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Development of Biodegradable Antioxidant Packaging Film from Beetroot (*Beta vulgaris*) Leaves for Food Preservation

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Abstract
One of the biggest problems facing the food industry is food spoilage caused by oxidative degradation and microbiological contamination. As an environmentally responsible substitute for traditional plastic packaging, the development of biodegradable and functional packaging materials has received increased attention. The present work focuses on combining natural biopolymers with beetroot (*Beta vulgaris*) leaf extract to create a biodegradable, antioxidant packaging film. The antibacterial, antifungal, and antioxidant properties of beetroot leaf extract prepared using an aqueous extraction technique were assessed. Antibacterial activity was evaluated using the disc diffusion method against *Staphylococcus albus*, *Klebsiella aerogenes*, and *Escherichia coli*. Antioxidant activity was measured using the DPPH radical scavenging assay. Gelatin was used as the polymer matrix, glycerol as a plasticizer, and beetroot leaf extract was incorporated to form a biodegradable film. The aim of this study was to develop and evaluate a biodegradable antioxidant film incorporating beetroot (*Beta vulgaris*) leaf extract for potential use in food preservation. The prepared film was further tested for physical properties such as thickness and water solubility. The film demonstrated strong antioxidant potential in the DPPH assay and discernible antibacterial activity against tested bacterial strains. Soil burial experiments revealed that the film gradually degraded over time, confirming its biodegradable nature. The findings suggest that beetroot leaf extract contains bioactive components with antioxidant and antibacterial effects. These results show that beetroot (*Beta vulgaris*) leaf extract can be successfully added to biodegradable films to improve antimicrobial and antioxidant qualities, providing a promising sustainable alternative for active food packaging applications.

Keywords: Biodegradable film; Beta vulgaris; Gelatin film; Beetroot extract; Antioxidant activity; Food preservation; Active food packaging

Introduction

A major problem in the food industry is food rotting brought on by oxidative reactions and microbiological infection, which results in large financial losses and a decline in food quality. Unwanted changes in food products' flavor, texture, and nutritional value can result from microbial growth and lipid oxidation. As a result, efficient packaging techniques are necessary to shield food from the elements and increase its shelf life (Robertson, 2016). Petroleum-based plastics are primarily used to make conventional food packaging materials because of their resilience, flexibility, and barrier qualities. Nevertheless, these materials are not biodegradable and greatly contribute to the buildup of plastic trash and environmental degradation. Researchers are investigating sustainable and biodegradable substitutes for food packaging applications because to growing environmental concerns (Siracusa et al., 2008). As environmentally friendly packaging materials, biodegradable films made from natural biopolymers including gelatin, starch, cellulose, and chitosan have drawn a lot of interest. These biodegradable polymers can create films with good mechanical and barrier qualities, are renewable, and are safe for the environment. Furthermore, the development of active packaging solutions that offer food items antibacterial and antioxidant protection has resulted from the inclusion of natural bioactive components into biodegradable films (Han, 2014). It is well known that plant-based extracts are a natural source of bioactive substances such as flavonoids, phenolics, and other phytochemicals with potent antibacterial and antioxidant properties. These natural substances can enhance the functional qualities of biodegradable packaging sheets and lessen microbiological contamination and oxidative deterioration in food items (Appendini and Hotchkiss, 2002). The Amaranthaceae family includes

beetroot (*Beta vulgaris*), which is well-known for its therapeutic and nutritional qualities. Numerous bioactive substances found in beetroot leaves, including phenolic compounds, flavonoids, vitamins, and betalains, have strong antibacterial and antioxidant properties. Beetroot leaves are a viable natural source for creating biodegradable antioxidant packaging materials because of their qualities (Clifford et al., 2015).

