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Plant-extract-infused edible films as natural antimicrobial and antioxidant packaging for chicken meat

Kanimozhi N. V., Sivabharathi M., Ragulya K., Livetha G. and Sukumar M. *

This study reports the development of starch-based edible films enriched with bioactive extracts from *Pongamia pinnata* and *Psidium guajava* leaves, valorized as underutilized agro-waste resources. Ultrasound-assisted ethanolic extraction followed by partial purification yielded phenolic- and flavonoid-rich fractions, with Fourier Transform Infrared Spectroscopy (FTIR) confirming quercetin and karanjin as key constituents. The extracts exhibited high bioactivity, with a total phenolic content of 800 μg mL^{-1} GAE and a flavonoid content of 1295 μg mL^{-1} QE. Edible films incorporating 5% (v/v) extracts demonstrated improved mechanical and barrier properties; *P. guajava* films achieved the highest tensile stress (2.57 MPa), while *P. pinnata* films showed the lowest Water Vapor Transmission Rate (WVTR) (1251.7 g m^{-2} 24 h). Antioxidant activity was confirmed *via* DPPH assay (IC_{50} of 49.32 and 54.76 μg mL^{-1} for *P. guajava* and *P. pinnata*, respectively), alongside strong antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Application to chicken meat reduced moisture loss, preserved pH and color, and suppressed microbial growth during short-term storage. Importantly, both plants are traditionally used in food and medicine, and no toxicity concerns were evident at the concentrations tested, supporting their safe use in edible films. Beyond chicken, these films have potential application in other perishable foods such as fish, fruits, and vegetables. Overall, the study demonstrates a sustainable valorization approach for agro-waste leaves and contributes to the development of clean-label, biodegradable packaging aligned with circular economy goals.

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Sustainability spotlight

This study presents an eco-friendly alternative to conventional plastic food packaging by developing biodegradable starch films incorporated with polyphenol-rich *Psidium guajava* and *Pongamia pinnata* leaf extracts. The films demonstrated strong antioxidant and antimicrobial properties, effectively extending the shelf life of chicken meat. By valorizing underutilized plant by-products and promoting biodegradable packaging, the work directly supports UN Sustainable Development Goals 12 (Responsible Consumption and Production), 13 (Climate Action), and 3 (Good Health and Well-being). The integration of green extraction methods and waste-to-value strategies underscores the sustainable advancement of this research in reducing plastic pollution and enhancing food safety.

1 Introduction

Meat is highly perishable due to its high moisture content, nutrient composition, and susceptibility to microbial contamination and oxidation.[1] Spoilage leads to economic losses, foodborne illnesses, and quality deterioration, necessitating effective packaging solutions. Traditional plastic-based packaging, while effective, contributes to environmental pollution due to its non-biodegradability, creating an urgent need for sustainable alternatives.[2]

Biodegradable edible films are gaining attention as eco-friendly alternatives to synthetic packaging.[3] Starch-based films, in particular, are widely studied due to their low cost, abundance, and biodegradability. However, they suffer from poor mechanical strength and high water sensitivity, necessitating modifications. One effective approach is incorporating plant-based bioactive compounds to enhance the antimicrobial and antioxidant properties of edible films, making them more effective in preserving perishable foods like meat.[4]

Plant-derived extracts are rich in phenolics, flavonoids, and tannins, which offer natural antimicrobial and antioxidant benefits.[5] This study utilizes bioactive extracts from *Pongamia pinnata* and *Psidium guajava* leaves, two underutilized plant resources rich in natural antimicrobials. *P. guajava* is particularly known for its high quercetin content, a flavonoid with strong free radical scavenging and antibacterial properties.[6] *P. pinnata*, traditionally used in ethnomedicine, contains

Centre for Food Technology, A. C. Tech., Anna University, Chennai-600025, India. E-mail: sukumarcbt@gmail.com

karanjin, a furanoflavonoid with documented antimicrobial efficacy against foodborne pathogens.[7] While these plants have individually shown preservative potential, their application in starch-based films for direct meat preservation has not been comparatively evaluated in depth.

Although various biodegradable films have been reported, limited studies have examined the incorporation of *Pongamia pinnata* and *Psidium guajava* leaf extracts into starch-based films. These underutilized leaves, often treated as agro-waste, are rich in bioactive compounds such as karanjin and quercetin, which can impart antioxidant and antimicrobial properties to packaging materials. The present study aims to valorize these plant resources by developing and characterizing extract-infused starch-based edible films for meat preservation. The objectives include:

- Extracting and characterizing bioactive compounds from *Pongamia pinnata* and *Psidium guajava* leaves.
- Developing and optimizing edible films infused with plant extracts.
- Analyzing their antioxidant and antibacterial activities against common meat spoilage bacteria.
- Evaluating their effectiveness in extending meat shelf life.

This research offers a sustainable, cost-effective, and biodegradable packaging alternative with significant benefits: (i) enhancing meat preservation by inhibiting spoilage and oxidation, (ii) reducing plastic waste, contributing to environmental sustainability, and (iii) promoting natural preservatives, reducing reliance on synthetic additives in food packaging.

This study aims to advance sustainable food preservation by developing starch-based edible films enriched with bioactive extracts from *Pongamia pinnata* and *Psidium guajava*. Although both plants have been individually studied for their phytochemical and medicinal properties, their application in starch-based food packaging remains limited, and no prior work has compared their functional performance within the same film matrix. The present study introduces two key novelties: (i) the use of partially purified, quercetin and karanjin rich leaf fractions to enhance film functionality and (ii) a direct evaluation of their antimicrobial and physicochemical effects on meat preservation. By valorizing underutilized agro-waste leaves and integrating their bioactive compounds into biodegradable films, this research provides a focused proof-of-concept for clean-label, plant-derived alternatives to synthetic packaging materials.

2 Materials and methods

2.1 Materials

2.1.1 Plant materials. The leaves of *Pongamia pinnata* and *Psidium guajava* were collected from Madras University, Chennai. The fresh leaves of this species were washed under running tap water and the leaves of *Pongamia pinnata* and *Psidium guajava* were dried using a hot air oven for 30 min at 60 °C.

2.1.2 Microorganisms. *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC6538 were used as test microorganisms. Cultures of each bacterial strain were maintained on Luria broth (LB) agar medium at 4 °C.

2.1.3 Meat sample. Fresh chicken meat was procured from a local retail market in Chennai on the day of the experiment. The samples were transported to the laboratory under hygienic conditions in sterile, insulated containers and used within 1 h of procurement to minimize initial microbial variation.

