



Evaluation of Bio-composite as a Packaging Material from Agricultural Waste

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ABSTRACT

This study aims to explore the potential of repurposing agricultural waste to develop a biodegradable bio-composite with antimicrobial properties. The research carried out focuses on utilizing pineapple leaves, a rich source of fiber, as the primary component for the bio-composite. Fibers were extracted from pineapple leaves through chemical degradation using Sodium hydroxide (NaOH) to form the base material. Tamarind seed polysaccharide and guar gum polysaccharide were incorporated for their binding capabilities, while limonene, extracted from sweet lime peels, was added to impart antimicrobial properties. By integrating these natural and biodegradable components, the study demonstrate to create an eco-friendly alternative material that not only reduces agricultural waste but also offers functional benefits. The proposed bio-composite has potential applications across various industries, contributing to sustainable waste management and environmental conservation efforts.

Keywords: Antimicrobial properties, Bio-composite, Biodegradable, Natural fibers, Polysaccharide, Sustainable development.

INTRODUCTION

The growing awareness of environmental degradation caused by synthetic polymers has steered the scientific and industrial communities toward the development of biodegradable and sustainable alternatives. Among the most pressing issues is the accumulation of non-degradable plastic waste, particularly from packaging materials, which are widely used and discarded daily. In this context, fruit processing industries generate an immense amount of organic waste in the form of peels, seeds, and fibrous residues that often remain underutilized. The utilization of bio-fibre for reinforcement in bio-composites used in the large-scale additive manufacturing industry creates a high-value co-product for the bioenergy industry. Bio-composite carbon-fiber-reinforced composites enhance the economic viability of the bioenergy industry by diversifying its product stream [1]. When improperly managed, this organic waste contributes to land and water pollution, greenhouse gas emissions, and loss of potential bio resources. However, with appropriate recycling strategies, fruit waste can be transformed into valuable raw materials for bio-composite production, helping to reduce environmental impact and promote sustainable innovation [2].

Recent studies highlight transforming agricultural residues and fruit peels into sustainable alternatives to conventional plastics as packaging materials. Researchers have evaluated multiple biopolymers derived from these wastes, including cellulose, hemicellulose, starch, etc., highlighting their biodegradability, mechanical performance, and barrier properties for food packaging. With improvements in processing methods, waste valorization, and biomaterial development, agricultural-waste bio-composites are emerging as potential candidates to replace conventional plastics [3].

Fruits such as pineapple, orange, and sweet lime are not only essential in human nutrition but also hold significant potential as sources of biodegradable raw materials. For instance, the leaves of the pineapple plant, which are usually discarded during fruit processing, are rich in lignocellulosic fibers. These pineapple leaf fibers (PALF) are strong, lightweight, and biodegradable, making them ideal for reinforcing biopolymeric matrices in the development of composite materials. Similarly, peels of citrus fruits, typically considered waste, contain limonene, an essential oil known for its potent antimicrobial and antioxidant properties. Instead of allowing these waste products to decay in landfills, they can be effectively repurposed to create functional and environmentally friendly materials [4].

Alongside natural fibers and bioactive agents, polysaccharides derived from plants play a crucial role in the formulation of biodegradable films and composites. Polysaccharides such as tamarind seed polysaccharide (TSP) and guar gum are renewable, non-toxic, and capable of forming films with desirable mechanical and barrier properties. TSP, extracted from tamarind kernel powder, is known for its thickening, gelling, and adhesive properties, while guar gum, sourced from guar beans, is valued for its high viscosity and stabilizing behavior. When combined with plasticizers such as glycerol, these polysaccharides can be processed into flexible and uniform films suitable for various packaging applications.

By incorporating pineapple leaf fibers into a matrix formed by TSP and guar gum, the mechanical properties of the bio-composite are significantly enhanced, particularly in terms of tensile strength and durability. [5] Moreover, the addition of limonene transforms the bio-composite into an active material with antimicrobial properties,

effectively inhibiting the growth of pathogenic microorganisms such as *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). This integration of antimicrobial agents not only adds value to the packaging material but also plays a critical role in food safety, shelf-life extension, and prevention of microbial contamination in perishable products.

