



ANALYSING *CARICA PAPAYA* LEAF EXTRACT, *CARICA PAPAYA* LEAF α -CELLULOSE, AND STARCH BASED BIOFILM ON PHYSICAL PROPERTIES AND ANTIMICROBIAL RESISTANCE AGAINST YEAST ASSAY

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Abstract: This study reports the extraction of α -cellulose from *Carica papaya* of genus *Carica*, of the family *Caricaceae* (*papaya*) leaves and its subsequent utilization in the fabrication of a biodegradable biofilm. *Carica papaya*, commonly known as the papaya tree, is a flowering tree found in modern-day southern Mexico and Central America and is grown in several countries with tropical climates. α -Cellulose was isolated through sequential alkaline treatment using sodium hydroxide (NaOH) for delignification, followed by sodium hypochlorite (NaOCl) bleaching remove hemicellulose and enhance purity. The extracted α -cellulose was integrated with natural biopolymers such as starch and gelatin to form a composite film via solution casting. The resulting biofilm exhibited improved film-forming ability, structural integrity, and flexibility due to synergistic interactions between cellulose fibers and the polymer matrix. To draw a comparative study, two additional biofilms were prepared using potato starch and papaya leaf extract directly. This work demonstrates the feasibility of valorizing agricultural leaf waste into sustainable, eco-friendly packaging and functional biomaterial alternatives to conventional petroleum-based plastics. The biofilms were evaluated through eight distinct tests including water degradation, soil burial degradation, gas production inhibition, vertical hanging test (tensile strength), oil resistance, opacity and UV-shielding, surface morphology (microscopy), and swelling ratio. Both the α -cellulose biofilm and papaya leaf extract biofilm showcased improved properties in comparison with the starch biofilm control.

Index Terms: α -Cellulose, *Carica papaya*, papaya leaves, biodegradable biofilm, bioplastic, starch, gelatin, antimicrobial resistance, yeast assay, tensile strength, solution casting.

I. INTRODUCTION

Bioplastic or biodegradable biofilm represents a newly developing class of materials aimed at replacing petroleum-derived plastics. Petroleum-derived plastics are long-chain synthetic polymers that are lightweight, durable, and can be moulded into various shapes. However, they are not biodegradable and thus persist in the environment for millions of years. The growing concerns related to plastic pollution have led to the emergence of bioplastics as a viable alternative.

Bioplastics are biodegradable alternatives to conventional plastics, derived from natural and renewable resources (organic matter). These plastics can be degraded just like any other organic refuse in the environment. However, bioplastics make up a small proportion of today's plastic production because of their high cost of production. This paper is aimed at preparing bioplastic from *Carica papaya*, a plant species widely found in India.

The leaves of *Carica papaya* are a rich source of cellulose, which can be extracted and utilized as a reinforcing agent in biopolymer matrices. α -Cellulose offers high crystallinity, good mechanical strength, and biodegradability, making it an ideal candidate for sustainable film formulations.

II. MATERIALS AND METHODS

2.1 Materials Required

The following materials were used in this study: 120 g of fresh *Carica papaya* leaves paste, sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), distilled water, pH paper, litmus paper, beakers (250 ml), volumetric cylinders (borosilicate glass, 1 L and 500 ml), water bath, cheese/muslin cloth, starch, gelatin, and glycerol.



Figure 2.1.1: Fresh *Carica papaya* leaves used as raw material.



Figure 2.1.2: *Carica papaya* tree from which leaves were sourced.

2.2 Extraction of α -Cellulose

A slurry/paste of fresh papaya leaves was prepared by adding a small amount of water (10 ml or less). Approximately 500 ml of 2% w/v NaOH solution was added to the paste, and the mixture was treated at 80°C in a water bath for 2.5 hours. This process served to delignify the entire solution. Subsequently, 750 ml of 3.5% NaOCl v/v was added to the treated mixture and heat-treated at 80°C for 30 minutes for bleaching purposes.

The mass was further treated with 500 ml of 17.5% w/v aqueous NaOH solution at 80°C for 1 hour. A cloudy mass of cellulose was obtained at the bottom of the container after this step, and the supernatant water was decanted. The resultant α -cellulose underwent a final stage of bleaching with 750 ml of 3.5% w/v NaOCl for 1 hour. The α -cellulose was washed thoroughly with distilled water until the pH and litmus readings were neutral. The obtained α -cellulose was then sun-dried for 2 hours.



Figure 2.2.1: α -Cellulose obtained after washing with distilled water.



Figure 2.2.2: Cloudy mass of cellulose obtained during treatment.