2.2 Ultrasonic extraction

The extraction of bioactive compounds from *Pongamia pinnata* and *Psidium guajava* leaves was carried out using ultrasound-assisted extraction (UAE) with a probe-type ultrasonicator.[8] Fresh leaves were thoroughly washed with distilled water to remove surface contaminants, shade-dried at room temperature (25 ± 2 °C) for 7–10 days, and ground into a fine powder. For extraction, 10 g of the powdered leaves were mixed with 100 mL of ethanol as the solvent in a 250 mL glass beaker. The mixture was placed in an ice bath to control temperature rise during sonication. A probe-type ultrasonicator (20 kHz, 500 W, 10 mm titanium alloy probe) was used at 40% amplitude in a pulse mode of 10 s ON and 5 s OFF for 30 minutes, maintaining the temperature below 40 °C to prevent the degradation of heat-sensitive compounds. After ultrasonication, the extract was filtered through Whatman no. 1 filter paper and centrifuged at 6000 rpm for 10 minutes to remove residual particulates. The supernatant was then concentrated using a rotary evaporator at 40 °C under reduced pressure to remove the solvent. The final extract was either freeze-dried for long-term storage or stored at 4 °C in an amber-colored bottle until further analysis. The extraction yield was calculated using the formula

$$\text{Yield } \% = \frac{\text{final dried extract weight}}{\text{initial plant powder}} \times 100$$

2.3 Characterization of the extract

2.3.1 Total phenolic content (TPC). The total phenolic content (TPC) of the leaf extracts was determined using the Folin–Ciocalteu colorimetric method with slight modifications. Briefly, 0.5 mL of plant extract was mixed with 2.5 mL of ten-fold diluted Folin–Ciocalteu reagent and incubated for 5 minutes at room temperature. Following this, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was then measured at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents (GAE) per gram of dry extract using the standard calibration curve.[9]

2.3.2 Total flavonoid content (TFC). The total flavonoid content (TFC) was estimated using the aluminum chloride colorimetric method. In this procedure, 0.5 mL of the extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was incubated at room temperature for 30 minutes. Absorbance was recorded at 415 nm using a UV-Visible spectrophotometer. Quercetin was used as the reference standard, and TFC was expressed as mg

quercetin equivalents (QE) per gram of dry extract based on the quercetin standard curve.[10]

2.3.3 Antioxidant activity of extracts. The antioxidant activity of the extracts was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A 0.1 mM DPPH solution was freshly prepared in methanol. Different concentrations of the plant extracts (10, 25, 50, 75, and 100 μg mL^{-1}) were mixed with 1 mL of DPPH solution and made up to 3 mL with methanol. The mixtures were vortexed and incubated in the dark at room temperature for 30 minutes. The decrease in absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH Inhibition } \% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the DPPH solution without the extract, and A_{sample} is the absorbance with the extract. The IC_{50} value (concentration required to inhibit 50% of the DPPH radicals) was determined by plotting percentage inhibition against extract concentrations using linear regression analysis.[11]

2.3.4 Antibacterial activity of leaf extracts. The antibacterial activity of *Psidium guajava* and *Pongamia pinnata* leaf extracts was evaluated using the Kirby–Bauer agar well diffusion method. Nutrient agar (NA) medium was prepared by dissolving 28 g of nutrient agar powder in 1000 mL of distilled water, followed by autoclaving at 121 °C for 15 minutes. Once solidified in sterile Petri plates, bacterial cultures of *Staphylococcus aureus* and *Bacillus subtilis* were uniformly inoculated onto the surface using sterile cotton swabs. Wells of 6 mm diameter were punched aseptically using a sterile cork borer.

Each well was loaded with 100 μL of extract solutions at different concentrations: 25 μg mL^{-1}, 50 μg mL^{-1}, and 100 μg mL^{-1}, prepared in DMSO or sterile distilled water. Gentamycin (10 μg mL^{-1}) was used as a positive control, while sterile distilled water served as the negative control. Plates were incubated at 37 ± 2 °C for 24 hours, and the zone of inhibition (mm) was measured using a digital Vernier caliper.[12]

2.4 Partial purification of bioactive compounds

2.4.1 Partial purification of karanjin from the *Pongamia pinnata* leaf extract. Crude ethanolic extracts of *P. pinnata* leaves were obtained *via* ultrasound-assisted extraction and concentrated using a rotary evaporator. The semi-solid extract was re-dissolved in distilled water and transferred to a separating funnel. Partitioning was carried out with ethyl acetate in a 1 : 1 ratio (v/v) and repeated three times (3 × 100 mL) to selectively extract moderately non-polar compounds such as furanoflavonoids. The pooled ethyl acetate fractions were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure at 40 °C using a rotary evaporator.[13] The resulting enriched fraction was dried and stored at 4 °C for further analysis. For compound confirmation, FTIR spectra of the partially purified extract were recorded in the range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1} using an ATR-FTIR spectrometer (Sigma-Aldrich, ≥98% purity).

2.4.2 Partial purification of quercetin from the *Psidium guajava* leaf extract. Crude ethanolic extracts of *P. guajava* leaves were similarly re-dissolved in distilled water and partitioned with ethyl acetate (3 × 100 mL). The pooled ethyl acetate layers were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under vacuum at 40 °C.[14] The resulting fraction, enriched in flavonoids such as quercetin, was stored in amber vials for further analysis. FTIR spectra of the partially purified extract were recorded in the range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1} using an ATR-FTIR spectrometer (Sigma-Aldrich, ≥95% purity).

2.5 Preparation of film incorporated with the leaf extracts

Starch-based edible films were prepared using a solution casting method with modifications. Initially, 3 g of corn starch was gradually dispersed in 100 mL of distilled water and heated to 90 °C with continuous stirring for 30 minutes to allow complete gelatinization. Subsequently, 0.8 mL of glycerol was added as a plasticizer to enhance film flexibility. After thorough mixing, varying concentrations (1%, 3%, 5% and 7% v/v) of *Pongamia pinnata* or *Psidium guajava* leaf extracts were incorporated into the starch solution. The mixture was then heated again at 65 °C for 15 minutes to ensure proper integration of the extract. The resulting film-forming solutions were homogenized using a high-speed homogenizer for 2–5 minutes to achieve uniform dispersion. Air bubbles in the homogenized solutions were removed using a vacuum oven. The degassed solutions were poured into sterile Petri dishes and dried under controlled conditions at 25 ± 1 °C and 50 ± 2% relative humidity (RH) for 24 hours to ensure reproducible film formation. Once dried, the films were carefully peeled off and stored at 25 °C in desiccators until further analysis and application.[15]

2.6 Characterization of the starch film

2.6.1 Film thickness. The thickness of the films was measured using a digital micrometer screw gauge with an accuracy of 0.01 mm. Measurements were taken at five random positions on each film sample (center and four edges), and the average value was recorded. This ensured uniformity and minimized variability in the thickness readings.

2.6.2 Tensile strength. The mechanical properties of the films, including tensile strength (TS) and elongation at break (EAB), were determined according to ASTM D882 standards using a universal testing machine (UTM).[16] Film strips were cut to dimensions of 10 mm × 100 mm, and the initial grip separation and crosshead speed were set to 50 mm and 30 mm min^{-1}, respectively. Each sample was conditioned at 50% relative humidity and 25 °C for 48 hours before testing. Tensile strength was calculated using the formula

$$\text{Tensile strength(MPa)} = \frac{\text{maximum force(N)}}{\text{cross sectional area(mm}^2\text{)}}$$

2.6.3 Water vapor transmission rate (WVTR). The WVTR was determined using a modified ASTM E96-00 gravimetric method.[17] Circular film samples were sealed over the mouth of glass cups containing desiccant (anhydrous calcium chloride) and stored in a controlled humidity chamber at 75% relative humidity (using saturated sodium chloride solution) and 25 °C. The weight of each cup was recorded at regular intervals (every 12 hours) for 72 hours. The WVTR was calculated from the slope of the weight gain *versus* time curve using the following equation:

$$\mathrm{WVTR}\left(\mathrm{g\ m^{-2}\ day^{-1}}\right) = \frac{\Delta W}{A \times t}$$

where ΔW is the weight gain (g), A is the exposed film area (m^2), and t is the time (days).

