www.ijarp.com ISSN 2456-9992 Page: 01-13

International Journal Advanced Research Publications

Research Article

Volume: 02
Issue: 09

POMEGRANATE PEEL BIOFILM: A GREEN AND ANTIBACTERIAL ALTERNATIVE TO CONVENTIONAL FOOD PACKAGING

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Article Received: 06 August 2026, Article Revised: 26 August 2026, Published on: 16 September 2026

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Doi: https://doi-doi.org/101555/ijarp.8105

ABSTRACT

The increasing demand for sustainable and effective food packaging materials has led to the exploration of natural, bioactive compounds as alternatives to synthetic additives. In this study, an antibacterial thin biofilm was developed using pomegranate (*Punica granatum*) peel extract due to its known antimicrobial properties. The biofilm was prepared using corn starch as the base material, along with glycerol, vinegar, distilled water, and pomegranate peel extract to provide antimicrobial activity. The antibacterial activity of the pomegranate-based biofilm was evaluated against common foodborne microorganisms, including Pseudomonas sp. The effectiveness of the biofilm as a food packaging material was further assessed by microbiological analysis of raw food samples stored under three different conditions: polythene-wrapped, pomegranate biofilm-wrapped, and unwrapped samples over a specified period. Several physical properties of the biofilm were also evaluated, including water solubility, thermal stability, biodegradability, and tensile strength. The results indicated that the incorporation of *P. granatum* peel extract enhanced the antibacterial activity of the biofilm and showed effective inhibition against the tested pathogen. The biofilm also demonstrated suitable mechanical and physical properties, indicating its potential as an eco-friendly and antimicrobial food packaging material capable of improving food safety and extending shelf life.

KEYWORDS: Biofilm, Antimicrobial activity, Punica granatum, food spoilage.

INTRODUCTION

Environmental pollution and food safety concerns have accelerated the development of biodegradable antimicrobial packaging materials as sustainable alternatives to conventional plastics. Unlike synthetic packaging, biopolymer-based films are eco-friendly, biodegradable, and can be incorporated with natural antimicrobial agents to enhance food preservation and reduce reliance on synthetic preservatives (Malhotra *et al*., 2019; Siracusa *et al*., 2018). Antimicrobial thin films are protective coatings that inhibit the growth of bacteria, fungi, and viruses on food packaging and other surfaces. They improve food safety, extend shelf life, and also serve as effective communication and marketing tools in the food industry (Boluda et al., 2020; Vilas *et al*., 2020; Shruthy *et al*., 2020; Surendhiran *et al*., 2020). Although several antimicrobial agents, including essential oils, silver nanoparticles, organic acids, and antibiotics, have been investigated, commercially available antimicrobial packaging remains limited. Replacing conventional plastics with renewable biopolymers is considered a sustainable solution (Arsenie *et al*., 2020; Feng *et al*., 2018; Cozmuta *et al*., 2017; Xing *et al*., 2020).Corn starch is widely used as the primary biopolymer for antimicrobial films because of its biodegradability and excellent barrier properties. Glycerol acts as a plasticizer, improving flexibility by reducing intermolecular interactions, while vinegar enhances UV barrier, antimicrobial, antioxidant, and elastic properties. Natural antimicrobial extracts from fruits and vegetables effectively inhibit foodborne pathogens, making them suitable for food packaging applications (Susmitha *et al*., 2021; Murni *et al*., 2013; Gao *et al.*, 2017; Alves *et al*., 2007; Bras *et al*., 2020; Wijngaard *et al*., 2009; Fancello *et al.*, 2019).

Pomegranate peel (*Punica granatum*) contains tannins, flavonoids, alkaloids, organic acids, gallic acid, and punicalagin, which exhibit strong antioxidant, anti-inflammatory, antimicrobial, anticancer, and hepatoprotective activities. Its phenolic compounds act synergistically to improve biological activity and effectively extend the shelf life of food products (Tanaka *et al.*, 1990; Kasimsetty *et al.*, 2010; Orgil *et al*., 2014). Antimicrobial packaging effectively controls spoilage and pathogenic microorganisms, thereby improving food quality, safety, and shelf life. It is particularly useful for protecting perishable foods, including fruits, vegetables, meat, and dairy products. In this study, antimicrobial thin films incorporating *Punica granatum*, rich in punicalagin were developed to inhibit food spoilage microorganisms isolated from *Solanum lycopersicum* (tomato) (Sablani *et al*., 2011; Sofi *et al*., 2018).