The resulting bio-composite is thus multifunctional, it is biodegradable, reinforced with sustainable fibers, and exhibits antimicrobial activity. Such materials have wide-ranging applications, particularly in food packaging, pharmaceuticals, and biomedical fields. For example, an antimicrobial film can be used to wrap fresh produce, meat, or bakery items to prevent spoilage and extend shelf life without the need for synthetic preservatives. Additionally, these films can be employed in wound dressings or hygienic products where microbial control is essential.

The research carried out focuses on the eco-friendly synthesis and characterization of a bio-composite film developed from pineapple leaf fibers, tamarind seed polysaccharide, guar gum, glycerol, and limonene. By utilizing agricultural waste and plant-derived polymers, the project aims to demonstrate a sustainable approach to material development that supports waste valorization, promotes circular economy principles, and contributes to environmental preservation through the reduction of plastic pollution.

MATERIALS AND METHODS

Collection of raw materials

Fresh pineapple leaves were obtained directly from a local fruit-juice vendor immediately after fruit processing to ensure minimal degradation of the fibers. Only intact and mature leaves showing no signs of pest damage or fungal infection were selected. Approximately 2 pineapple crowns of leaves were collected per batch, placed in clean, ventilated plastics trays to avoid mechanical bruising, and transported to the laboratory as shown in Fig. 1.



Fig 1: removal of pineapple leaves from the crown



Fig. 2: Mechanical scraping of pineapple fibers from the leaves using a scalpel



Fig 3: alkali treatment with sodium hydroxide



Fig 4: bleaching treatment with hydrogen peroxide



Fig. 5: Preparation of tamarind seed Polysaccharide (TSP) solution

Extraction of pineapple fibres from pineapple leaves

Pineapple leaf fiber (PALF) was obtained from fresh *Ananas comosus* leaves sourced from a local fruit-juice vendor. Leaves were first rinsed thoroughly in running tap water to remove adhering soil and fruit residues, then air-dried at room temperature for 24 hours. The midrib regions were manually separated from the leafy lamina and cut into 5 to 10 cm segments. Two distinct extraction routes: mechanical separation and chemical degradation, were evaluated to isolate the fiber bundles, and both sets of fibers were finally oven-dried to constant mass [6].

Mechanical separation

Pre-cleaned leaf segments were subjected to manual scraping using a stainless-steel scalpel: the leaf cuticle and parenchyma were gently peeled away along the fiber axis in multiple passes until most non-fibrous tissue was removed and the fiber bundles were exposed as shown in Fig 2. Although this decortication approach requires no chemical reagents, it is labor-intensive and produces fibers of variable length with some remaining impurities. These mechanically separated fibers were subsequently washed in distilled water to neutral pH and pre-dried at room temperature.

Chemical degradation

Cut leaf segments were immersed in a 5% (w/v) NaOH solution maintained at 80°C under constant stirring for 2 hours to saponify pectin and solubilize hemicellulose as shown in Fig. 3, thereby loosening the lignocellulosic matrix. After alkali treatment, samples were rinsed with distilled water until washing reached neutrality. The alkali-treated fibers were then bleached in a 2% (v/v) H₂O₂ solution at 60°C for 30 minutes to remove residual lignin and brighten the fiber

surface as shown in Fig. 4 [7]. Following bleaching, fibers were again washed to neutral pH and gently pressed to remove excess moisture.

Drying

Fibers obtained from both mechanical and chemical extraction routes were oven-dried at 40°C for 15 to 20 minutes to eliminate bound moisture. The dried fibers were then stored in a desiccator over silica gel before further characterization or composite preparation.

Formation of polymer matrix

Preparation of Tamarind Seed Polysaccharide (TSP) Solution

Tamarind seed polysaccharide powder was accurately weighed (typically 4% w/v, e.g., 2 g TSP per 100 mL distilled water) and transferred into a 250 mL beaker. The suspension was heated to boiling (~100°C) with constant stirring (300 rpm) using a glass rod. As the temperature rose, the polysaccharide gradually hydrated, and the mixture became increasingly viscous. After 10 minutes at reflux, ensure complete dispersion and dissolution of the TSP as shown in Fig. 5. The hot solution was then cooled to 50 °C under continuous stirring to prevent gelation before matrix assembly [8].