2.7 Meat wrapping and shelf life analysis

Fresh raw chicken meat samples were cut into uniform pieces weighing 5 grams each. The starch-based edible films incorporated with *Pongamia pinnata* and *Psidium guajava* leaf extracts were wrapped tightly around the meat samples, while an unwrapped meat sample served as the control. All samples were kept at room temperature (25 ± 2 °C), and the following parameters were analyzed at 30-minute intervals for a total duration of 3 hours.

2.7.1 pH measurement. The surface pH of each meat sample was determined by homogenizing 5 g of meat in 45 mL of distilled water using a mechanical homogenizer. The resulting slurry was filtered and the pH of the filtrate was measured using a digital pH meter[18] (pre-calibrated with standard buffer solutions of pH 4.0 and 7.0).

2.7.2 Moisture content. Moisture content was measured by the hot air oven drying method. Accurately weighed meat samples (2–3 g) were placed in pre-weighed crucibles and dried in a hot air oven at 100 °C for 2 hours until a constant weight was achieved. The moisture content was calculated using the formula[19]

$$\text{Moisture content}(\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

2.7.3 Water activity (a_w). Water activity was measured using a water activity meter (Aqualab, India). Small portions of each meat sample were placed into the sample chamber, and readings were taken once equilibrium was reached. All measurements were conducted in triplicate for accuracy.

2.7.4 Color analysis. Color parameters (L^*, a^*, b^*) of the meat surface were analyzed using a 3nh portable colorimeter. Measurements were taken directly on the film-wrapped meat surface at three different spots per sample. The L^* value indicates lightness, a^* denotes redness/greenness, and b^* indicates yellowness/blueness. Average values were calculated and expressed as mean ± standard deviation.

2.7.5 Microbial analysis (plate count method). Microbial load was assessed using the standard plate count method. A 1 g portion of each sample was aseptically transferred into 9 mL of sterile peptone water and homogenized. Serial dilutions (10^{-1} to 10^{-5}) were prepared and 1 mL aliquots were plated on nutrient agar using the pour plate technique. Plates were incubated at 37 °C for 24 hours, and colonies were counted and expressed as log CFU per g. For the refrigerated shelf-life study, wrapped and control samples were stored at 4 ± 1 °C and analyzed at predetermined intervals. All analyses were performed in triplicate and results were recorded to assess the preservation efficiency of the extract-incorporated edible films.[20]

2.8 Sensory evaluation

A basic sensory assessment was conducted using eight semi-trained people (n = 8) familiar with sensory methods. Samples (control film-wrapped chicken, *P. pinnata* film-wrapped, and *P. guajava* film-wrapped) were coded with three-digit random numbers and presented in randomized order. Evaluations were performed in individual booths under neutral lighting and room temperature (≈25 °C). Panelists rated odor, surface color, texture (mouthfeel/firmness) and overall acceptability using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Water and plain crackers were provided for palate cleansing between samples. Data were collected anonymously; the procedure followed institutional ethical guidelines for voluntary participant testing.

3 Results and discussion

3.1 Extraction yield

The efficiency of ultrasound-assisted extraction (UAE) was assessed based on the percentage yield of dried crude extracts obtained from *Pongamia pinnata* and *Psidium guajava* leaves. The results showed that UAE yielded 76% for *P. pinnata* and 78% for *P. guajava*. These values indicate a high extraction efficiency for both plant materials using UAE.

Ultrasound-assisted extraction is known for its ability to enhance mass transfer and disrupt plant cell walls through acoustic cavitation, which facilitates the release of intracellular bioactive compounds. The high yields observed in this study support the effectiveness of UAE in recovering phytochemicals from medicinal leaves. Compared to conventional methods such as Soxhlet or maceration, UAE significantly reduces extraction time and energy consumption while increasing yield.

The slightly higher yield of *P. guajava* extract compared to *P. pinnata* may be attributed to its softer leaf structure and higher natural moisture content, which could enhance solvent penetration and compound diffusion. Additionally, the polarity of the ethanol used and its compatibility with polyphenolic compounds might have favored the extraction of bioactive compounds from guava leaves.

These findings are consistent with previous studies,[21–24] which reported similar extraction yields ranging from 70% to 80% for UAE of polyphenol-rich plant materials.

3.2 Characterization of the leaf extracts

3.2.1 Total phenolic content (TPC). Total phenolic content was determined by the Folin–Ciocalteu colorimetric method. As

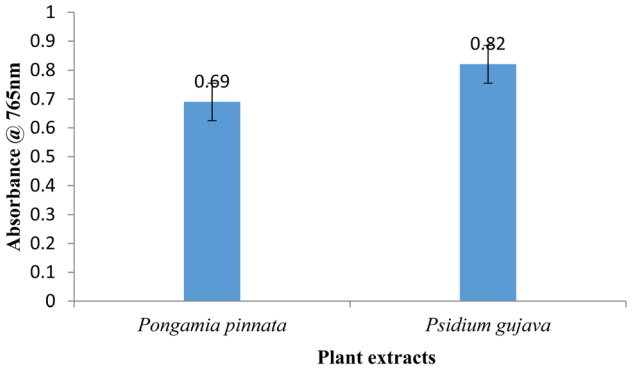

Fig. 1 Total phenolic content (TPC) of *Pongamia pinnata* and *Psidium guajava* leaf extracts.

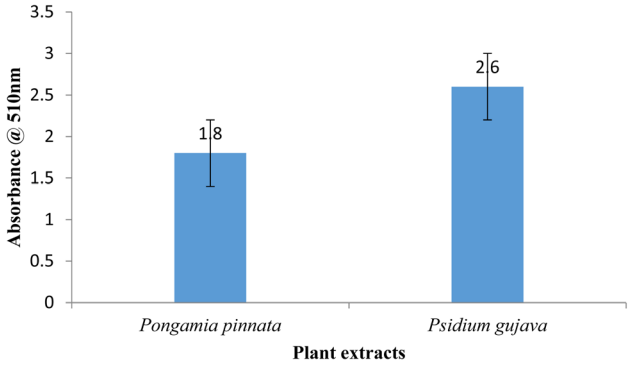

Fig. 2 Total flavonoid content (TPC) of *Pongamia pinnata* and *Psidium guajava* leaf extracts.

depicted in Fig. 1, absorbance values correspond to phenolic concentrations that were calculated using a gallic acid standard curve (typically, $y = 0.001x + 0.02$, $R^2 \approx 0.998$).