MATERIALS AND METHODS

1. Sample Collection

Fresh **pomegranate (*Punica granatum*)** fruits were collected from local markets in Palakkad. The fruits were washed, shade-dried at room temperature for 3–4 weeks, and ground into fine powder using an electric blender.

2. Preparation of Antimicrobial Biofilm

Corn starch, glycerol, vinegar, distilled water, and orange/pomegranate peel extracts were used as raw materials. Peel extracts were prepared by extracting 5 g of dried peel powder with isopropyl alcohol under magnetic stirring for 1 h, followed by filtration (Berenice et al., 2017). Corn starch (25 g) was dissolved in 100 mL distilled water, heated at 80°C, mixed with glycerol and vinegar, followed by incorporation of 5 mL peel extract. The mixture was cast on aluminium sheets and dried at room temperature for 3–4 days to obtain antimicrobial films (Chhatariya et al., 2022).

3. Isolation and Identification of Microorganisms

Bacteria were isolated from spoiled tomato surfaces using the spread plate technique on nutrient agar. Pure cultures were obtained after incubation at 37°C for 24 h. Identification was carried out using Gram staining (Beveridge et al., 1983) and standard biochemical tests including Indole, Methyl Red, Voges–Proskauer, Citrate utilization, Triple Sugar Iron (TSI), Mannitol Motility, Oxidase, and Catalase tests (MacFaddin et al., 2000; Forbes et al., 2007; Tilley et al., 2014; Jorgensen et al., 2015; Karen et al., 2010).

4. Antimicrobial Activity

The antimicrobial activity of peel extracts was evaluated using the agar well diffusion method on Mueller–Hinton agar, while antimicrobial biofilms were tested using the disc diffusion method. Zones of inhibition were measured after incubation at 37°C for 24 h (Shleghm et al., 2024; Bauer et al., 1996).

5. Film Characterization

The prepared films were evaluated for:

- Organoleptic properties using apple, bread, and tomato stored under film, polythene, and uncovered conditions (Vrindas et al., 2018).
- Water solubility by determining percentage weight loss after immersion in distilled water (Ismail et al., 2024).
- Mechanical strength using a hanging weight test.
- Thermal stability by exposing films to 80–120°C for 1 h.

● Biodegradability by measuring weight loss after burial in soil for one week (Lisuzzo et al., 2020).

6. Microbial Analysis

1. Total Plate Count (TPC) – ISO 4833-1:2013

● Apple, cucumber, and bread samples were tested before and after wrapping with biofilm, polythene, and without wrapping.

● Samples were serially diluted in phosphate-buffered saline (PBS) and plated on Plate Count Agar (PCA) using the pour plate method.

● Plates were incubated at 35°C for 48 ± 2 h, and colonies were counted.

● Results were expressed as CFU/g or CFU/mL.

2. Total Coliform Count – ISO 4832:2006

● Serially diluted samples were plated on VRBLA (Violet Red Bile Lactose Agar).

● Plates were incubated at 37°C for 24 h.

● Typical purplish-red colonies were counted as presumptive coliforms.

● Confirmation was performed using BGLB broth, with gas production and turbidity indicating coliform presence.

RESULTS AND DISCUSSION

1. Extraction of *Punica granatum* (Pomegranate) Peel

Fresh pomegranate fruits (*Punica granatum*) were collected from local markets in Palakkad. The peels were separated, washed with distilled water, shade-dried at room temperature for 3–4 weeks, and finely powdered. The powdered peel was extracted using isopropyl alcohol under continuous stirring to enhance extraction efficiency. The extract was filtered using filter paper, and the filtrate obtained was used for biofilm preparation and antimicrobial studies.

Fig 1. pomegranate peel and its extraction.

2. Preparation of Pomegranate Peel Biofilm

The biofilm was prepared using corn starch as the base material along with glycerol, vinegar, distilled water, and pomegranate peel extract. The prepared mixture was poured onto a flat aluminium sheet and dried at room temperature for 3–4 days to obtain a thin, flexible antibacterial film suitable for food packaging applications.

