Namwase [72] confirmed its efficacy against *S. mutans*.

It is generally observed that Gram-positive bacteria are more resistant to antibacterial agents than Gram-negative bacteria, which may be attributed to the thick peptidoglycan layer in their cell walls that hinders penetration and confers mechanical resistance. Plant extracts exert antimicrobial effects by disrupting phospholipid components in cell membranes, increasing membrane permeability and causing cytoplasmic leakage, or by interacting with enzymes located in the cell wall [73].

Antioxidant activity determination

As shown in Table 5, the antioxidant activity of the biocomposite films increased with higher *C. olitorius* extract content, as expected. Films containing the highest extract concentration exhibited antioxidant activity ($80.5 \pm 0.1\%$) approaching that of the pure extract itself ($89.5 \pm 0.2\%$), indicating effective incorporation of the extract into the chitosan/gelatin matrix. These results are consistent with previous reports in the literature [17].

In the DPPH free-radical scavenging assay, all extract-containing films demonstrated substantial antioxidant activity. Films with higher extract content showed enhanced activity, in agreement with contact angle measurements, as increased hydrophilicity facilitates greater release of the extract. No antioxidant activity was detected in the pure chitosan/gelatin films (CGC0).

It was also observed that the antioxidant activities of the extract-incorporated films were lower than the activity of the free extract, likely due to limitations in extract release from the cross-linked polymer network. Previous studies have reported that the addition of cross-linking agents can reduce film antioxidant activity by approximately 50% [74, 75]. During cross-linking, carboxyl groups from citric acid and tannic acid may interact with hydroxyl and amino groups of gelatin and chitosan, as well as with active sites on the *C. olitorius* extract, thereby reducing the number of functional sites available for antioxidant activity.

Conclusion

In conclusion, chitosan/gelatin biocomposite films containing *C. olitorius* extract were successfully fabricated, exhibiting enhanced hydrophilicity and antioxidant activity. Although optical and SEM analyses indicated some heterogeneity in the distribution of the extract within the polymer matrix, this was accompanied by a corresponding decrease in film transparency. FT-IR spectra showed minimal changes due to the low extract content, confirming that no chemical bonds were formed between the polymers and the extract. The thermal stability of the films slightly decreased upon extract incorporation, likely due to the natural extract.

The extract improved the wettability of the films and enhanced their antibacterial activity, with CGC4 showing the highest inhibition zones of 9 mm against both *E. coli* and *S. aureus*. Furthermore, incorporation of the extract increased the antioxidant activity from 0% in pure films to $80.5 \pm 0.1\%$ in the highest-loaded films. Mechanical testing revealed that the tensile strength of the films ranged from 5.95 ± 1.70 to 1.74 ± 0.2 MPa, demonstrating suitable mechanical properties for wound dressing applications.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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