• *Pongamia pinnata*: absorbance = 0.69 → concentration = (0.69 − 0.02)/0.001 = 670 μg mL^{-1} GAE

• *Psidium guajava*: absorbance = 0.82 → concentration = (0.82 − 0.02)/0.001 = 800 μg mL^{-1} GAE

The total phenolic content (TPC) was found to be significantly higher in the *Psidium guajava* leaf extract (800 μg mL^{-1} GAE) when compared to *Pongamia pinnata* (670 μg mL^{-1} GAE). This observation highlights guava leaves as a richer source of polyphenolic compounds, which are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties. Phenolic compounds act as primary antioxidants by donating hydrogen atoms or electrons and neutralizing free radicals. The elevated TPC in *P. guajava* aligns with its superior DPPH radical scavenging capacity observed in this study, suggesting a direct correlation between phenolic content and antioxidant efficacy. These findings are consistent with previous reports demonstrating that phenolic-rich plant extracts enhance oxidative stability and can be effectively incorporated into biodegradable films for food preservation.[25,26] Thus, the higher phenolic content in the guava extract substantiates its application as a functional additive in the development of antioxidant-enriched active packaging materials.

3.2.2 Total flavonoid content (TFC). TFC was evaluated by the aluminum chloride colorimetric assay and expressed as quercetin equivalents (QE). Fig. 2 depicts the absorbance values corresponding to flavonoid concentrations, plotted with the standard curve: $y = 0.002x + 0.01$, $R^2 \approx 0.996$.

• *Pongamia pinnata*: absorbance = 1.8 → concentration = (1.8 − 0.01)/0.002 = 895 μg mL^{-1} QE

• *Psidium guajava*: absorbance = 2.6 → concentration = (2.6 − 0.01)/0.002 = 1295 μg mL^{-1} QE

The total flavonoid content (TFC) analysis revealed a significantly higher flavonoid concentration in the *Psidium guajava* leaf extract (1295 μg mL^{-1} QE) compared to *Pongamia pinnata* (895 μg mL^{-1} QE). Flavonoids, known for their potent antioxidant and radical scavenging properties, contribute substantially to the prevention of oxidative deterioration in food systems. The elevated TFC in *P. guajava* supports its enhanced DPPH scavenging ability observed in the current study, indicating a strong correlation between flavonoid concentration and antioxidant activity. These findings align with earlier reports that emphasize the role of flavonoid-rich plant extracts in prolonging shelf-life and improving oxidative stability in food products.[27–29] Consequently, the higher flavonoid content in the guava extract underscores its potential application in the formulation of active packaging films intended for meat preservation and other perishable commodities.

3.2.3 Antioxidant activity. The antioxidant potential of *Pongamia pinnata* and *Psidium guajava* leaf extracts was evaluated using the DPPH free radical scavenging assay. As shown in Fig. 3, both extracts exhibited a concentration-dependent increase in % inhibition of DPPH radicals. Among the tested samples, the *Psidium guajava* extract demonstrated superior antioxidant activity at all concentrations, with maximum inhibition of 89% at 100 μg mL^{-1}, followed closely by *Pongamia pinnata* with 86.23%.

The antioxidant potential of *Pongamia pinnata* and *Psidium guajava* leaf extracts was assessed using the DPPH radical scavenging assay. At lower concentrations (25 μg mL^{-1}), *P. pinnata* exhibited 42.28% inhibition, outperforming *P. guajava*, which showed 28.12% inhibition, indicating a relatively stronger scavenging potential of *P. pinnata* at initial dose levels. Interestingly, with increasing concentration, *P. guajava*

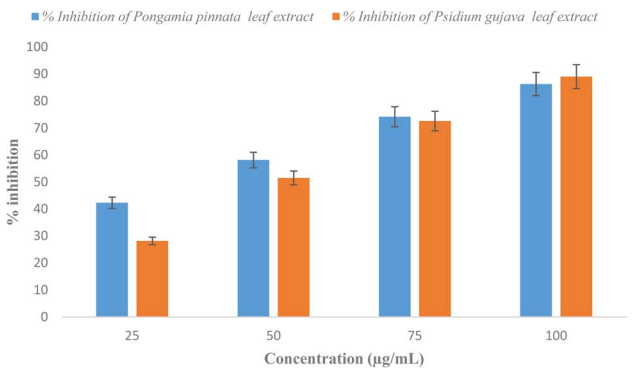

Fig. 3 DPPH radical scavenging activity (% inhibition) of *Pongamia pinnata* and *Psidium guajava* leaf extracts at different concentrations.

demonstrated a marked improvement in activity, surpassing *P. pinnata* at 75 and 100 μg mL^{-1}, suggesting the presence of dose-dependent bioactive constituents contributing to its radical scavenging efficacy.

The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, was calculated *via* linear regression of the concentration *vs.* % inhibition curve. The IC_{50} for *P. guajava* was found to be 49.32 μg mL^{-1}, while for *P. pinnata* it was slightly higher at 54.76 μg mL^{-1}, indicating that *P. guajava* possesses marginally superior antioxidant activity. These results may be attributed to its higher total phenolic and flavonoid content, as observed in previous reports,[30,31] supporting the hypothesis that antioxidant activity is positively correlated with polyphenolic concentration.

Overall, the findings highlight the potential application of both extracts as natural antioxidants in food preservation and biomedical formulations, offering a sustainable alternative to synthetic additives.

3.2.4 Antibacterial efficacy of the extracts. Table 1 shows the antibacterial potential of *Pongamia pinnata* and *Psidium guajava* leaf extracts evaluated against *Staphylococcus aureus* and *Bacillus subtilis*. The *P. pinnata* extract exhibited a concentration-dependent inhibition against both bacteria, with the maximum zone of inhibition observed at 100 μg mL^{-1}: 16 mm for *S. aureus* and 17 mm for *B. subtilis*. In contrast, the *P. guajava* extract showed no activity against *S. aureus* at any tested concentration, while moderate activity was observed against *B. subtilis* (10–12 mm). Gentamycin (positive control) exhibited zones of 31 mm and 29 mm for *S. aureus* and *B. subtilis*, respectively, validating the assay.

The present study demonstrated that the *Pongamia pinnata* leaf extract possesses notable antibacterial activity, especially against *Bacillus subtilis* and *Staphylococcus aureus*, with the activity increasing at higher concentrations. These results align with previous reports,[32–34] which documented significant antimicrobial effects of *P. pinnata* due to its rich phytochemical profile, including flavonoids and karanjin compounds.

In contrast, the *Psidium guajava* extract showed selective activity, effectively inhibiting *B. subtilis* but not *S. aureus*. This selective activity may be attributed to differences in bacterial cell wall permeability or the concentration of active antimicrobial constituents like quercetin and tannins in the extract. Similar findings were reported,[6,35] where the guava extract exhibited higher efficacy against Gram-positive *Bacillus* species than *Staphylococcus*.

It is important to note that the *P. guajava* extract showed no inhibitory activity against *Staphylococcus aureus* at any tested concentration, which represents a limitation in its broad-spectrum antimicrobial applicability. This lack of efficacy has been similarly observed in previous studies and may be due to the lower permeability of *S. aureus* cell walls to quercetin-rich extracts. Therefore, *P. guajava* based films may be more suitable for applications targeting Bacillus species rather than universal pathogen control.

While the activity of both extracts was lower than that of gentamycin, the natural extracts still present promising alternatives, especially for applications in food preservation or packaging, where synthetic antibiotics are not preferred. The results suggest that *P. pinnata* may serve as a more potent natural antimicrobial agent compared to *P. guajava*.