Preparation of Guar Gum Polysaccharide (GGP) Solution

Guar gum powder was weighed to achieve a 2% w/v concentration (e.g., 2 g per 100 mL distilled water) and sprinkled slowly into 80 mL of boiling water in a separate 250 mL beaker [9]. The slow addition minimized lump formation and facilitated uniform hydration. The mixture was maintained at 95–100°C for 5 minutes to fully activate the galactomannan chains, then held at a boil for an additional 5 minutes. Once a clear, viscous solution formed. The GGP solution was allowed to cool to 50°C while stirring [10].

Matrix Formation with Glycerol

With both polysaccharide solutions equilibrated at 50°C to avoid thermal shock, glycerol (as a plasticizer) was added at 10% w/w



Fig: 6 Casting the composite film

relative to the combined dry polysaccharide mass. The mixture was homogenized for 15 minutes to ensure uniform plasticizer distribution and to reduce air entrapment. The resulting gel-like matrix exhibited a smooth, castable consistency, ready for subsequent casting or crosslinking steps.

Antimicrobial Susceptibility Testing of Limonene

Limonene, a naturally occurring monoterpene predominantly found in the peels of citrus fruits such as oranges and sweet limes, is renowned for its potent antimicrobial properties. Its efficacy against a broad spectrum of microorganisms has garnered significant interest in the development of bio-based antimicrobial agents [11]. In this study, limonene was extracted from citrus peels and subjected to antimicrobial susceptibility testing to evaluate its effectiveness against both gram-positive and gram-negative bacteria. The testing involved two standard methods: the disk diffusion method and the agar cup (well diffusion) method. Furthermore, the incorporation of limonene into a polymer matrix composed of tamarind seed polysaccharide, guar gum, and glycerol, reinforced with dried pineapple fibers, was explored to develop a biodegradable composite with enhanced antimicrobial properties.

Media Preparation

Mueller–Hinton agar (MHA) was prepared, followed by autoclaving at 121°C for 15 minutes to ensure sterility. The sterilized medium was then poured into sterile Petri dishes and allowed to solidify.

Preparation of Inoculum and Subculture

Two bacterial strains were selected for the study: *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Each strain was inoculated into separate tubes containing sterile nutrient broth and incubated at 37°C for 18 to 24 hours to achieve optimal growth [8].

Inoculation with Sterile Swabs

Sterile cotton swabs were immersed in the standardized bacterial suspensions, and excess fluid was removed by pressing the swab against the inner wall of the tube. The swabs were then used to evenly spread the bacterial suspension over the entire surface of the Mueller-Hinton agar plates in three directions to ensure a uniform lawn of bacterial growth.

Preparation of Limonene Solutions

Pure limonene was diluted to obtain four different concentrations: 100, 75, 50, and 25% (v/v). Each solution was thoroughly mixed to ensure homogeneity before application in the susceptibility tests.

Disk Diffusion Method

Sterile filter paper disks, each measuring 6 mm in diameter, were impregnated with 20 µL of the respective limonene concentrations. These disks were then carefully placed onto the surface of the inoculated Mueller-Hinton agar plates using sterile forceps.

Agar Cup (well diffusion) method

Using a sterile cork borer, wells of 6 mm diameter were punched into the agar of the inoculated MHA plates. Each well was filled with 30 µL of the respective limonene concentrations. The plates were then left undisturbed at room temperature for 30 minutes to allow the limonene to diffuse into the agar before incubation.

Incubation and Zone Measurement

All prepared plates were incubated in an inverted position at 37°C for 48 hours. Post incubation, the zones of inhibition around each disk and well were measured in millimeters. The diameter of the clear zones indicated the extent of bacterial growth inhibition by limonene.

Incorporation of Limonene into the Polymer Matrix with Dried Pineapple Fibers

Dried pineapple fibers, previously extracted and oven-dried at 40°C for 15 to 20 minutes, were incorporated into the cooled (approximately 50°C) polymer matrix composed of tamarind seed polysaccharide, guar gum, and glycerol. Gentle stirring ensured uniform dispersion of the fibers throughout the matrix.

Limonene addition

The addition was done by gently mixing the limonene into the cooled polysaccharide mixture to ensure uniform dispersion without causing volatility loss due to high temperatures. This step was crucial, as limonene is sensitive to heat and could degrade or evaporate if introduced at high temperatures. The incorporation of limonene not only enhanced the functional properties of the bio-composite but also transformed it into an active packaging material capable of inhibiting microbial growth. This antimicrobial action is particularly beneficial for food packaging applications, where it helps in extending shelf life and maintaining hygiene, thus adding significant value to the biodegradable film [12].