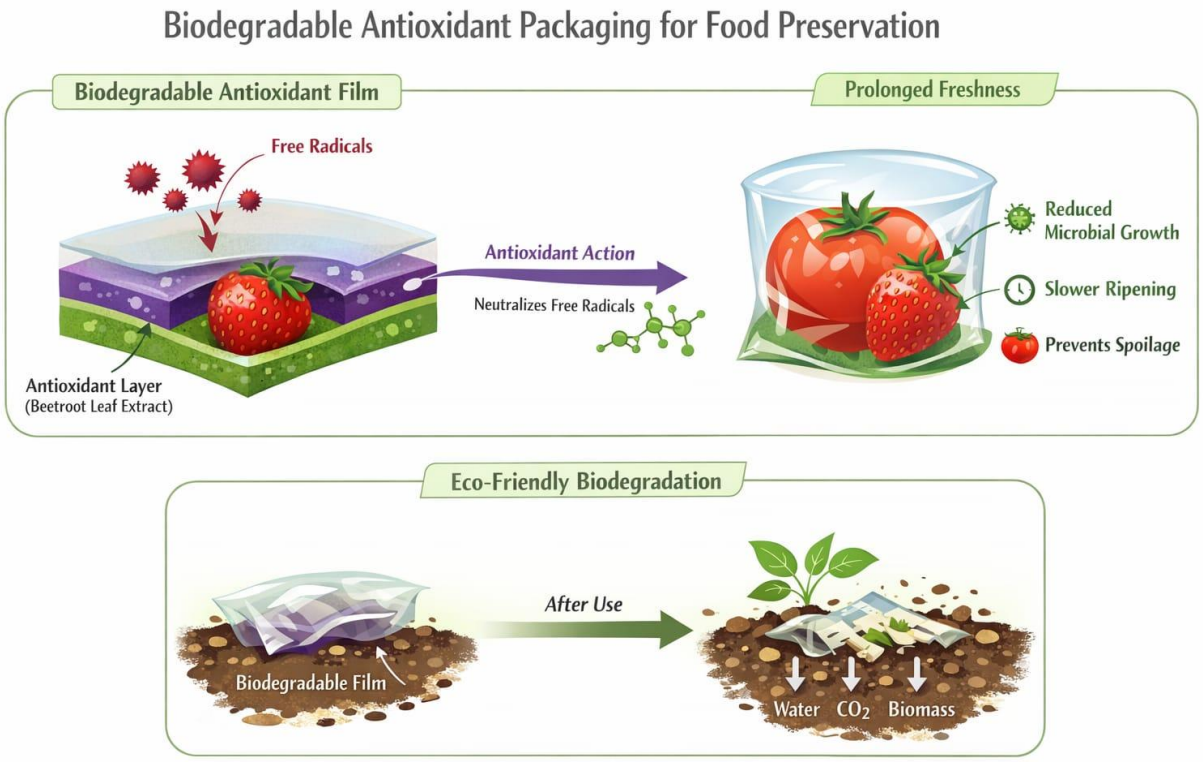

Fig. 1. A conceptual representation of biodegradable antioxidant packaging that demonstrates food preservation, antioxidant activity, and environmentally friendly Disintegration.

Literature Review

The development of biodegradable packaging materials as environmentally friendly substitutes. Traditional plastic packaging has been the focus of numerous studies. Natural polymers such as starch, gelatin, and chitosan have been widely used to produce biodegradable films because of their film-forming ability, biodegradability, and compatibility with food products. Kumar and Singh (2020) reported that biodegradable films made from natural polymers offer an environmentally beneficial approach to food packaging and help reduce plastic waste. To enhance functional properties, these films can be modified by incorporating natural bioactive Substances. The antibacterial and antioxidant properties of natural plant extracts have also been widely investigated. Sharma (2019) noted that plant-based antioxidants, including flavonoids and products. These compounds can efficiently scavenge free radicals and delay spoilage. Recent research has also demonstrated the potential application of natural pigments and extracts in active packaging systems. Sun et al. (2013) reported that incorporating plant extracts into Biode The gradable films can enhance antibacterial activity and extend the shelf life of food products. To improve the functional qualities of biodegradable films, a number of researchers have looked at adding plant extracts. Strong antioxidant and antibacterial properties of natural substances including phenolics, flavonoids, and betalains help prevent food spoiling. Sánchez-González et al. (2017) claim that biodegradable films enhanced with plant extracts can function as active packaging materials that can prolong food shelf life by preventing oxidative deterioration and microbiological development.

