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Antimicrobial zein/glucomannan edible films with pomegranate polyphenols and meadow sage essential oil for enhanced food preservation

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ABSTRACT

Background: Edible coatings have garnered considerable attention in recent years as sustainable alternatives to conventional food packaging, owing to their safety, biodegradability, and environmental compatibility. Natural polymers, such as proteins and polysaccharides, are widely used to develop edible films with favorable mechanical strength and gas barrier properties.

Objective: This study investigates the incorporation of bioactive compounds from Meadow Sage (*Salvia pratensis*) and polyphenols from pomegranate peel (*Punica granatum* L.) into a biopolymer matrix composed of zein (Z) and glucomannan (GM). The mechanical, moisture barrier, and antimicrobial properties of the resulting composite films were evaluated to assess their potential in functional food applications.

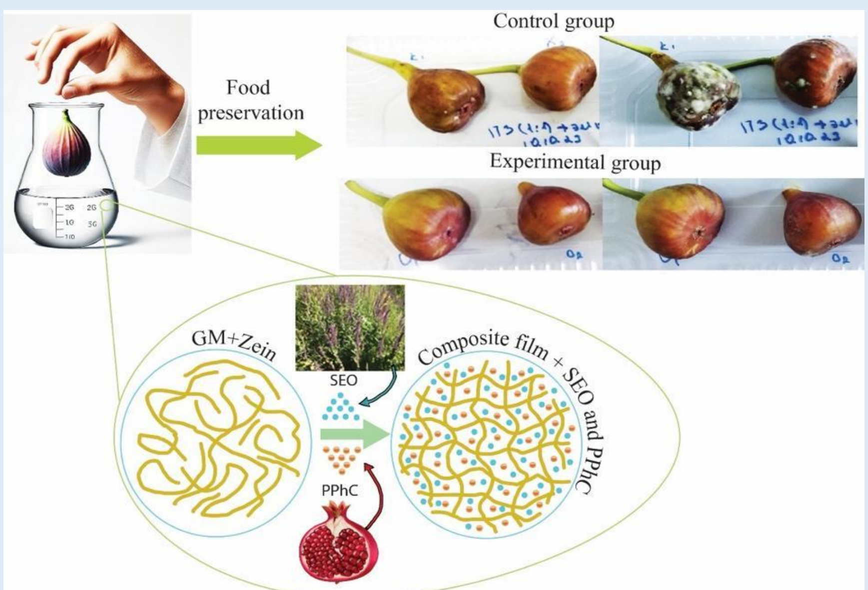
Methods: Essential oils from *S. pratensis* (SEO) and polyphenolic extracts from *P. granatum* (PPhC), sourced from plants grown in Tajikistan, were incorporated into Z/GM-based edible films. The structural properties, antioxidant activity, mechanical behavior, and antimicrobial effects of the films were systematically characterized.

Results: The bioactive films demonstrated strong radical scavenging activity. The addition of SEO enhanced the hydrophobicity of the films, resulting in improved moisture barrier performance. While the films exhibited moderate tensile strength, they showed increased flexibility; notably, SEO significantly increased elongation at break to 184.2% ($P < 0.05$). Films containing BAC concentrations of 20–50 µg/mL displayed broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria, as well as yeast strains.

Novelty and Contribution: This study presents a novel approach to developing biodegradable edible films incorporating plant-derived bioactive compounds for active food packaging. The functional enhancement of the films, particularly in terms of mechanical resilience and antimicrobial efficacy, underscores their potential as carriers of bioactive ingredients in the context of functional foods.

Conclusion: Z/GM edible films enriched with PPhC and SEO show promising properties for food coating applications. Their biodegradability, combined with enhanced antimicrobial and barrier functions, positions them as eco-friendly, functional alternatives for next-generation food packaging systems.

Keywords: glucomannan, zein, edible films, polyphenols, essential oils, antibacterial activity.



Graphical Abstract: Antimicrobial zein/glucomannan edible films with pomegranate polyphenols and meadow sage essential oil

INTRODUCTION

Fruits and vegetables have increasingly attained the focus of many recent studies. They serve as a rich source of nutrients that significantly benefit consumers, helping to prevent chronic diseases. They contain biologically active compounds (BAC) such as polyphenols, polysaccharides, and dietary fibers, which are particularly effective in preventing cardiovascular diseases and diabetes. Additionally, they contribute to antioxidant effects due to their polyphenol content. However, fruits and vegetables are susceptible to microbial infections, environmental factors, and storage conditions [1,2]. In addition to production, the storage of produce presents a considerable challenge—specifically, the prevention of postharvest loss—which significantly hinders food and nutritional security. This can lead to excessive waste production, threatening the ecosystem and limiting availability during the off-season [3].