Final film setting and casting:

After incorporating tamarind seed polysaccharide, guar gum, glycerol, limonene, and dried pineapple fibers into a homogeneous mixture, the composite solution underwent a final homogenization step. This involved constant stirring for approximately 10 minutes, followed by high-speed homogenization at the same speed for an additional 15 minutes. This process ensured uniform distribution of all components and minimized air entrapment, which is crucial for achieving films with consistent thickness and mechanical properties.

Casting the Composite Film

The degassed composite solution was then poured into a stainless steel mold to form films of uniform thickness as shown in Fig. 6. The casting process was conducted under controlled conditions to prevent the introduction of air bubbles and to ensure even spreading of the solution. Care was taken to maintain a consistent thickness across the entire film surface, as variations could lead to differences in mechanical strength and barrier properties.

Drying and Film Formation

The cast films were dried in a convection oven set at 40 to 45°C for 20 to 25 minutes. This drying process facilitated the evaporation of water, leading to the solidification of the film matrix. The temperature and duration were optimized to prevent thermal degradation of the polysaccharides and limonene while ensuring complete drying. After drying, the films were carefully peeled from the molds and conditioned at room temperature (approximately 25°C) and 50% relative humidity for 48 hours. This conditioning step allowed the films to equilibrate, enhancing their mechanical flexibility and stability.

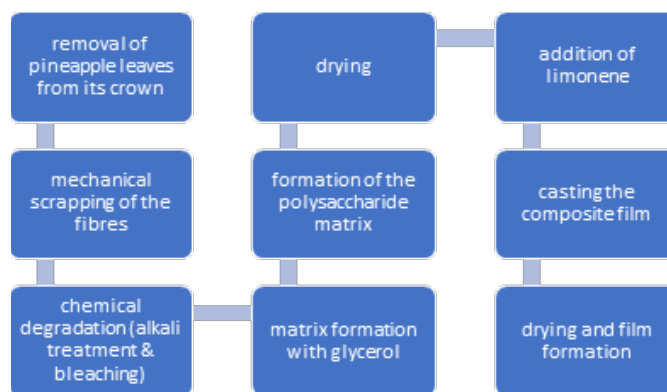


Fig 7: Procedural flowchart of the methodology used in the development of the limonene-based agricultural-waste biomaterial.

Final film characteristics

The resulting bio-composite films exhibited a smooth, uniform surface with well-integrated pineapple fibers and evenly distributed limonene. The incorporation of glycerol as a plasticizer imparted flexibility, while the natural fibers provided reinforcement, enhancing tensile strength. The presence of limonene endowed the films with antimicrobial properties, making them suitable for applications in food packaging and biomedical fields. The films demonstrated good handling characteristics, with sufficient elasticity and durability for practical use.

RESULTS

Based on the comprehensive procedures and analyses conducted, the following detailed results encapsulate the findings from each stage of the bio-composite film development:

Pineapple Leaf Fiber Extraction

Mechanical Scraping Method

Manual mechanical scraping yielded dry pulp of pineapple leaves. This method, while straightforward, is labor-intensive and less efficient in purifying the fibers.

Chemical Degradation Method

Chemical treatment using sodium hydroxide (NaOH) concentrations of 6% or higher effectively removed impurities, resulting in clean and smooth fibril surfaces. The process involved treating crushed pineapple leaves with NaOH at elevated temperatures, followed by bleaching with hydrogen peroxide. This method produced a higher yield of purified fibers with enhanced mechanical properties, making them more suitable for composite applications.

Polysaccharide matrix formation

Tamarind Seed Polysaccharide (TSP)

TSP, a neutral xyloglucan extracted from tamarind seed kernels, was prepared by mixing tamarind seed powder with boiling water to form a viscous solution. TSP exhibits excellent thickening, emulsifying, and gelling properties, making it a valuable component in film formation.

Guar Gum Polysaccharide

Guar gum, a galactomannan polysaccharide derived from guar beans, was similarly prepared by dissolving guar gum powder in boiling water. It is known for its high viscosity and stabilizing properties, contributing to the mechanical strength and flexibility of the composite film.

Matrix Formation with Glycerol

The TSP and guar gum solutions were combined in specific ratios, and glycerol was added as a plasticizer to enhance flexibility. The mixture was homogenized to ensure a uniform matrix, providing a suitable medium for incorporating fibers and antimicrobial agents.