Materials And Methods

Preparation of Beetroot Leaf Extract (Aqueous Extraction Method)

Beetroot Leaf Extract Preparation (Aqueous Extraction) To get rid of dust and contaminants, fresh beetroot leaves (*Beta vulgaris*) were gathered and carefully cleaned with distilled water. After being allowed to air dry at ambient temperature, the leaves were ground into a fine powder using a lab grinder. In a conical flask, 100 mL of distilled water and about 10 g of powdered beetroot leaves were combined. To aid in the extraction of bioactive chemicals, the mixture was slowly heated to 50–60°C for 30–40 minutes while being constantly stirred. Following extraction, the mixture was allowed to settle to room temperature before solid residues were filtered out using Whatman No. 1 filter paper. To get a clear extract, the filtrate was centrifuged for ten minutes at 5000 rpm. After being collected, the supernatant was kept at 4°C. In order to conduct additional antioxidant, antibacterial, and film preparation tests, the supernatant was gathered and kept in airtight containers at 4°C.

Disc Diffusion Method (Antibacterial Activity)

The disc diffusion method outlined by Bauer et al. (1996) was used to assess the antibacterial activity of beetroot leaf extract. The nutrient agar medium was made and autoclaved for 15 minutes at 121°C to sterilize it. Under aseptic circumstances, the sterilized medium was transferred into sterile Petri plates and left to harden. Using a sterile spreader and laminar airflow, fresh bacterial cultures of *Escherichia coli*, *Klebsiella aerogenes*, and *Staphylococcus albus* were uniformly distributed throughout the agar surface. The inoculated agar plates were carefully covered with sterile filter paper discs that had been saturated with beetroot leaf extract. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the discs were measured in millimeters (mm). The presence of a clear zone around the disc indicated antibacterial activity of the beetroot leaf extract against the tested bacterial strains.

DPPH Radical Scavenging Assay (Antioxidant Activity)

The antioxidant activity of beetroot leaf extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following a slightly modified method by Brand-Williams et al. (1995). A 0.1 mM DPPH solution was prepared by dissolving DPPH in methanol. The extract was prepared at different concentrations using methanol as the solvent. In a sterile test tube, 1 mL of beetroot leaf extract solution was mixed with 3 mL of DPPH solution. The mixture was gently mixed and kept in the dark at room temperature for 30 minutes to allow the reaction. The deep violet color of DPPH gradually changed to yellow, indicating radical scavenging activity. Absorbance was measured at 517 nm using a UV–visible spectrophotometer. Methanol with DPPH served as the control, and methanol without DPPH served as the blank. Antioxidant activity was expressed as percent DPPH inhibition using the following formula:

DPPH Scavenging Activity (%) = $(A_0 - A_1) / A_0 \times 100$

Where ,

A_0 is the absorbance of the control

A_1 is the absorbance of the sample.

A higher percentage of inhibition indicates stronger antioxidant activity of the beetroot leaf extract.

Antifungal Activity Using Potato Dextrose Agar (PDA)

The antifungal activity of beetroot leaf extract was assessed using PDA Potato Dextrose Agar (PDA) medium by the agar well diffusion method procedure modified by Perez et al (1990) was prepared and autoclaved at 121°C for 15 minutes, then aseptically poured into sterile Petri dishes and allowed to solidify. The fungal culture of *Aspergillus niger*, *Penicillium* sp., and *Rhizopus* sp. was evenly spread across the agar method surface using a sterile cotton swab. Wells of approximately 6 mm diameter were made using a sterile cork borer, and a measured amount of beetroot leaf extract was added to each well. Plates were incubated at 28°C for 48 hours to allow fungal growth. After incubation, the zone of inhibition around the wells was measured in millimeters. The presence of a clear zone indicated antifungal activity of beetroot leaf extract against the tested fungal strains.

Fungal Strain	Inhibition Zone (mm) - Control	Inhibition Zone (mm) – Beetroot Extract
Aspergillus niger	0	10
Penicillium sp.	0	12
Rhizopus sp.	0	8

Preparation of Gelatin-Based Packaging Film

The solvent casting method was used to prepare the antioxidant packaging film. Approximately 10 g of gelatin was dissolved in 100 mL of distilled water and heated to 50–60°C with continuous stirring until a clear, homogeneous solution was obtained. Once the gelatin solution was formed, 5 mL of beetroot leaf extract was added and mixed thoroughly to ensure uniform distribution of antioxidant compounds within the polymer matrix. Glycerol (1 mL) was added as a plasticizer to improve the flexibility and mechanical properties of the film. The mixture was stirred continuously to obtain a uniform film-forming solution. The solution was poured evenly into sterile Petri dishes and allowed to dry at room temperature for 24–48 hours. After drying, the gelatin films were carefully peeled off and stored in airtight containers for further characterization and experimental analysis.