3.3 FTIR confirmation of bioactive compounds in plant extracts

To validate the presence of specific bioactive compounds – quercetin in *Psidium guajava* and karanjin in *Pongamia pinnata* – the crude extracts were subjected to partial purification using ethyl acetate partitioning. This step enriched the phenolic and furanoflavonoid fractions by removing highly polar and non-phenolic impurities. The resulting fractions were then analyzed using Fourier-transform infrared (FTIR) spectroscopy, a widely accepted method to identify functional groups and confirm the presence of key phytochemicals based on their characteristic vibrational frequencies.

The FTIR spectrum of the *Psidium guajava* ethyl acetate fraction (Fig. 4) showed characteristic absorption peaks corresponding to quercetin and related flavonols. A broad band at ~3300 cm^{-1} indicated O–H stretching vibrations of phenolic hydroxyl groups. The strong peak observed at ~1655 cm^{-1} corresponded to the C=O stretching of the flavonol backbone. Prominent aromatic C=C stretching bands were noted at ~1600 cm^{-1} and ~1515 cm^{-1}, confirming the presence of conjugated benzene rings. Additional peaks at ~1460 cm^{-1} (C–O–H bending), ~1250 cm^{-1} (C–O stretching of phenolic ethers), and ~1050 cm^{-1} (C–O–C stretching) further supported the

Table 1 Antibacterial activity of *Pongamia pinnata* and *Psidium guajava* leaf extracts against *Staphylococcus aureus* and *Bacillus subtilis* evaluated by the Kirby–Bauer well diffusion method

		Zone of inhibition (mm)			
S. no.	Tested organisms	25 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	Gentamycin
Pongamia pinnata					
1	*Staphylococcus aureus*	12	12	16	31
2	*Bacillus subtilis*	13	15	17	29
Psidium guajava					
1	*Staphylococcus aureus*	0	0	0	29
2	*Bacillus subtilis*	10	10	12	25

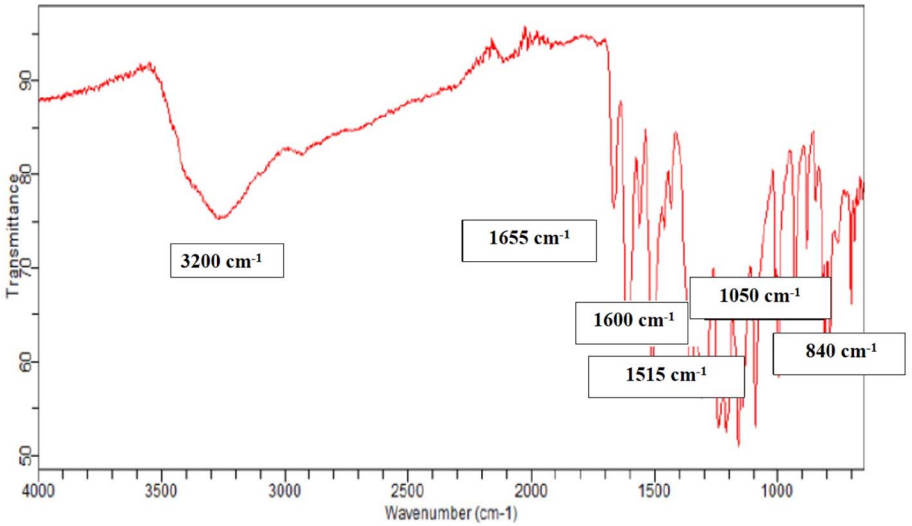

Fig. 4 FTIR spectra of quercetin enriched plant extracts. Spectrum A shows characteristic quercetin peaks in the ethyl acetate fraction of *Psidium guajava* (~3200 cm^{-1} O–H, ~1650 cm^{-1} C=O, and ~1600–1520 cm^{-1} aromatic C=C)

presence of flavonoid glycosidic linkages. A distinct band around ~840 cm^{-1} represented the C–H out-of-plane bending of substituted aromatic rings. These functional group vibrations collectively confirm that the extraction and partial purification successfully retained quercetin-rich phytochemicals within the *P. guajava* fraction.[36,37]

The FTIR spectrum of the *Pongamia pinnata* ethyl acetate fraction (Fig. 5) displayed distinct absorption peaks characteristic of karanjin, a methoxy-furanoflavone abundant in Pongamia leaves. A strong peak at ~1650 cm^{-1} corresponded to the C=O stretching of the γ-pyrone (flavone) ring system. Prominent bands at ~1600 cm^{-1} and ~1510 cm^{-1} were attributed to aromatic C=C stretching vibrations, confirming the presence of a conjugated benzene framework. The peak at ~1260–1250 cm^{-1} represented the C–O stretching of methoxy substituents, a signature functional group of karanjin. Additional peaks at ~1100–1050 cm^{-1} indicated the C–O–C stretching of the furan ring, while the band around ~840 cm^{-1} corresponded to the C–H out-of-plane bending of substituted aromatic rings. These spectral features closely match the reported FTIR profiles of karanjin, confirming that the partial purification effectively enriched the bioactive methoxy-flavonoid constituents in the extract.[38,39]

The FTIR-based confirmation of quercetin and karanjin supports their contribution to the observed antioxidant and antimicrobial activities in this study. The partial purification step further enhanced the interpretability of spectral data by minimizing spectral interference from other matrix components.

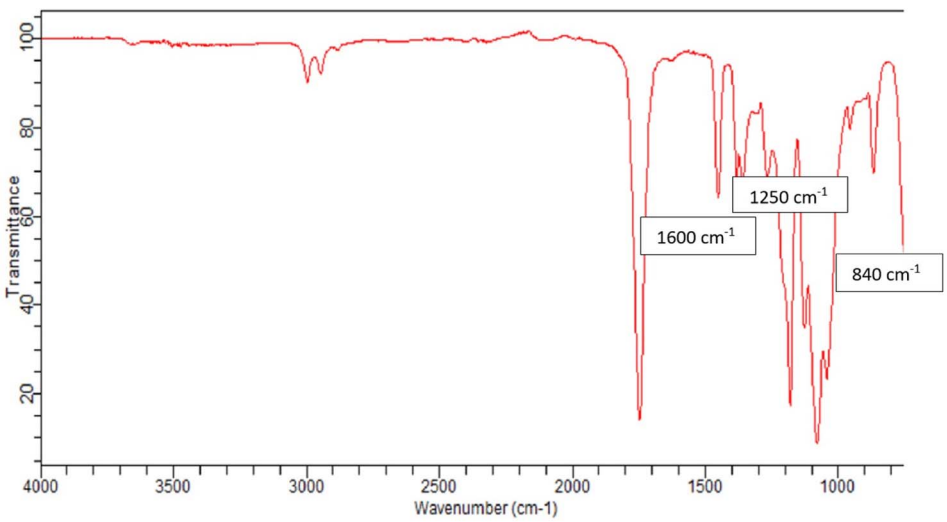

Fig. 5 FTIR spectra of karanjin enriched plant extracts. Spectrum B confirms the presence of karanjin in *Pongamia pinnata* with bands near ~1600 cm^{-1} (C=C), ~1260 cm^{-1} (C–O stretch), and ~840 cm^{-1} (C–H bending).

Fig. 6 Preparation of bioactive films with varying concentrations of leaf extracts.

3.4 Preparation of bioactive films

Films were developed as shown in Fig. 6: control film without extract, 5% *Pongamia pinnata* leaf extract film, and 5% *Psidium guajava* leaf extract film. The 5% extract films exhibited superior transparency and a uniform structure compared to other tested concentrations.