Antimicrobial susceptibility testing of limonene:

Limonene has been widely used for its antibacterial activity. A review highlights the MIC values for D-limonene against various bacteria, ranging from tens of $\mu\text{g}/\text{mL}$ to several mg/mL , highlighting the concentration-dependent nature of its antibacterial effect.[13] Another recent study demonstrated a zone of inhibition of 21.67 mm against *E. coli* and up to 40 mm against *Clostridium perfringens* in agar diffusion assays [14].

Media and inoculum preparation:

Sterile Mueller-Hinton agar plates were prepared for antimicrobial testing. Gram-positive *S. aureus* and Gram-negative *E. coli* were subcultured in nutrient broth to serve as test organisms [15].

- *Testing methods*
- *Disk Diffusion Method: Sterile discs were impregnated with varying concentrations of limonene (100, 75, 50, 25%) and placed on inoculated agar plates.*
- *Agar Cup Method: Wells were created in the agar using a sterile cork borer, and 30 μL of limonene solutions at different concentrations were added to each well.*

After 48 hours of incubation, the zone of inhibition was measured. Limonene exhibited a clear concentration-dependent antibacterial effect in both the assays (agar-cup and disc diffusion); however, agar-cup exhibited a better zone of inhibition as shown in Fig. 8. In the agar-cup assay against *E. coli*, all four concentrations (100, 50, 25, and 12.5%) produced measurable inhibition, with the mean zone diameters (mm) decreasing progressively with dilution ($n = 4$; mean \pm SD) as shown in Table 1, Fig. 9. While in the disc diffusion assay for *E. coli* demonstrated inhibition only at higher concentrations. Both 100 and 50% discs produced distinct inhibition zones, but 25 and 12.5% showed no detectable activity ($n = 2$; mean \pm SD), indicating a significant loss of efficacy in this method compared to the agar-cup assay as shown in Table 2, Fig. 10. For *S. aureus*, the disc diffusion results showed a similar concentration-dependent pattern, with measurable inhibition zones recorded at 100, 50, and 25, while 12.5% did not produce any measurable zone of inhibition ($n = 3$; mean \pm SD) as shown in Table 3, Fig. 11. Overall, the data consistently highlight that limonene's antibacterial activity decreases with dilution, with lower concentrations, particularly 12.5% with the lowest concentration, often losing its activity entirely, depending on the type of organism and assay that were tested.



Fig 8: Zone of inhibition around the colonies of E. coli & S. aureus due to the presence of limonene in the media

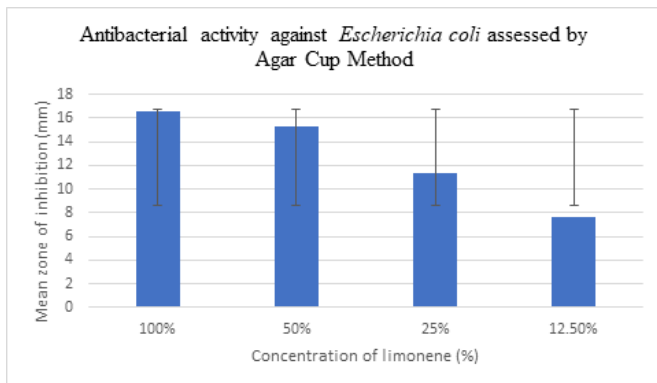


Fig 9: Antibacterial activity against E. coli assessed by agar cup method.

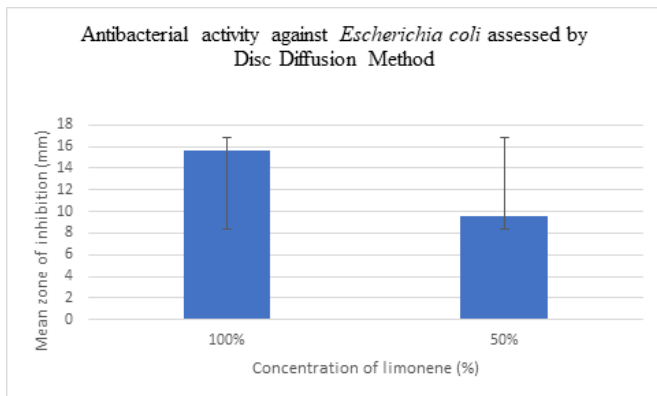


Fig 10: Antibacterial activity against E. coli assessed by disc diffusion method.