Characterization of Geletin-Based Antioxidant Film

The physical and functional characteristics of the manufactured gelatin-based film containing beetroot leaf extract, such as thickness, water solubility, moisture content, water vapor permeability, and food preservation effectiveness, were assessed.

Thickness of Film

A digital micrometer screw gauge was used to determine the produced films' thickness. To guarantee uniformity, measurements were made on each film sample at five distinct locations. Millimeters (mm) were used to indicate the estimated average value.

Solubility in Water

To evaluate the film's stability in aqueous conditions, its water solubility was ascertained. Two-by-two-centimeter film samples were weighed and left in room-temperature distilled water for a full day. The leftover film remnants were dried and weighed again after immersion. After drying, the leftover film remnants were weighed again. The weight loss of the samples was used to compute the percentage solubility.

Content of Moisture

The oven-drying method was used to determine the films' moisture content. Film samples that had already been weighed were dried at 105°C in a hot air oven until they reached a consistent weight. The difference between the samples' initial and final weights was used to compute the moisture content.

Permeability of Water Vapor (WVP)

The films' moisture barrier qualities were assessed by measuring their water vapour permeability (WVP). Film samples were kept at room temperature in controlled environments after being sealed over containers filled with distilled water. and kept at ambient temperature under controlled circumstances.

Test for Food Preservation (Film Wrapping Test)

A film wrapping procedure was used to assess the prepared films' capability for food preservation. The gelatin-based film was used to wrap perishable food samples (such sliced fruits), while unwrapped samples served as controls. Over the course of many days, all samples were kept at room temperature and observed for changes in appearance, moisture loss, and microbiological deterioration.

Results And Discussion

Disc Diffusion Method Antibacterial Activity

Using the disc diffusion method, the antibacterial activity of beetroot leaf extract was assessed against three bacterial strains: *Staphylococcus albus*, *Klebsiella aerogenes*, and *Escherichia coli*. Clear zones of inhibition surrounding the discs containing beetroot leaf extract were seen following a 24-hour incubation period at 37°C, showing antibacterial activity.

Table 1: Zone Of Inhibition Produced by Beetroot Leaf Extract

Bacterial Strain	Zone Of Inhibition	Activity
Escherichia coil	14	Moderate
Klebsiella aerogenes	15	Strong
Staphylococcus albus	12	Moderate

The disc diffusion method was used to assess the biodegradable film's antibacterial activity. The assay showed that microbial growth in the agar medium was inhibited by the creation of clear zones around the discs. Plates A and B had obvious zones of inhibition surrounding the discs, as shown in Figure 2, indicating the produced film extract's antimicrobial activity against the tested bacterial culture. The antibacterial activity seen in plates A and B was linked to the presence of bioactive chemicals in the film, while plate C (control) did not show a discernible inhibitory zone. The existence of inhibitory zones suggests that the produced biodegradable film has antimicrobial qualities, which could be advantageous for active food packaging applications by lowering microbial contamination and extending the shelf life of perishable food items.

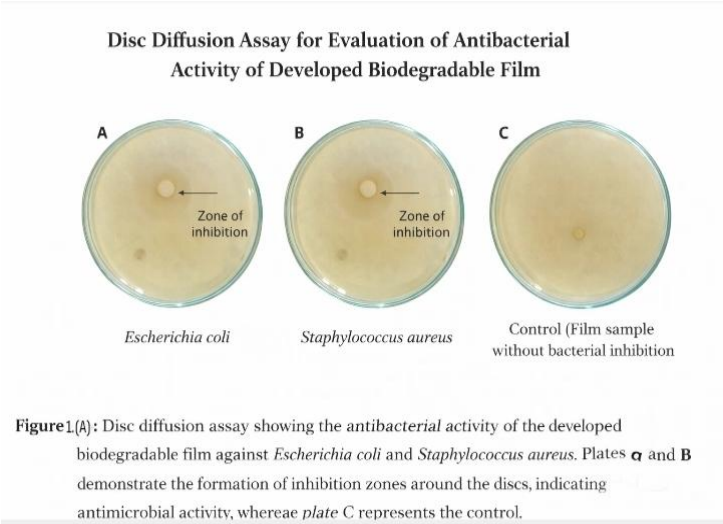

Fig. 2. Photographic representation of inhibition zones against *Escherichia coli* and *Staphylococcus aureus*