The active packaging of food products is considered a superior choice for preservation as it provides a good barrier against contamination from gases, moisture, pathogens, and light. Concerning the rising threat to the ecosystem and health, synthetic packaging films/coatings or chemical-based treatments are not recommended. Typically, the modified atmospheric packaging and wax coatings approach to fruit preservation could be used for fruit storage [4-6]. Active packaging has emerged as an effective preservation technology to protect food from environmental and microbial influences, offering significant economic and health benefits and attracting increasing attention [5,6].

Petroleum-based polymers such as ethylene vinyl alcohol, polyvinyl alcohol, and low-density polyethylene dominate film-forming materials because they offer superior barrier and mechanical properties, are easy to process, and possess other advantageous features. However, with the implementation of global carbon-neutral initiatives and a rising consumer focus on environmental sustainability and health concerns, there is increasing interest in developing food packaging using natural and biodegradable materials like polysaccharides, phospholipids, and proteins. These sustainable alternatives are expected to become significant contributors to film-forming materials in the future [6].

Although polysaccharides have significant advantages as edible coatings due to their biocompatibility and biodegradability, they also have certain limitations depending on their type and structure, such as poor moisture barrier properties, low mechanical strength, and sensitivity to humidity, which may restrict their application in food packaging. The correct selection of the ratio and type of biopolymers, in this instance, polysaccharides and proteins or the inclusion of extracts containing BAC in the composites, can effectively change the properties of the coating to overcome these limitations, which is the goal of this study. Moreover, natural antioxidants and antimicrobials derived from herbal sources enhance the functionality of the coatings and meet consumer demand for edible coatings [3]. Polysaccharide and protein blend materials have been tested for their biocompatibility in forming packaging films. Generally, in composite films, each biopolymer imparts its unique characteristics, thereby improving the properties of the films compared to single-material systems, provided there is compatibility between the used biopolymers [7].

Glucomannan polysaccharide is suitable for producing edible films due to several significant advantages, including availability, water solubility, hydrophilicity, biocompatibility, non-toxicity, and suitable rheological and mechanical properties. Plants of the family *Eremurus*, belonging to the Liliaceae family, grow worldwide, including in Central Asia. The tubers of these species contain a water-soluble glucomannan-type polysaccharide. Methods for extracting glucomannan from *Eremurus* roots, its structure, and its functional properties are described in numerous studies [7-12].

Zein, a hydrophobic protein commercially produced from corn flour during starch production, is insoluble in water due to its high non-polar amino acid content [13]. Zein possesses excellent water-barrier properties and unique film-forming capabilities. Therefore, zein-based films have excellent moisture barrier ability and water resistance compared to other protein films. Additionally, the U.S. FDA has certified zein as a pharmaceutical film-forming polymer under the Generally Recognized As Safe (GRAS) designation. However, natural zein films are fragile and have poor flexibility, which is not conducive to industrial processing and limits their application [14].

Our previous studies have shown that films obtained from hydrophobic zein and glucomannan extracted from the *Eremurus hissaricus* plant grown in Tajikistan exhibit moderate swelling in water, vapor permeability [15], and possess the required mechanical properties suitable for long-term transportation and storage of food products [16].

Active edible coatings of natural origin are new approaches for enhancing the shelf life of foods and have recently been preferred by consumers over synthetic films. Incorporating antimicrobial and antioxidant agents can enhance the inhibitory effects of edible films. Many plant extracts and essential oils have been increasingly considered natural preservatives [2-6, 17].

Essential oils are GRAS and contain BACs, including terpenes, terpenoids, phenylpropenes, and polyphenolic compounds. These compounds are lipophilic and exhibit strong antimicrobial and antioxidant properties [18]. Polyphenol-rich extracts can enhance films' antibacterial and antioxidant potential and increase their strength and flexibility while reducing brittleness. They can also improve mechanical and barrier properties and enhance thermal stability by increasing crosslinking between the polyphenols and the film-forming polymers [19]. Nastasi et al. found that the tensile strength of polyphenol films increased by up to 40% [20]. However, the elongation at break differed based on the concentration and molecular type of the polyphenols. [19]. In contrast, incorporating essential oils into edible films can improve hydrophobicity, mechanical properties, antimicrobial activity, and facilitate the delayed release of active components on the food surface, extending shelf life [20].