The formulation trials for film development revealed that the incorporation of the 5% leaf extract resulted in the most stable and visually uniform bioactive film. Films prepared with lower concentrations (1% and 3%) lacked adequate film-forming integrity, appearing fragile or incomplete, while those with higher concentrations (7%) showed brittleness and non-uniform textures. The 5% formulation maintained transparency, elasticity, and mechanical cohesion, making it ideal for further testing. These findings align with earlier reports where the inclusion of plant extracts at 3–5% concentration enhanced the physical properties and bioactivity of biopolymer-based films.[40–42] The optimization trials further indicated that 5% extract loading provided the best balance of mechanical strength, film uniformity, and bioactivity, consistent with previous studies emphasizing that 3–5% incorporation of plant extracts enhances the performance of polysaccharide-based edible films.[9,43,44] Thus, the 5% extract concentration was selected for the subsequent analysis of functional properties.

3.5 Characterization of the bioactive film

Preliminary formulation trials were conducted to determine the optimal concentration of *P. pinnata* and *P. guajava* leaf extracts for incorporation into starch-based edible films. Films prepared with 1% and 3% extracts appeared structurally weak, exhibiting fragile or partially formed matrices, whereas the 7% extract produced brittle, non-uniform films due to excessive disruption of the starch-glycerol polymer network. In contrast, the 5% (v/v) extract films demonstrated the most desirable characteristics, including uniform surface morphology, good flexibility, and higher mechanical stability. These observations were supported by tensile strength measurements, where 5% extract films recorded the highest stress values for both plants. A detailed comparison of visual attributes, integrity scores, and tensile strength across all concentrations is provided in SI Tables S1 and S2. Based on these results, the 5% extract loading was selected for all subsequent physicochemical, antimicrobial, and meat-wrapping evaluations.

3.5.1 Film thickness. The thickness of the starch-based films was measured to evaluate the effect of plant extract incorporation on the film structure. The control starch film exhibited the highest average thickness of 209 μm, whereas the films incorporated with 5% *Pongamia pinnata* and *Psidium guajava* leaf extracts showed reduced thicknesses of 157 μm and 162 μm, respectively (Table 2). The decrease in film thickness upon extract addition may be attributed to the interaction of phenolic and flavonoid compounds with the starch matrix, potentially promoting tighter molecular packing and reduced film expansion during drying.

Similar reductions in thickness upon bioactive incorporation have been reported,[43,45,46] suggesting that the integration of plant-based compounds modifies the microstructure of biopolymer films. Additionally, thinner films are often associated with improved transparency and flexibility, which are desirable traits in food packaging. The consistent thickness among all extract-based films indicates good reproducibility in

Table 2 Thickness of starch-based films with and without leaf extract incorporation

S. no.	Sample	Trial 1 (μm)	Trial 2 (μm)	Trial 3 (μm)	Average thickness (μm)
1	Starch film (control)	212	206	210	209
2	5% *P. pinnata* leaf extract film	164	156	151	157
3	5% *P. guajava* leaf extract film	162	164	161	162

Table 3 Tensile properties of starch-based films with and without leaf extract incorporation

S. no.	Sample	Length (mm)	Width (mm)	Max load (N)	Extension at max load (mm)	Tensile stress (MPa)	Tensile strain (mm mm^{-1})	Modulus (MPa)
1	Starch film (control)	90	20	7.44	10.25	1.74	10.87	28.14
2	5% *P. pinnata* leaf extract film	90	20	5.51	12.10	2.47	13.44	67.19
3	5% *P. guajava* leaf extract film	90	20	8.12	21.79	2.57	24.21	62.54

formulation, supporting their suitability for further mechanical and barrier property evaluations.

3.5.2 Tensile strength. The mechanical characteristics of the developed films were assessed by evaluating tensile strength, strain, and Young's modulus. As shown in Table 3 and Fig. 7, the control starch film exhibited a tensile stress of 1.74 MPa and Young's modulus of 28.14 MPa, indicating moderate stiffness and limited elasticity. Upon incorporation of the 5% *Pongamia pinnata* extract, tensile stress increased slightly to 1.83 MPa, and the modulus improved significantly to 67.19 MPa, suggesting enhanced rigidity. Interestingly, the *Psidium guajava* extract-based film showed the highest tensile strength (2.57 MPa) and strain (24.21 mm mm^{-1}), indicating significantly improved elasticity and mechanical flexibility.

The incorporation of plant extracts is known to alter the polymer matrix due to interactions between phenolic compounds and starch chains, which may lead to a more plasticized and cohesive network. The higher strain and tensile values for the *P. guajava* film may be attributed to the higher flavonoid content, contributing to improved film integrity and resistance to breakage. These results are in agreement with previous studies,[47–49] where bioactive compounds enhanced the mechanical flexibility of biopolymer films.

Overall, the findings indicate that extract incorporation, particularly *P. guajava*, not only improves antioxidant and antimicrobial properties but also enhances the mechanical performance of the films – making them suitable candidates for sustainable food packaging applications.

The tensile properties of the starch film (control) and films incorporated with 5% *Pongamia pinnata* and *Psidium guajava* extracts are evaluated. The graph illustrates tensile stress (MPa) and Young's modulus (MPa), indicating enhanced flexibility and mechanical performance with extract incorporation.

3.5.3 Water vapor transmission rate. The water vapor transmission rate (WVTR) is a critical parameter in evaluating the moisture barrier properties of edible films. As presented in Table 4 and Fig. 8, the control starch film exhibited a WVTR of 1358.08 g m^{-2} 24 h, indicating high water permeability due to the hydrophilic nature of native starch. Upon incorporation of the 5% *Pongamia pinnata* extract, the WVTR decreased to 1251.70 g m^{-2} 24 h, suggesting improved water barrier performance. In contrast, the *Psidium guajava* extract-based film exhibited a WVTR of 1350.99 g m^{-2} 24 h, which was similar to that of the control.

The reduction in the WVTR observed in the *P. pinnata* film may be attributed to the presence of phenolic and hydrophobic compounds that could interact with the starch matrix, thereby reducing free hydroxyl groups available for water absorption. This aligns with previous findings,[48] where plant extracts altered the moisture barrier by filling voids in the polymer network or creating denser film structures.

Although the *P. guajava* extract did not significantly reduce the WVTR, its strong antioxidant and antimicrobial effects compensate for this limitation. Thus, the *P. pinnata* extract may offer added advantage for moisture-sensitive food packaging, while the *P. guajava* extract provides functional benefits in terms of bioactivity.

Although incorporation of plant extracts reduced the WVTR compared to the control film, the overall values remained high (1251–1358 g m^{-2} 24 h), consistent with the hydrophilic nature of starch-based matrices. These values are considerably higher than those of commercial synthetic packaging materials such as LDPE (5–15 g m^{-2} 24 h) or PVC (10–20 g m^{-2} 24 h), indicating that the developed films offer only limited moisture barrier functionality. This represents a notable limitation for applications involving high-moisture foods such as fresh meat, where rapid water vapor transmission may accelerate surface dehydration. However, since the goal of this study was to explore antimicrobial and antioxidant active packaging rather than replace moisture-proof synthetic packaging, the films can still function as short-term preservative wraps or be combined with secondary moisture-barrier layers in future applications.