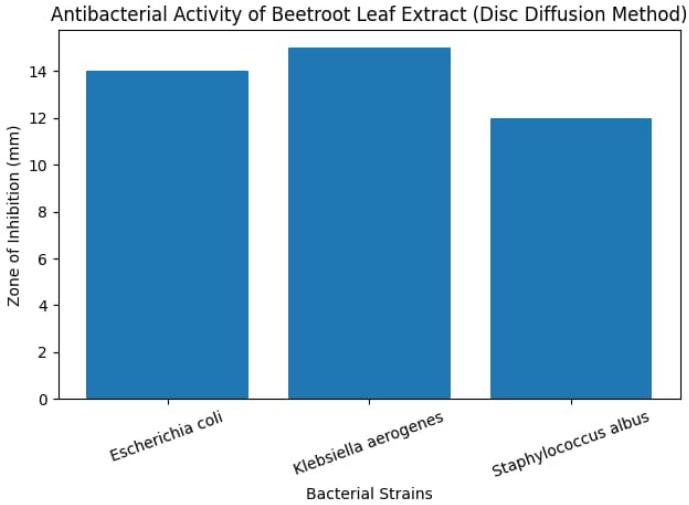

Fig. 3. Beetroot leaf extract's antibacterial efficacy against particular bacterial strains

DPPH Radical Scavenging Assay for Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment, a widely used technique to assess the free radical scavenging ability of natural compounds, was utilized to assess the antioxidant activity of the produced film. By lowering the DPPH radicals, the results showed that the film extract had discernible antioxidant activity. The following formula was used to determine the percentage of DPPH radical scavenging Activity:

$$RSA(\%) = \left(\left(A_{control} - A_{Sample} \right) \div A_{Control} \right) \times 100$$

Where,

$A(control)$ Absorbance of the control and

$A(\ sample)$ presents the absorbance of the sample.

According to the findings, the produced film has a moderate level of antioxidant activity, which could help reduce oxidative deterioration in food items. Because they aid in postponing lipid oxidation and preserving food quality and shelf life, their antioxidant qualities are advantageous for active food packaging applications. Figure 4.2 shows the biodegradable film's ability to scavenge DPPH radicals.

Sample Concentration	Absorbance (A)	% Radical Scavenging (Inhibition)
Control (0 mg/ml)	0.980	-
1	0.842	14.1
2	0.720	26.5
3	0.610	37.8
4	0.500	48.9
5	0.410	58.3

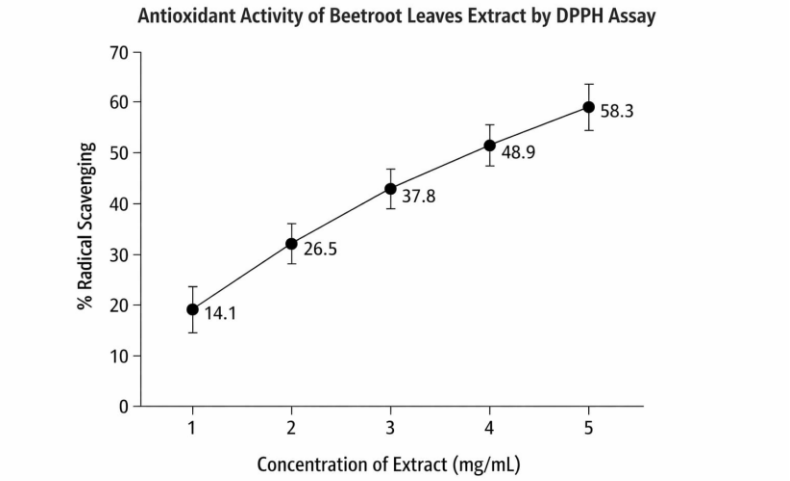

Fig. 4. shows a graphical depiction of the film's antioxidant activity

Interpretation: The findings demonstrate the extract's strong antioxidant activity, which qualifies it for use as an active ingredient in biodegradable films for food packaging.