In this work, we studied the formation of biodegradable polymer composite materials based on zein and glucomannan, incorporating biologically active compounds from polyphenol extracts and essential oils to impart antibacterial activity to the studied edible films.

This study aims to investigate the incorporation of biologically active compounds from Meadow Sage (*Salvia pratensis*) and polyphenols from pomegranate peel (*Punica granatum* L.), both cultivated in Tajikistan, into a polymer composite matrix based on zein and glucomannan, and to evaluate the mechanical and antibacterial properties of the resulting films to assess their potential application in functional foods and active packaging systems.

MATERIALS AND METHODS

Materials: Zein (food additive corn protein) was obtained from Kobayashi Zein DP-N, Tokyo, Japan. Glucomannan was extracted from the *Eremurus hisaricus* plant grown in Tajikistan according to the method described in [12].

Extraction of Bioactive Compounds: Essential oil from Meadow Sage (*Salvia pratensis*) (SEO) grown in Tajikistan was extracted by applying the steam distillation method with a Clevenger apparatus. The polyphenolic compounds (PPhC) from pomegranate peel were isolated as follows: thirty grams (30 g) of dried pomegranate peel powder were boiled under reflux in a Soxhlet extractor to remove fats and waxes. Subsequently, 250 mL of water, 70% aqueous-alcoholic solution, and 90% aqueous-alcoholic solution were added to each residue of pomegranate peel and left for six days to ensure complete extraction of PPhC. The remaining alcoholic liquid phase was then used to measure the total PPhC content using the Folin-Ciocalteu reagent [21]. Total flavonoid compounds were determined, and antioxidant capacity was evaluated by the DPPH assay [22].

Preparation of Films: Biopolymer composite films were prepared from corn zein (Z) and glucomannan (GM) in different biopolymer ratios according to the method described in [15]. Unlike the previous work, the films were prepared by mixing an alcohol solution of zein with an aqueous solution of GM and adding SEO and PPhC from pomegranate. Composite films were formed by applying the solution to a glass plate. The resulting films were dried in a cabinet for 14 hours at temperatures of 50–60 °C, then kept at room temperature for 24 hours before analysis.

FT-IR Spectroscopy Analysis: The interactions between biopolymers and bioactive compounds (BAC) in the resulting films were studied using IR spectroscopy on a Spectrum 65 FT-IR device (Perkin Elmer, Switzerland). After drying, a small sample was taken from the films, further dried at 90 °C, and analyzed for chemical interactions of polymer chains by comparing them with the spectra of individual biopolymers and BAC. During the analysis, film samples were placed in contact with a plate using an ATR attachment with a ZnSe prism. Each spectrum was obtained by 16–32 scans of the region 4000–600 cm^{-1}. Each spectrum was recorded by Perkin Elmer Spectrum software, version 10.03.07.

Moisture Absorption Capacity: The films' moisture absorption capacity (W) was determined according to the procedures presented in [16]. Anhydrous $CaCl_2$ (3 g) was placed in a cup (d = 1.8 cm), carefully sealed with Z/GM film, and placed in a desiccator at 25 °C. The experiment was conducted at equilibrium pressure and humidity equal to 75% over a NaCl solution at room temperature (25 °C). W ($mg/cm^2 \cdot h$) at atmospheric pressure was calculated using the following equation:

$$W = \frac{m - m_0}{t \times S}$$

where m and m_0 (g) are the final and initial weights of the cup, S (cm^2) is the contact area of the composite film, and t (h) is the time duration.

Tensile Test: The mechanical properties of the investigated films were measured using a Shimadzu AGS-X testing machine equipped with a 50 N load cell at room temperature. Samples were cast in rectangular shapes with a width of 10 mm, dried, and cut a gauge length of 35 mm. The sample thickness was measured using a Mitutoyo Litematic VL-50 thickness tester (measuring force 0.01 N). Films were stretched at a 1 mm/min rate on the testing machine. Stress and strain parameters were calculated using Trapezium X software. Average values and standard deviations for both parameters were determined from six similar measurements.