Fig 2. Pomegranate film.

3. Isolation and Identification of Bacteria from Spoiled Vegetables

Microorganisms were isolated from spoiled vegetable samples using the spread plate technique. Distinct colonies obtained based on colony morphology were selected and subcultured to obtain pure cultures. The isolated bacteria were further characterized using morphological and biochemical tests for identification (Table.1) and to evaluate the antimicrobial activity of the pomegranate peel extract.

Fig 3. pure culture of bacteria isolated from spoiled vegetable.

Table :1 Morphological and Biochemical tests for identification.

S. No.	Test	Result
1	Gram's Stain	Gram-negative
2	Shape	Rod
3	Indole Test	Negative
4	Methyl Red Test	Negative
5	Voges–Proskauer Test	Negative
6	Citrate Utilization Test	Positive
7	Triple Sugar Iron (TSI) Test	K/K
8	Motility Test	Motile
9	Catalase Test	Positive
10	Oxidase Test	Positive

4. Antimicrobial Activity

The pomegranate peel extract exhibited significant antimicrobial activity, producing a zone of inhibition measuring 36 mm against the tested microorganism.

Fig.4. Well diffusion shows zone of inhibition.

5. Organoleptic Evaluation of Pomegranate Biofilm

The sensory characteristics of the pomegranate biofilm, including appearance and texture, were evaluated. Food samples wrapped with pomegranate biofilm, polythene film, and unwrapped samples were observed for three days (Table. 2). The pomegranate biofilm maintained acceptable physical properties and showed better preservation of food appearance compared to unwrapped samples.

Table 2: Food samples wrapped with pomegranate biofilm, polythene film, and unwrapped samples were observed for three days.

Sample	Sensory Parameter	Polythene Wrapped	Antibacterial Film Wrapped	Unwrapped
Apple	Appearance / Texture	Slightly hard	Good structure	Very hard
	Colour	No colour change	Slight brown colour	Slight brown colour
	Aroma / Smell	Normal	Acceptable aroma	Acceptable aroma
	Overall Acceptability	Moderately acceptable	Moderately acceptable	Acceptable
Tomato	Texture	Slightly dried texture	Hard	Dried
	Colour	Normal	No colour change	Faded
	Aroma / Smell	Normal	Normal	Acceptable aroma
	Overall Acceptability	Moderately acceptable	Acceptable	Unacceptable
Bread	Texture	Wonderful texture	Slightly hard	Hard
	Colour	No colour change	No colour change	No colour change
	Aroma / Smell	Normal	Normal	Normal
	Overall Acceptability	Extremely acceptable	Moderately acceptable	Acceptable

Fig 4. Day 3 observation (left to right) control ,pomegranate film, polythene.

6. Total Coliform Analysis

Total coliform analysis was performed using Violet Red Bile Lactose Agar (VRBLA) on apple, tomato, and bread samples stored under three conditions: pomegranate biofilm-wrapped, polythene-wrapped, and unwrapped were tabulated below:

Table: 3 Total coliform analysis: pomegranate biofilm-wrapped, polythene-wrapped, and unwrapped.

Sample	Polythene Wrapped (CFU/ml)	Pomegranate Film (CFU/ml)	Unwrapped (CFU/ml)	Orange Film (CFU/ml)
Apple	49×10^1	31×10^1	17×10^1	25×10^1
Tomato	137×10^1	50×10^1	61×10^1	42×10^1
Bread	72×10^1	57×10^1	168×10^1	46×10^1

Polythene-wrapped samples showed higher coliform counts, with apple and tomato recording 49×10^1 CFU/g and 137×10^1 CFU/g, respectively. In contrast, pomegranate biofilm-wrapped samples showed reduced counts of 31×10^1 CFU/g (apple), 50×10^1 CFU/g (tomato), and 57×10^1 CFU/g (bread), indicating the antimicrobial effect of the pomegranate peel extract. After 24 hours of storage, polythene-wrapped apple and tomato samples showed

Too Numerous To Count (TNTC) results, indicating significant microbial growth. Pomegranate biofilm-wrapped samples exhibited lower microbial counts. Bread samples wrapped with pomegranate biofilm recorded 30×10^1 CFU/g, whereas polythene-wrapped bread showed 77×10^1 CFU/g. These results demonstrate the ability of the pomegranate biofilm to retard microbial growth during storage.