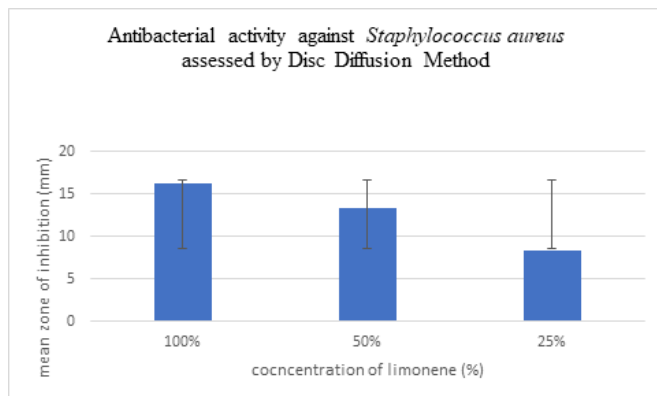


Fig11: Antibacterial activity against S. aureus assessed by disc diffusion method.

Incorporation of Limonene and Pineapple Fibers into Polymer Matrix

The cooled polysaccharide-glycerol matrix (~50°C) was blended with dried pineapple fibers to ensure uniform dispersion. Subsequently, limonene was incorporated into the mixture. The composite was subjected to constant stirring for 10 minutes, followed by high-speed homogenization at the same speed for an additional 15 minutes. This process ensured even distribution of fibers and limonene within the matrix, enhancing the structural integrity and antimicrobial properties of the final film.

Film casting and drying:

The homogenized composite mixture was poured into a stainless steel mold to achieve films of uniform thickness. The films were dried in a convection oven at 40 to 45°C for 20 to 25 minutes, to facilitate solvent evaporation and film formation. Post-drying, the films were conditioned at room temperature (~25°C) and 50% relative humidity for 48 hours to stabilize their mechanical properties.

Final bio-composite film characteristics

The resulting bio-composite films exhibited a smooth, uniform surface with well-integrated pineapple fibers and evenly distributed limonene. The incorporation of glycerol imparted flexibility, while the natural fibers provided reinforcement, enhancing tensile strength

Water Sensitivity and Beeswax Coating for Improved Water Resistance

Although the developed bio-composite film exhibits excellent biodegradability, flexibility, and antimicrobial activity, one of its major limitations is its high sensitivity to water. Being composed primarily of natural polysaccharides like tamarind seed polysaccharide and guar gum, the material readily absorbs moisture and tends to dissolve or lose structural integrity upon prolonged exposure to water. This significantly restricts its practical application, especially in packaging scenarios where moisture resistance is crucial. To overcome this drawback and enhance the water-repellent properties of the bio-composite, the surface of the dried film was coated with a thin layer of beeswax. Beeswax, a natural hydrophobic substance, forms a protective barrier over the film, effectively reducing water permeability and increasing the film's resistance to moisture. This coating not only preserves the integrity of the bio-composite under humid conditions but also retains its biodegradability and safety for food contact, making it more suitable for real-world packaging applications.

Fig 1: Antibacterial activity against *E. coli* assessed by agar cup method.

Concentration of limonene (%)	Mean zone inhibition (mm)	Standard deviation
100	16.6	0.58
50	15.3	0.57
25	11.3	0.47
12.5	7.6	0.57

Table 2: Zone of inhibition obtained by the disc diffusion method against *E. coli*

Concentration of limonene (%)	Mean zone inhibition (mm)	Standard deviation
100	15.6	0.57
50	9.6	0.58

Table 3: Zone of inhibition obtained by the disc diffusion method against *S. aureus*

Concentration of limonene (%)	Mean zone inhibition (mm)	Standard deviation
100	16.3	0.47
50	13.3	0.57
25	8.3	0.57

DISCUSSION

The development of an antimicrobial bio-composite film integrating pineapple leaf fibers, tamarind seed polysaccharide (TSP), guar gum, glycerol, and limonene represents a significant advancement in sustainable material science.

The extraction of pineapple leaf fibers through chemical degradation using sodium hydroxide (NaOH) proved more effective than mechanical scraping. This method efficiently removed non-cellulosic materials, resulting in cleaner fibers with enhanced mechanical properties. The purified fibers exhibited improved tensile strength, making them suitable for reinforcement in composite materials.