Antibacterial Activity of Films: The antibacterial activity of film solutions was evaluated in the independent laboratory "Intestinal Infections and Parasitology" of the Tajik Research Institute of Preventive Medicine, Ministry of Health and Social Protection of the Republic of Tajikistan. The antimicrobial activity of the composite films against food pathogens, including *Staphylococcus epidermidis* (G^+), *Staphylococcus aureus* (G^-), *Escherichia coli* (G^-), *Klebsiella spp.*, *Pseudomonas aeruginosa* (G^-), *Citrobacter spp.*, and *Proteus vulgaris* (G^-), was determined using the method described in [23]. Briefly, a well was pre-made in the center of the cup on frozen nutrient media using a sterile test tube with a diameter of 12–15 mm. Using a sterile pipette, 0.1 mL of the tested preparations were applied to the wells on the surface of the agars. The results were evaluated after 24 hours of incubation at 37 °C in a thermostat. The Z/GM (BAC) film-casting solution was tested against all listed bacterial strains. The diameters of the inhibition zones around the wells were measured using a digital caliper. Control treatments were also performed for each microbe without adding film samples. All tests were performed in triplicate.

Statistical Analysis: All experiments were performed in triplicate, and differences between means were determined using Duncan's test at $p < 0.05$ using SPSS 19 Statistics. The significance level was 2-5% ($p < 0.05$), and results were expressed as mean ± SEM.

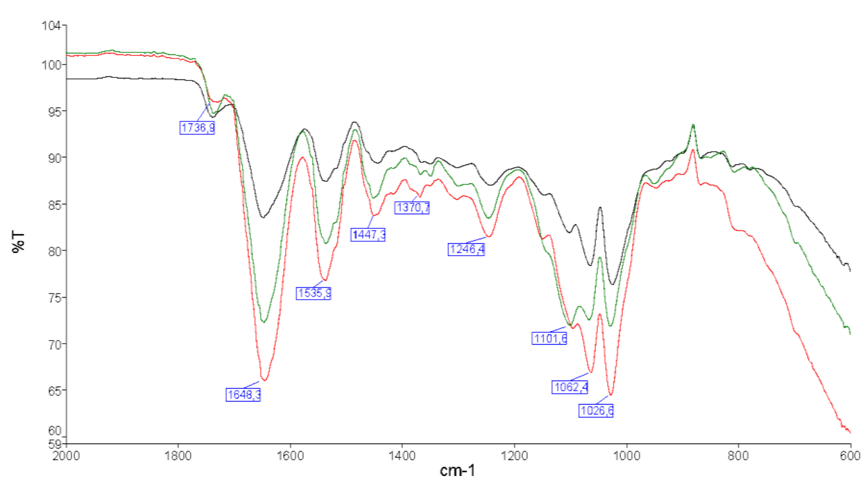
RESULTS AND DISCUSSION

Fabrication of biofilms: Previously [12,15], we developed composite films based on zein (Z) protein and glucomannan (GM) polysaccharide using the casting method and characterized their physicochemical and mechanical properties. To maintain the physical and mechanical properties of edible films and impart water-repellent characteristics to the polysaccharide film, we utilized corn zein protein, which possesses excellent film-forming properties. The optimal composition of biopolymers was established at a Z/GM ratio of 1.5, which demonstrated the best physicochemical and mechanical properties. FTIR spectroscopic analysis confirmed the presence of molecular interactions between zein and GM [15].

This work introduced natural antimicrobial components into the composition to functionalize the obtained composites with the optimal polymer ratio. A polyphenol-rich 70% ethanol extract from non-feed residues (pomegranate peel) and SEO as antibacterial agents were incorporated. The maximal encapsulated concentrations of SEO and PPhC were 25 ± 2.5 μg and 50 ± 3.0 μg, respectively.

Z/GM(SEO) composite films are formed based on biopolymer coacervation technology. The formation and stabilization of films are facilitated by the high viscosity of the biopolymer solution and the formation of hydrogen bonds between zein and GM biopolymers. The addition of zein also can increase the thermal properties and hydrophobicity of the films [24].

Intermolecular interactions and structural changes in the film matrix at the molecular level were detected by FTIR spectroscopy using spectral analysis [25]. After the incorporation of BAC (SEO and PPhC), a broad peak in the range of 3300 cm^{-1} to 3280 cm^{-1}, associated with O-H stretching vibrations, became flatter and shifted to a lower wavenumber at 3287–3291 cm^{-1} (Figure 1S.). This shift indicates deformation vibrations of free O-H bonds due to the formation of hydrogen bonds between EO or PPhC and the GM and zein components.



Wavelength (cm^{-1})

Figure 1. FT-IR spectra of a composite film containing EO and PPhC: – Z/GM 1.5; – Z/GM 1.5+SEO; – Z/GM 1.5+PPhC (2000-600 cm^{-1}).