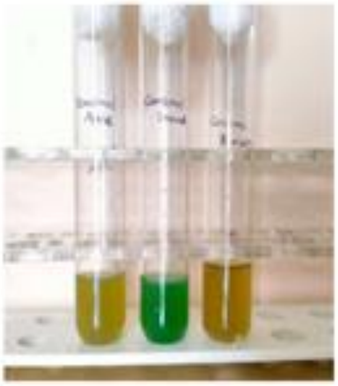

Fig 5. 1.Control and Fig. 5.2 Confirmatory test results of pomegranate biofilm (apple, tomato, bread; from left to right)

Positive colonies obtained on VRBLA were subjected to confirmatory testing using Brilliant Green Lactose Bile Broth (BGLB). Polythene-wrapped samples showed strong turbidity and gas production, confirming the presence of coliforms. Unwrapped samples also showed positive results with turbidity. In pomegranate biofilm-wrapped samples, bread showed a negative result, while apple and tomato showed only slight turbidity with minimal gas production, indicating a lower level of coliform contamination compared to polythene-wrapped samples.

7. Physical property analysis

The water solubility of the biofilm was found to be 23.33% after one hour of stirring. In the normal water immersion method, the biofilm remained insoluble for more than one week. The strength of the biofilm was evaluated using the hanging method, and the results were tabulated and film was able to carry up to 55g weight. The biodegradability of the biofilm was also evaluated under natural or controlled conditions by measuring the amount of CO_2 produced by microorganisms during the degradation of the film.

Fig. 7.Film subjected to stirring

Fig.8. strength of biofilm (hanging method)

Fig. 9 film is stable up to 100°C and at 120°C it is unstable.

DISCUSSION

The present study focused on the preparation and evaluation of an antimicrobial biodegradable film using food-grade materials for food-packaging applications. Pomegranate (*Punica granatum*) peel extract was incorporated to provide antimicrobial activity and was evaluated by the well-diffusion method. Pomegranate peel contains bioactive compounds such as phenolics, flavonoids and tannins, which contribute to its antimicrobial and antioxidant properties (Mphahlele et al., 2016; Ali et al., 2019). The antimicrobial activity observed was consistent with previous findings that pomegranate peel enhances the antimicrobial properties of starch-based films (Ali et al., 2019), mainly due to its phenolic compounds (Friedman, 2007). The film showed satisfactory mechanical properties and remained thermally stable at 80°C and 100°C for one hour, while instability occurred at 120°C; plant extracts can influence the thermal and structural properties of biodegradable films (Priyadarshi & Rhim, 2020). The film showed 23.33% water solubility after one hour of stirring and remained relatively insoluble in normal water immersion for more than one week, although water sensitivity remains a limitation of starch-based films (Müller et al., 2008). Its biodegradability was assessed through microbial degradation and CO_2 production, highlighting its potential as a sustainable packaging material (Siracusa et al., 2008; Mphahlele et al., 2016). Evaluation using apple, tomato and bread indicated the potential of the pomegranate film to reduce microbial growth compared with polythene wrapping, consistent with previous studies (Mousavi Khaneghah et al., 2018; Ali et al., 2019). Overall, the film represents a promising eco-friendly alternative to conventional plastic packaging, with future research needed to improve mechanical strength, optimize extract concentration, evaluate shelf life and assess large-scale commercial applications.

CONCLUSION

The present study demonstrated that the pomegranate peel extract-based biofilm possesses promising antimicrobial and biodegradable properties, making it a potential eco-friendly alternative to conventional polythene packaging. The film showed antimicrobial activity against *Pseudomonas* sp. and exhibited satisfactory physical, mechanical and thermal properties, along with biodegradability. Its use can help reduce plastic pollution while improving food safety and shelf life. Future research should focus on optimizing extract concentration, improving mechanical strength, developing smart packaging systems, and scaling up production for commercial food-packaging applications. The biofilm may also have potential applications in wound dressings, drug delivery and other biomedical and industrial fields.

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