A review highlights that agricultural wastes such as pineapple leaf fibers, husk fibers, fruit residues, and plant-derived polysaccharides can be effectively transformed into bio-composites with improved mechanical and thermal behavior. This provides a strong niche for utilizing waste-derived materials in the present time. [16]

The polysaccharide matrix formed by combining TSP and guar gum provided a robust and flexible foundation for the bio-composite film. TSP, a galacto xyloglucan, contributes to the viscosity and gel-forming capabilities of the matrix, while guar gum enhances film-forming.

Properties and stability: The addition of glycerol as a plasticizer further improved the flexibility and handling characteristics of the film.

Our findings align with the development of agricultural-waste-derived biomaterials, which often require functional additives, such as essential oils, to achieve effective antimicrobial activity. The incorporation of limonene in the biomaterial follows this trend and contributes to the antibacterial performance observed [17].

Incorporating limonene, a natural antimicrobial agent extracted from citrus peels, into the matrix imparted significant antimicrobial

properties to the bio-composite film. Antimicrobial susceptibility testing using both disk diffusion and agar cup methods demonstrated that the agar cup method yielded more pronounced zones of inhibition against *S. aureus* and *E. coli*, indicating effective diffusion and activity of limonene within the film. The final bio-composite film exhibited a smooth, uniform surface with well-integrated pineapple fibers and evenly distributed limonene. The film's mechanical properties, including tensile strength and flexibility, were enhanced by the synergistic effects of the natural fibers and polysaccharide matrix. The presence of limonene endowed the film with antimicrobial properties, making it suitable for applications in food packaging and biomedical fields. The film demonstrated good handling characteristics, with sufficient elasticity and durability for practical use.

Overall, this study highlights the potential of utilizing agricultural waste and natural polymers to develop sustainable, biodegradable, and functional materials with applications in various industries.

CONCLUSION

This research successfully demonstrates the development of a biodegradable and antimicrobial bio-composite film utilizing agricultural and fruit-processing waste, highlighting an innovative approach toward sustainable material development. By incorporating naturally sourced components pineapple leaf fibers, tamarind seed polysaccharide (TSP), guar gum, glycerol, and limonene the study offers an eco-friendly alternative to conventional plastic-based packaging materials.

The process began with the collection of raw pineapple leaves, obtained from a local fruit juice vendor, a step that reflects the practical application of waste valorization. Two fiber extraction methods were explored: mechanical scraping and chemical degradation using sodium hydroxide and hydrogen peroxide. The chemical method proved more effective, yielding cleaner and more uniform fibers with improved mechanical integrity, making them suitable for reinforcing biodegradable matrices.

This research shows that the biomaterial having limonene resonates with the current limonene antibacterial systems and suggest that further refinement and repeated trials may help it achieve effectiveness similar to the encapsulated formulations [18].

Simultaneously, a polymeric matrix was formulated using TSP and guar gum. Both polysaccharides were individually dissolved in boiling water to ensure complete hydration and gelation. Once cooled slightly, the mixtures were blended with glycerol, which acted as a plasticizer to improve flexibility and film-forming capability. The combination of these natural polysaccharides provided a robust, biodegradable matrix with good structural properties and environmental compatibility.

A major enhancement to the composite was achieved through the incorporation of limonene, an essential oil with well-documented antimicrobial properties, extracted from citrus fruit peels such as oranges and sweet lime. Antimicrobial susceptibility testing conducted using both disc diffusion and agar cup methods confirmed the effectiveness of limonene, with the agar cup method showing clearer and larger zones of inhibition against *S. aureus* and *E. coli*. This addition gave the final film active antimicrobial functionality, opening its application in food packaging and healthcare.

The final composite was prepared by integrating the treated pineapple fibers into the polysaccharide-limonene-glycerol blend, followed by casting and drying to form a uniform film. The resulting material exhibited desirable properties such as flexibility, strength, biodegradability, and antimicrobial activity.

In conclusion, the study not only demonstrates a viable method to repurpose fruit and agricultural waste but also underlines the potential of bio-composites as functional materials for active packaging. This work supports the principles of a circular economy by transforming waste into value-added products that are both eco-friendly and commercially applicable.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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