3.6 Meat wrapping and analysis

The moisture content of chicken meat after two hours of room-temperature storage is presented in Table 5. The unwrapped control retained the highest moisture level (78.2%), while the samples wrapped with *P. pinnata* and *P. guajava* films showed slightly lower values (74% and 75.6%, respectively). This reduction indicates that the starch-based films did not fully prevent moisture migration and allowed some dehydration, which is consistent with their relatively high WVTR values. However, the moisture loss in wrapped samples remained moderate compared to the rapid surface drying typically observed in unwrapped meat, suggesting that the polyphenol-enriched films provided partial, though not complete, moisture regulation. Similar findings were observed,[50,51] indicating that plant-based antimicrobial films can slow spoilage even when their moisture barrier properties are limited.

Color parameters (L^*, a^*, b^*) are critical quality indicators, as consumers associate freshness with bright appearance and redness. The meat wrapped in the *P. pinnata* film showed

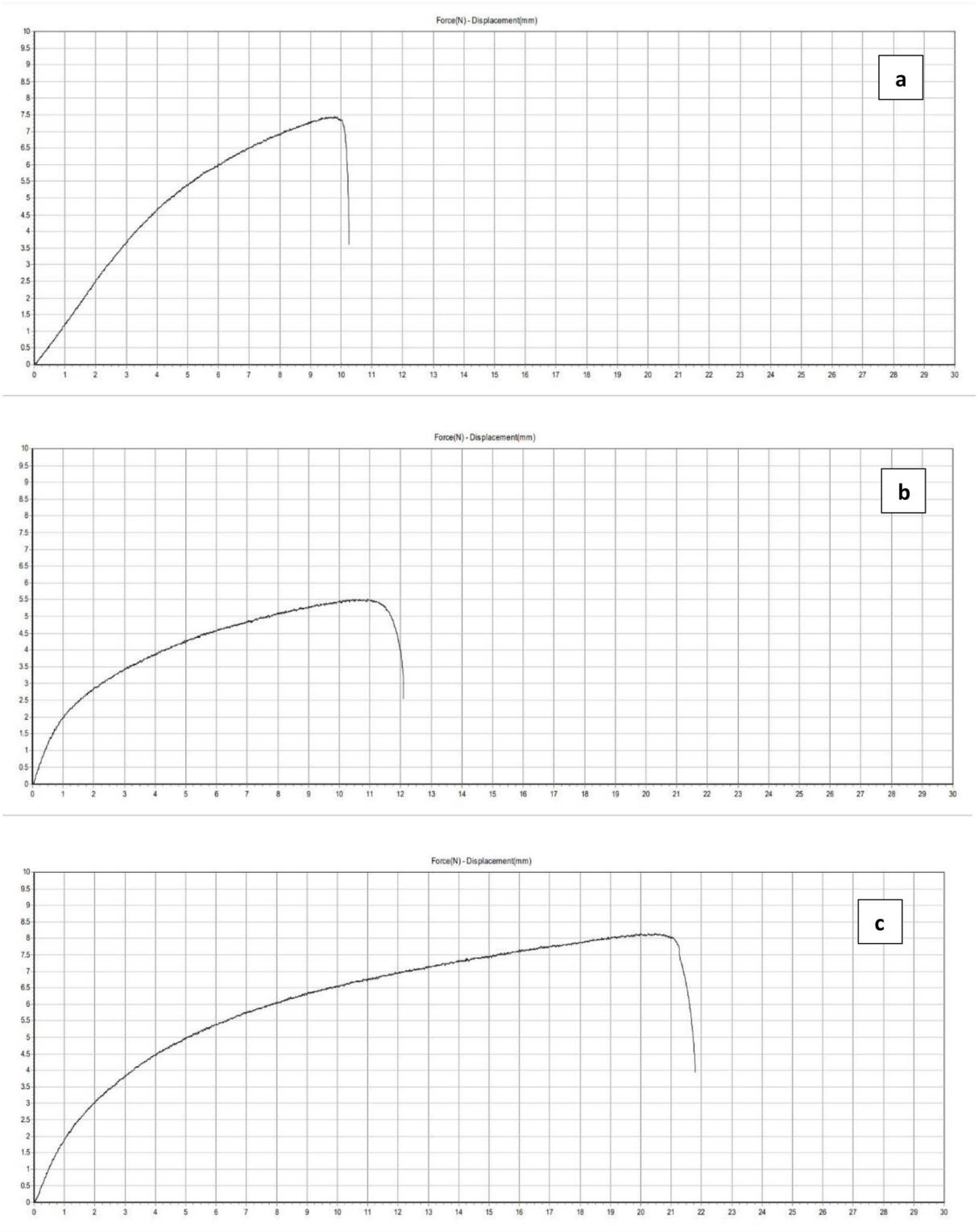

Fig. 7 Tensile stress and Young's modulus of control and extract-based films – (a) control film; (b) *P. pinnata* leaf extract incorporated film; (c) *P. guajava* leaf extract incorporated film.

notably improved lightness ($L = 47.14$) and redness ($a = 8.82$) compared to the control ($L = 29.23$, $a = 2.30$), indicating enhanced oxidative stability and reduced discoloration. This effect is attributed to the antioxidant properties of flavonoids and polyphenols, as previously reported,[52] demonstrating color stabilization in meat products with plant-derived phenolics. Conversely, *P. guajava*-wrapped meat showed a modest increase in L^* and a^* values (32.31 and 2.35, respectively), indicating

Table 4 Water vapor transmission rate (WVTR) of starch-based films with and without leaf extract incorporation

S. no.	Sample	Relative humidity (% RH)	WVTR (g m^{-2} 24 h)
1	Starch film (control)	30	1358.08
2	5% *P. pinnata* leaf extract film	30	1251.70
3	5% *P. guajava* leaf extract film	30	1350.99

slightly less pigment stabilization, though still better than the control.

In terms of pH, both wrapped samples exhibited pH 6.0, compared to 5.0 in the unwrapped control, which suggests lower microbial activity and slower spoilage in the treated samples. Maintenance of near-neutral pH is essential for delaying proteolytic degradation and bacterial proliferation in meat systems, as supported by previous findings,[53] which

Fig. 8 Water vapor transmission rate (WVTR) of control and extract-based starch films – (a) control film; (b) *P. pinnata* leaf extract incorporated film; (c) *P. guajava* leaf extract incorporated film.

Table 5 Effect of plant extract-incorporated starch films on moisture content, color values (*L*, *a*, *b**), and pH of chicken meat stored at room temperature

S. no	Samples	Moisture content (%)	*L** (lightness)	*a** (redness)	*b** (yellow)	pH	Water activity
1	Meat at room temperature after 2 hours	78.2	29.23	2.30	10.45	5	0.974
2	Meat wrapped in the *P. pinnata* leaf extract incorporated film	74	47.14	8.82	20.27	6	0.932
3	Meat wrapped in the *P. guajava* leaf extract incorporated film	75.6	32.31	2.35	10.65	6	0.935

reported similar pH stabilization in meat packaged with active films.

Collectively, these results demonstrate the effectiveness of plant-extract-based biofilms in preserving chicken meat quality and appearance under ambient conditions, supporting their potential use in active food packaging applications.