The bands at 1647 cm^{-1}, 1536 cm^{-1}, and 1246 cm^{-1} are characteristic of zein, and the contributions from the stretching vibrations of C=O and C–N in zein overlap with GM's characteristic carbonyl (C=O) band at 1642 cm^{-1} (Figure 1). Recent studies [25], through comparative analysis of IR Fourier spectra, demonstrated that the inclusion of bioactive compounds—PPhC in Z/GM composite films—occurs through the formation of new bonds involving the carbonyl and free hydroxyl groups of the polysaccharide and PPhC. This confirms the hypothesis of obtaining composite films with active antibacterial compounds.

Moreover, adding SEO and PPhC to the Z/GM composite film led to increased peaks at 2928 cm^{-1} and 2876 cm^{-1}, with lower frequencies than films without PPhC (Figure 1S), indicating increased hydrophobization of the films. When BAC is introduced into composite films, the absorption peak at 1736 cm^{-1}, related to esterified OH groups, increases significantly ($P < 0.05$), more so for PPhC than EO). In SEO-containing films, the adsorption bands at 1102.0 cm^{-1} and 1246.4 cm^{-1} correspond to the ether group, 1447.8 cm^{-1} and 1536.0 cm^{-1} represent the aromatic group, and 2933.38 cm^{-1} indicate aliphatic CH of lipids. The peak at 1102 cm^{-1} in the composite film with EO was more intense due to the O-H bond, while the intensity of the prominent peaks in the spectral regions of 1800–600 cm^{-1} in films with both BAC decreased (Figure 1). Compared with the FT-IR spectrum of the control composite, the introduction of both EO and PPhC demonstrated the attenuation of the bands at 1648 cm^{-1}, 1536 cm^{-1}, and 1246 cm^{-1} belonging to amides I-III of zein. Also, there is a decrease in low-intensity bonds of 1447 cm^{-1} and 1370 cm^{-1}, attributed to the methyl and acetyl groups of polysaccharides, which was observed. Moreover, the introduction of both EO and PPhC resulted in the attenuation of the bands at 928 cm^{-1}, 850 cm^{-1}, and 808 cm^{-1}, which were attributed to the glycosidic linkages in polysaccharides and the typical aromatic rings of terpenes and PPhC. These findings confirm that EO and PPhC bind to the polysaccharide-protein matrix of the composite, which is consistent with the literature [17,20,22].

The total PPhC, flavonoids, and antioxidant activity: The total amount of PPhC extracts was determined by gravimetrically drying an aliquot of the extracts and using the Folin-Ciocalteu (FC) method. The resulting data are presented in Table 1.

As shown in Table 1, the highest amount of extractable substances was obtained using a 70% alcohol medium (34.75%), followed by a 90% alcohol medium, and the lowest amount was found when using water (22.82%). In addition to PPhC, the extracts may contain neutral sugars, polysaccharides, tannins, and other extractable substances from the pomegranate peel.

Table 1: Total extracted substances, PPhC and flavonoid content, and free radicals scavenging activity.

Extraction medium	The yield of isolated substances, % ±SD	The total amount of PPhC (mg GA/g dray weight) ± SD	The quantity of Flavonoids (mg Rt/g dry weight) ± SD	Free radicals scavenging activity, % ± SD
Water	22.82 ± 2.43	190.9 ± 8.34	45.7 ± 2.65	86.7 ± 2.70
Ethanol 70%	34.75 ± 3.54	245.8 ± 7.63	58.5 ± 1.89	89.5 ± 2.84
Ethanol 90%	27.67 ± 3.20	178.6 ± 11.47	43.5 ± 1.67	78.3 ± 2.83

The amount of PPhC, calculated as milligrams of gallic acid per gram of dry weight, was higher in the extract obtained with a 70% alcohol solution (245.8 ± 7.63 mg/g) compared to those using water and a 90% alcohol solution (178.6 ± 11.47 mg/g) as extractants. The content of flavonoids, expressed as rutin equivalent per gram of dry weight of pomegranate peel, was also highest in the 70% alcohol extract (58.5 ± 1.89 mg) compared to water (45.7 ± 2.65 mg) and the 90% alcohol solution (43.5 ± 1.67 mg).

The antioxidant activity of the PPhC was determined using the 2,2-diphenyl-1-picrylhydryl (DPPH) radical method, as described in [23]. The extract obtained with a 70% alcohol solution exhibited high inhibitory activity against the DPPH free radical, equal to 89.5 ± 2.84%, which was statistically different ($p < 0.05$) from the aqueous extract's activity of 86.7 ± 2.83% ($p > 0.05$). Overall, the antioxidant activity of the pomegranate peel extract was significantly higher than that of the pomegranate seeds and juice. This finding is consistent with studies demonstrating the high antioxidant activity of pomegranate peel compared to other parts of the fruit [24], highlighting the peel's potential as a raw material for PPhC extraction.