PDA Media Antifungal Activity

Test Potato Dextrose Agar (PDA) medium was used to assess the biodegradable film's antifungal efficacy. The produced film samples were aseptically deposited on the surface of the agar medium after PDA plates were inoculated with fungal culture. The temperature at which the plates were incubated was appropriate for the growth of fungi. The plates were examined for fungal growth inhibition surrounding the film sample following incubation. The produced biodegradable film's antifungal capability was demonstrated by the clear zone that surrounded it. The bioactive substances in the film matrix may obstruct the growth and proliferation of fungal cells, as indicated by the inhibition of fungal growth. The produced biodegradable film showed discernible antifungal activity, which could aid in reducing fungal contamination in food products, according to the data. Because it can help prolong the shelf life of perishable goods like the tomatoes utilized in this study's wrapping experiment, this feature is especially advantageous for food packing applications. Figure 4.4 presents a photographic depiction of the antifungal activity shown on PDA plates, demonstrating the suppression of fungal growth surrounding the film sample.

Characterization of the Developed Biodegradable Film

Phytochemical characterization of Gelatin-Based Biodegradable Film

The beetroot leaf extract-infused gelatin-based biodegradable film has a homogeneous, flexible, and smooth structure. It has a noticeable scarlet hue due to the presence of natural pigments like betalains from the beetroot leaves. The film was easily pulled from the casting surface without causing any harm, and it demonstrated good mechanical integrity.

Thickness Analysis of Gelatin-Beetroot Leaf Extract Film

To guarantee consistency, the thickness of the created gelatin-beetroot film was measured at several spots using a micrometer screw gauge. Consistent film formation was indicated by the average film thickness of 0.35 mm. Appropriate distribution of the polymer matrix and extract inside the film structure is indicated by uniform film thickness. The produced film appears to have adequate structural integrity for possible use in food packaging based on its observed thickness.

The Film's Water Solubility

To assess the gelatin-beetroot film's stability in damp environments, its water solubility was ascertained. The film had a modest water solubility of 21.5 ± 1.2%. This degree of solubility implies that the film can still biodegrade in the environment while retaining structural integrity during brief food storage.

The Film's pH Sensitivity

When exposed to various pH conditions, the produced film showed discernible color variations. The film looked pink in acidic environments and eventually turned yellowish in alkaline ones. The film has potential uses as an intelligent packaging material that may identify food spoiling through pH changes, according to the observed pH-responsive behavior.

PH Buffer	Initial Colour	Final Colour	Time(Min)
2	Red	Pink	5
4	Red	Light Red	5
6	Red	Reddish Orange	5
8	Red	Orange	5
10	Red	Yellow	5

The Film's Permeability to Water Vapor

The gelatin-based film's water vapor permeability (WVP) was assessed to ascertain its moisture barrier qualities. With a WVP value of 1.85, the produced film demonstrated a moderate level of resistance to water vapor transmission. In food packaging applications, less moisture transfer is advantageous since it preserves product quality and increases shelf life.

The Developed Film's Antioxidant Activity

The DPPH radical scavenging assay was used to assess the produced film's antioxidant properties. The antioxidant components in beetroot leaf extract were successfully preserved within the gelatin matrix, as evidenced by the film's 52.3 ± 2.0% radical scavenging activity. When utilized as a packaging material, this antioxidant characteristic may lessen oxidative deterioration in food goods.

Conclusion

This study successfully developed a biodegradable antioxidant film by integrating beetroot (*Beta vulgaris*) leaf extract into a gelatin-based biopolymer matrix. The incorporation of glycerol as a plasticizer yielded a flexible, uniform, and structurally stable film capable of hosting bioactive compounds. Our findings indicate that the extract

provides robust antimicrobial activity against pathogens such as *Escherichia coli*, *Klebsiella aerogenes*, and *Staphylococcus albus*, alongside notable antifungal properties. Furthermore, DPPH radical scavenging assays confirmed the film's strong antioxidant capacity, driven by the presence of phytochemicals that neutralize free radicals. These results suggest that beetroot-enhanced gelatin films serve as a viable, eco-friendly alternative to conventional petroleum-based plastics, potentially extending the shelf life of perishable goods through active packaging mechanisms. To fully establish industrial feasibility, future research should focus on quantifying mechanical tensile strength, water vapor permeability, and real-world performance in food storage trials.

References

Abdolmaleki K, et al. (2026) The use of red beetroot betalain with gelatin/HPMC film in smart food packaging. Food Science and Technology International.

Ali A, et al. (2019) Edible films enhanced by pomegranate peel extract have antibacterial properties. International Food Research Journal.