3.7 Shelf-life evaluation of chicken meat wrapped in bioactive films

Visual microbial analysis of chicken meat stored under ambient conditions revealed significant differences in spoilage progression between the control and bioactive film-wrapped samples. The control (unwrapped) meat (Fig. 9a) showed visible fungal and bacterial growth after 3 hours, indicating rapid microbial deterioration. In contrast, chicken meat samples wrapped with starch films incorporated with *Pongamia pinnata* (Fig. 9b) and *Psidium guajava* (Fig. 9c) leaf extracts remained visibly unspoiled, showing no evident microbial colonies within the same period.

To address the limitation of short-term ambient testing, a supplementary refrigerated study was performed at 4 ± 1 °C. During cold storage, the control meat exhibited a gradual increase in microbial load, exceeding 6 log CFU per g by day 3, consistent with typical spoilage kinetics in raw poultry. In contrast, samples wrapped with extract-incorporated films showed significantly slower microbial proliferation. Meat wrapped with *P. pinnata* films remained below 4 log CFU per g until day 3 and reached only 5.2 log CFU per g by day 5, while *P. guajava* films maintained counts below 5 log CFU per g through day 4.

This marked delay in spoilage in treated samples can be attributed to the antimicrobial efficacy of polyphenolic compounds present in the leaf extracts, which disrupt microbial cell membranes and inhibit enzymatic activity. These results align with the findings, which reported that plant-based antimicrobial packaging films significantly suppressed microbial growth on perishable foods.[54] Similarly, reports demonstrated enhanced shelf stability in meat products when wrapped with films containing botanical extracts.[55,56]

The absence of visual spoilage in the extract-treated films underscores their potential as effective bioactive packaging materials for extending the shelf life of fresh meat under room temperature conditions. Notably, *P. pinnata*-wrapped meat exhibited slightly better clarity and microbial suppression compared to *P. guajava*, possibly due to higher antimicrobial activity associated with its specific phytochemical profile. Although the films do not match the long-term barrier properties of commercial packaging, they provide meaningful short-term microbial protection, supporting their application as clean-label, biodegradable meat wraps.

3.8 Sensory analysis

To assess whether the bioactive films imparted any undesirable sensory changes to chicken meat ($n = 8$), odor, color, texture,

Fig. 9 Visual observation of spoilage in chicken meat stored at room temperature: (a) unwrapped control showing microbial growth after 3 hours; (b) meat wrapped in the *P. pinnata* extract-incorporated film after 24 hours; (c) meat wrapped in the *P. guajava* extract-incorporated film after 24 hours.

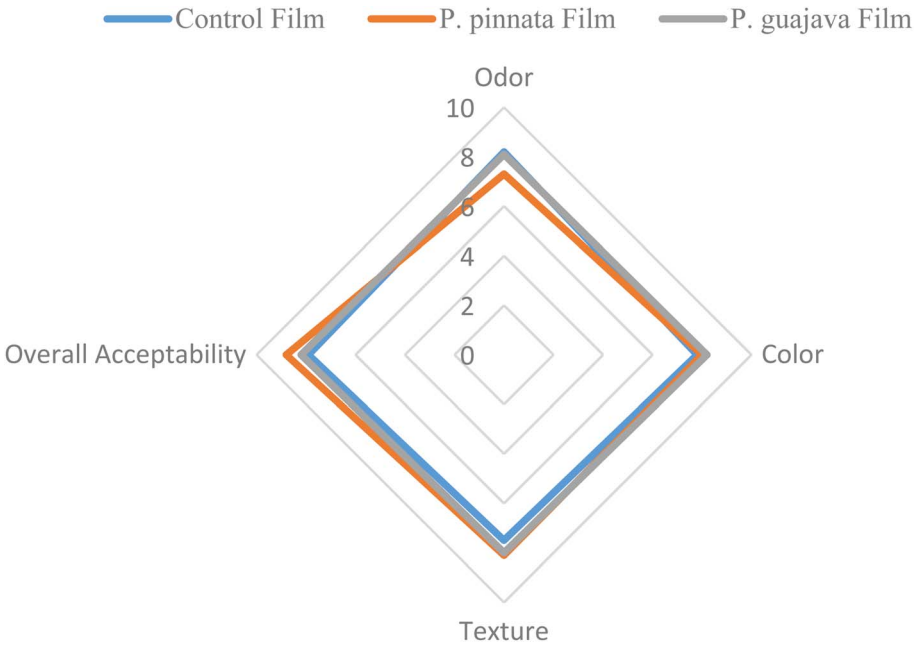

Fig. 10 Radar plot showing sensory scores (odor, color, texture, and overall acceptability) of chicken meat wrapped with control, *P. pinnata*, and *P. guajava* extract-infused edible films (9-point hedonic scale, $n = 8$).

and overall acceptability were evaluated using a 9-point hedonic scale. The sensory scores showed no adverse impact from the incorporation of *P. pinnata* or *P. guajava* extracts. Sensory evaluation as depicted in Fig. 10 revealed that neither extract-infused film imparted undesirable odor, color, or texture to the chicken meat.

The *P. guajava* film exhibited slightly higher scores for odor (8.8) and overall acceptability (8.2), reflecting its mild aromatic profile and effective preservation of meat freshness. The *P. pinnata* film also maintained acceptable sensory characteristics, with odor and texture scores of 8.1 and 8.3, respectively. All films preserved the natural color of the meat, with scores between 8.0 and 8.4, demonstrating the films' ability to inhibit surface discoloration during short-term storage.

Texture scores for treated samples were marginally higher than that of the control, indicating reduced moisture loss and firmer meat surface structure consistent with the barrier properties observed in WVTR results. Overall, no off-odors or negative sensory attributes were reported, supporting the suitability of both plant-based films for meat packaging applications. These findings align with previous research showing that phenolic-rich coatings do not impart undesirable flavors when used at low incorporation levels and instead contribute to maintaining product freshness.

In this research, starch-based edible films incorporated with bioactive extracts of *Pongamia pinnata* and *Psidium guajava* leaves were successfully developed and evaluated for their physicochemical, antioxidant, and antimicrobial properties. The incorporation of plant extracts enhanced the functional attributes of the films, improving tensile strength, antioxidant activity, and microbial inhibition. Application to chicken meat effectively reduced moisture loss, stabilized pH and color, and lowered microbial load compared to unwrapped controls. Importantly, both plants are traditionally used in food and medicine, and no toxicity concerns have been reported at the tested concentrations, supporting their safe use in edible films. Beyond meat, these films hold potential for other perishable foods such as fish, fruits, and vegetables. Future research may focus on optimizing extract concentrations across different food matrices and exploring blends with other biopolymers or natural agents to enhance performance. These findings reinforce the promise of plant-based edible films as sustainable, biodegradable alternatives for food packaging.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

All data supporting the findings of this study are included in the manuscript and its supplementary information (SI). Supplementary information: optimization data for starch-based edible films incorporated with *Pongamia pinnata* and *Psidium guajava* leaf extracts, including comparative evaluation of different extract concentrations (1%, 3%, 5% and 7% v/v) based on visual appearance, film integrity, and tensile strength measurements, which were used to justify the selection of 5% (v/v) extract concentration for further analyses. See DOI: https://doi.org/10.1039/d5fb00553a.

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