Composition of SEO: The essential oil (EO) yield from *Salvia pratensis* (Meadow Sage) was 1.34%, with a density of 0.785 g/cm³ and a refractive index of 1.3840. Meadow Sage EO, growing in Tajikistan, contains the following components: 22.5% β-caryophyllene, 7.8% sclareol, 6.8% isocaryophyllene, 6.7% caryophyllene oxide, 4.9% logifolene, 4.8% α-terpineol, 3.9% δ-cadinene, 3.7% gurjunene, and up to 1.4% and 1.7% limonene and (E)-β-ocimene, respectively [26]. Additionally, the EO contains tannins, bitters, and gum, as previously studied by [26].

Moisture Absorption Capacity: The moisture absorption capacity of the films was determined by drying the film samples in an oven at 105 °C for 48 hours. The film thickness was measured using a digital caliper at four points and averaged. Film thickness can affect mechanical properties by increasing the density per cm² of the film. In this study, the thickness of the control film was 235±5.0 µm. With the addition of BAC, the film thickness increased to 240±5.5 µm and 250±5.5 µm with the addition of PPhC and SEO, respectively. The differences between the films were not statistically significant ($p > 0.05$). Therefore, thickness, as a film property that could significantly affect the mechanical properties of the films investigated in this study, was not considered a contributing factor.

Moisture Adsorption Isotherms: The moisture adsorption isotherms for the studied films in Figure 2 exhibit a complex pattern. As observed from the adsorption curves, the moisture content initially increased during the first 5 hours, then slowly decreased over the next 20 hours, followed by a substantial increase. This behavior is attributed to the initial swelling of the polysaccharide, which takes time to create a barrier to escaping vapors. The observed variability in moisture adsorption may stem from changes in the molecular structure of the film and the presence of hydrophobic zein, which influences water vapor interactions. The nonlinear nature of water sorption isotherms in biopolymer films makes modeling water transport through these materials particularly challenging. It is generally accepted that hydrophobic components and plasticizers reduce intermolecular attractive forces within the biopolymer matrix. Additionally, residual water within the film can act as a plasticizer in hydrophilic regions, which may contribute to a further reduction in water adsorption. Subsequently, the film material gradually dissolves, leading to the observed changes in moisture content.

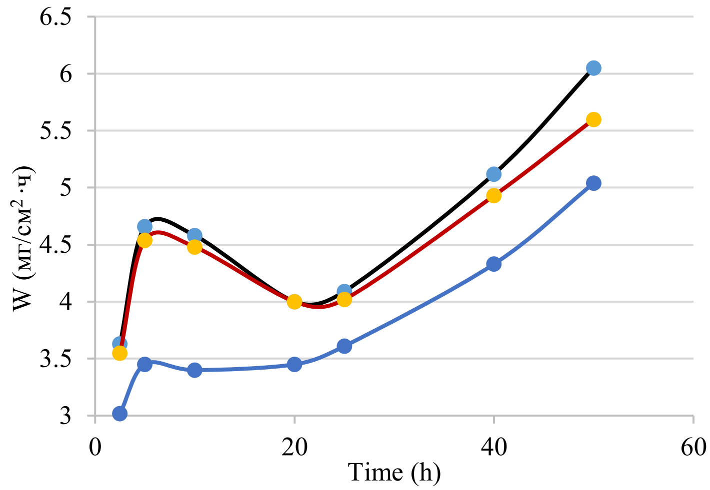


Figure 2. Moisture absorption capacity of Z/GM films: – Z/GM=1.5; – Z/GM/PPhC, – Z/GM/SEO.

The addition of PPhC did not significantly ($p > 0.05$) alter the adsorption behaviors of the films. However, the moisture content of active films significantly decreased after adding SEO ($p < 0.05$). This decrease in moisture content in films containing SEO is related to the hydrophobic nature of SEO and the reduction in the hydrophilicity of the film.

Edible coatings should meet specific basic requirements, including moisture barrier property, maintenance of appropriate gas levels to prevent an anaerobic environment, melting above 40 °C, non-stickiness, efficient drying characteristics, low viscosity, and translucency to opacity, but they should not negatively affect the quality of the food products to be coated. [24]. Thus, the results obtained in this study suggest that the moisture content of the storage environment for food products should be controlled after applying Z/GM or Z/GM (BAC) coatings.