Apostolidis E, Kwon YI and Shetty K (2008) Phenolic extracts and fibers inhibit foodborne pathogens. International Journal of Food Microbiology.

Arfat YA and Ahmed J (2017) Active packaging with chitosan and essential oils. Food Packaging and Shelf Life.

Arfat YA, Ahmed J and Kumar P (2015) Clove oil and chitosan-gelatin starch films for antioxidant packaging. Food Packaging and Shelf Life.

Bitencourt TA, et al. (2019) Curcuma extract functionalized gelatin films. Food and Bioprocess Technology.

Chaari M, Elhadef K, Akermi S, Ben Akacha B, Fourati M, Chakchouk Mtibaa A, Ennouri M, Sarkar T, Shariati MA, Rebezov M, Abdelkafi S, Mellouli L and Smaoui S (2022) Novel gelatin-sodium alginate-based active food packaging films with beetroot peel extract. Antioxidants 11:2095.

Chamas A, Moon H, Zheng J, et al. (2020) Plastics' rates of environmental degradation. ACS Sustainable Chemistry and Engineering.

Djenar NS, Fulazzaky MA, Rinaldi K, Yulianthina S, et al. (2024) An assessment of a gluten-based edible film with ZnO nanoparticles and beetroot extract as an active packaging system. ACS Food Science and Technology 4:1511–1526.

Dorta E, Lobo MG and González M (2012) A review of edible films and coatings with natural antioxidants in food science and technology. LWT - Food Science and Technology.

Guilbert S, Gontard N and Gorris LGM (1996) Prolamins as barriers to humidity control. Food Science and Technology Trends.

Guo Z, Ge X, Li W, Yang L, Han L and Yu Q (2021) An active-intelligent film that uses watermelon peel pectin and beetroot extract to track the freshness of packaged chilled beef. Food Hydrocolloids 119:106751.

Han JH (2014) Food packaging innovations. Academic Press.

Jiang L, et al. (2021) Films for food packaging made of polysaccharides and plant extracts. Food Hydrocolloids.

Kumar P and Gautam S (2019) Creating embedded starch biofilms with ZnO nanoparticles for packaging. arXiv.

La DD, et al. (2021) The impact of nanoparticles on banana coatings. Progress in Organic Coatings.

Liu J, Yao X, Yun D, Zhang M, Qian C and Liu J (2021) Chitosan-PVA and litchi pericarp extract-based active packaging films. Quality Assurance and Safety of Crops and Foods.

Martucci JF and Ruseckaite RA (2012) The effects of natural extracts on the properties and applications of gelatin films. Food Hydrocolloids.

Perez-Gago MB and Krochta JM (2001) Protein-based edible films: uses and function. Critical Reviews in Food Science.

Pranoto Y, et al. (2005) Rosemary extract's effects on meat packaging and edible films. Food Chemistry.

Pranoto Y, et al. (2007) Leaf extract-based films' antioxidant qualities. Food Chemistry.

Rhim JW, et al. (2015) Bioactive extracts are used to characterize gelatin/chitosan blend films. Food Science and Biotechnology.

Roy S, Ezati P and Rhim JW (2022) Grape seed extract is used to create pullulan/gelatin films that are antimicrobial and antioxidant. ACS Applied Bio Materials.

Siqueira de Oliveira L, Silva DVT, et al. (2025) Edible polysaccharides as carriers for phenolics and pigments. arXiv.

Sousa AS, et al. (2019) Natural extracts in active packaging systems as antioxidants. Food Science and Technology Trends.

Tolmasky ME, et al. (2023) The antimicrobial and antioxidant properties of nanocomposite films using natural plant extracts. Food Science and Technology.

Tolmasky ME, et al. (2023) The antimicrobial and antioxidant properties of nanocomposite films using natural plant extracts. Food Science and Technology.

Wang C, Liu Y, Chen WQ, et al. (2021) Global plastics stock and flow data: a review. Journal of Industrial Ecology.

Yadav VJ, Shukla VH and Zende RJ (2022) Starch, chitosan, and beetroot extract are used in an active packaging film to improve the quality and shelf life of chicken patties. Meat Science Journal.

Zheng L, et al. (2020) Methods and results of including natural antioxidants in active packaging. Comprehensive Reviews in Food Science.

Author Contributions
SS, DDI, SM and DV conceived the concept, wrote and approved the manuscript.

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Competing interest
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