Mechanical Properties of Films: A key function of edible coated films as an active food preservation method, alongside moisture control, is their ability to withstand high mechanical stress and temperatures. Therefore, edible coatings must be flexible and transparent while possessing good barrier and mechanical properties to reduce mechanical damage during loading and unloading operations and transportation. Figure 3 illustrates the relationship between tensile strength and elongation at the break of the biofilm samples. This relationship indicates that the deformation curves of Z/GM composites correspond to elastic systems that are only minimally strengthened under tension. These composites exhibit relatively low tensile strength values and higher elongation at break values. Additionally, the ultimate mechanical properties of the composite films showed slight variation among different samples.

The addition of bioactive compounds such as PPhC and SEO significantly affects the mechanical behavior of the biofilms. The Z/GM (PPhC) and Z/GM (SEO) composites exhibit relatively low tensile strength and higher tensile strain values. Specifically, the addition of PPhC increased the elongation at the break of the composite films to 96.3% ±6.2, while the addition of SEO extended this value to 184.2% ±8.4. Compared to commercially available plastic films for food packaging, the mechanical properties of these films are somewhat weaker [27], and they are mainly intended to protect products from bacteria. In addition, the biodegradable nature of biopolymer-based films makes them highly desirable as environmentally friendly alternatives for future food packaging applications.

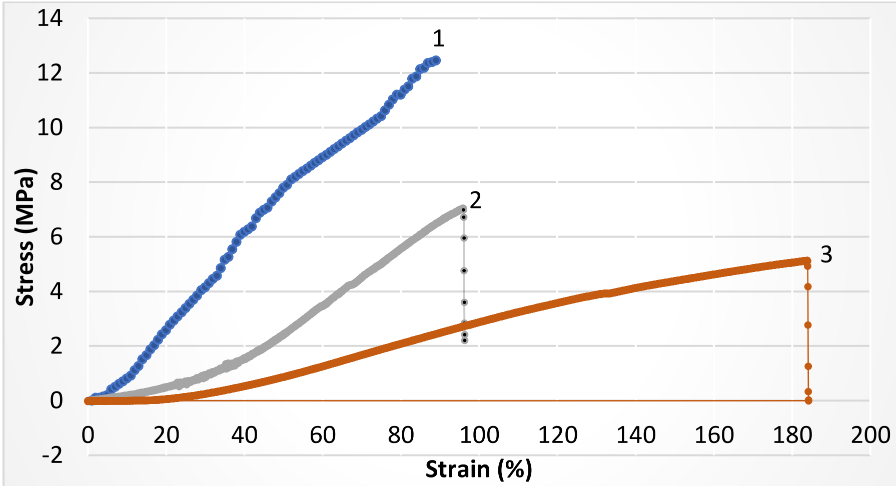


Figure 3, Stress-strain curves of selected Z/GM films: **1 –** Z/GM, **2 –** Z/GM (PPhC), **3 –** Z/GM (SEO)

Estimation of Antibacterial Activity of the Films: The bactericidal properties of the films were evaluated by measuring the inhibition zones of microbial growth in millimeters (mm). The presence of an inhibition zone around the films indicates the sensitivity of the microorganisms to the films. In contrast, the absence of an inhibition zone suggests resistance of the microorganisms to the films. The data is presented in Table 2.

Table 2: Antimicrobial activity of composite films of Z/GM (SEO) and Z/GM (PPhC) against microbial growth.

№	Bacterial strains	Inhibition zone of microbial strains ± SD (mm)		
		Z/GM	Z/GM (SEO) 25 μg/mL	Z/GM (PPhC) 50 μg/mL
1	Staph.epidermidis (+)	25.0 ± 2.4	22.0 ± 3.1	21.0 ± 2.6
2	Staph.aureus (+)	0	8.0 ± 1.2	17.0 ± 2.2
3	Streptococcus SPP (+)	0	23.0 ± 3.4	9.0 ± 1.0
4	E.coli (-)	31.0 ± 3.1	26.0 ± 3.5	35.0 ± 4.1
5	Klebsiella (-)	28.0 ± 2.7	30.0 ± 3.6	10.0 ± 2.2
6	Pseudomonas aeruginosa (-)	30.0 ± 3.4	31.0 ± 3.8	12.0 ± 3.7
7	Proteus vulgaris (-)	13.0 ± 1.7	32.0 ± 2.9	30.0 ± 1.2
8	Yeast fungi	24.0 ± 2.2	25.0 ± 2.1	30.0 ± 3.4

According to the results presented in Table 2, the Z/GM films exhibit antibacterial activity against the food-borne gram-positive *Staphylococcus epidermidis*, all tested gram-negative bacteria, and yeast fungi. However, when examining pure films, two gram-positive strains, *Staphylococcus aureus* and *Streptococcus spp.*, showed numerous bacterial colonies without obvious inhibitory zones around the agar disk. Incorporating minimal amounts of SEO and PPhC into the composite solution improved the antimicrobial properties of the films. Notably, adding both BACs to the film structure significantly ($p < 0.05$) increased the inhibitory activity of the active films against the two gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus spp.* The highest zone reductions (20–30 mm) in viable counts of both tested bacterial species were observed in the presence of films containing both SEO and PPhC against gram-negative bacteria *Escherichia coli*, *Klebsiella spp.*, and *Pseudomonas aeruginosa*. Nevertheless, the inhibitory effect of films containing PPhC against *Klebsiella spp.* was significantly less ($p < 0.05$) than that of control films and films containing SEO. A recent study illustrates that edible films made with zinc oxide nanoparticles and active phenol compounds extracted from pomegranate peel edible coatings prolong product shelf life, reduce the risk of pathogen growth, and improve the quality of fruit and vegetable surfaces [28].

Antibiotic resistance is a leading global issue, contributing to the increasing number and severity of bacterial infections. Therefore, multiple approaches are being taken to utilize plants and naturally occurring substances for their antibacterial and antioxidant effects [29]. The antibacterial activity of the Z/GM films containing SEO and PPhC at the smallest available concentrations (25 µg/mL and 50 µg/mL, respectively) was slightly higher in many cases than that of control films, likely due to the more significant and more manageable release of the active compounds because of their smaller size.

Scientific Innovation: The results obtained in this study on the antimicrobial activity of biopolymer composite films containing BAC were consistent with those reported in other studies cited in this work [1-10, 13, 14, 17-19, 27-30]. Literature reports that the antimicrobial activity of encapsulated essential oils is higher than that of free oils [30]. As stated in [30], essential oils encapsulated in nanoemulsion systems enhance their antibacterial effects by enhancing transfer across cell membranes, acting as a carrier agent, and facilitating penetration and release of BAC. These results indicate that SEO exhibits a better antibacterial effect due to its release and diffusion from the composite films. Thus, active edible coatings derived from natural proteins and polysaccharides, enriched with polyphenolic compounds (PPhC) and sage essential oil (SEO), demonstrate an innovative approach to active food packaging.

Practical Implications: The Z/GM (PPhC) and Z/GM (SEO) composite films exhibit favorable moisture barrier properties, good elasticity, and enhanced antibacterial activity. Owing to their biodegradable nature, these biopolymer-based films represent an eco-friendly and sustainable alternative to conventional packaging materials. They are poised to play a significant role in the future development of film-forming technologies for food preservation.

CONCLUSION

Fresh food products, particularly fruits, are highly prone to spoilage due to microbial contamination, environmental exposure, and inadequate storage conditions. This study successfully developed innovative active edible films by incorporating polyphenolic compounds (PPhC) from *Punica granatum* and essential oil from *Salvia pratensis* (SEO) into a biopolymer matrix composed of glucomannan (GM) and zein (Z). FTIR analysis confirmed molecular interactions between hydrophilic GM and PPhC and the association of hydrophobic SEO with zein. While the addition of PPhC did not significantly affect the films' moisture adsorption behavior ($p > 0.05$), the inclusion of SEO significantly reduced moisture content ($p < 0.05$), attributed to its hydrophobic nature. The composite films exhibited moderate tensile strength and improved flexibility, with SEO increasing the elongation at break to 184.2% ($p < 0.05$).

The integration of plant-derived bioactive compounds not only enhanced the functional properties of the films but also contributes to the growing demand for sustainable and health-promoting materials in the food industry. Given their biodegradability and ability to deliver bioactive agents, these composite films represent a promising step forward in the development of functional foods and eco-friendly food preservation technologies. Future work should focus on their performance in real food systems and on studying the controlled release behavior of bioactives under different storage conditions.

List of abbreviations: BAC: biologically active compounds; GM: glucomannan; GRAS: generally recognized as safe; (G+): Gram-positive; (G-): Gram-negative; $mg/cm^2 \cdot h$: milligram per square centimeter; PPhC: polyphenolic compounds; SEO: Saliva essential oil; Z: zein.

Supplemented materials: Figure 1S.

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