2.3 Formation and casting of α -Cellulose Biofilm

A solution was prepared with 2.93 g of cellulose, 7.07 g of starch in 100 ml of water, and 5 g of gelatin powder was added. Approximately 5 ml of glycerol was added for flexibility. The mixture was heated slowly in a vessel until a thick, homogeneous mixture was obtained. This mixture was then cast onto a tray lined with aluminium foil and allowed to dry for 72 hours.

2.4 Biofilms Obtained

Three types of biofilms were prepared (all measurements based on 100 ml of water):

Sample 1 (α -Cellulose Biofilm): A biofilm with 2.93 g of papaya leaf-derived α -cellulose and 7.07 g of starch.

Sample 2 (Starch Biofilm – Control): A biofilm with 10 g of starch content.

Sample 3 (Papaya Leaf Extract Biofilm): A biofilm with 10 g of ground papaya leaf and 5 g of starch.

All the biofilms obtained were following a ratio of 2 [cellulose + starch] : 1 [gelatin] : 1 [glycerol]

2.5 Characterization Tests

The biofilms were subjected to eight distinct tests to evaluate their properties: (1) degradation in water, (2) soil burial degradation (micro-scale), (3) gas production inhibition (volumetric analysis), (4) vertical hanging test (tensile strength), (5) oil resistance (grease permeability), (6) opacity and UV-shielding, (7) surface morphology (microscopy), and (8) swelling ratio.

III. RESULTS AND DISCUSSION

The shelf life of all three films was observed to be more than one month under dry conditions.

3.1 Degradation in Water

Biofilm samples were cut into 5 cm × 5 cm squares and placed in beakers containing 250 ml of pond water at constant room temperature without agitation. The time taken for complete dissolution was recorded. The α -cellulose biofilm took 3 days to degrade completely in water. During this period, the soil in the water settled and the water turned less clear due to the dissolved biofilm. The starch film took 4 days for degradation, while the papaya leaf extract biofilm took 2 days to degrade in water.



Figure 3.1.1: Biofilm samples in pond water on Day 1 — intact squares.



Figure 3.1.2: Biofilm samples on Day 2 — partial dissolution visible; papaya leaf extract film fully degraded.



Figure 3.1.3: Biofilm samples on Day 3 — α -cellulose film fully dissolved; starch film persisting.

3.2 Soil Burial Degradation (Micro-Scale)

Samples were cut into 2 cm diameter disks and buried at a depth of 2 cm from the surface in a 250 ml beaker filled with moist soil. Moisture was maintained daily. Samples were retrieved each day and gently cleaned with a soft brush. The α -cellulose biofilm took 4 days to degrade in soil with maintained moisture, demonstrating that these films can be decomposed in home gardens. The papaya peel extract film took 3 days for degradation, while the starch film took 5 days.



Figure 3.2.1: Biofilm disks after Day 1 of soil burial — surface still largely intact.



Figure 3.2.2: Biofilm disks after Day 2 — visible surface erosion and fragmentation.



Figure 3.2.3: Biofilm disks after Day 3 — papaya leaf extract film fully degraded; others partially decomposed.

3.3 Gas Production Inhibition (Volumetric Analysis)

5% w/v sugar solution was prepared in 15 ml of water and small amount of yeast was added to it. 1.5 cm x 2 cm of each sample were added to the test tubes with the solution. Balloons were secured on the top of the test tubes, and the set up was left for 2 hours.

The α -cellulose biofilm showed the highest inhibition of yeast fermentation due to its dense and uniform structure. The starch film (control) showcased least inhibition to microbial activities because of its porous and weak matrix. The papaya leaf extract film showed moderate inhibition due to both structural and antimicrobial properties.

3.4 Vertical Hanging Test (Tensile Strength)

Strips (1 cm × 5 cm) were attached to a clamp stand and loaded with increasing metal mass until breakage occurred. Tensile strength (σ) was calculated using the formula $\sigma = F/A$, where $F = mg$ (force at breaking point) and $A = \text{width} \times \text{thickness}$ (cross-sectional area).

Biofilm Sample	Mass (kg)	Force F (N)	Thickness (mm)	Area A (mm ²)	σ (MPa)
α -Cellulose Biofilm	0.35	3.430	0.025	0.025	137.2
Starch Biofilm	0.225	2.205	0.025	0.025	88.2
Papaya Leaf Extract	0.125	1.225	0.0125	0.0125	98.0

Table 1: Tensile Strength of Biofilm Samples

The α -cellulose biofilm exhibited the highest tensile strength (137.2 MPa), significantly outperforming the other samples, indicating superior structural bonding and load-bearing capacity. The papaya leaf extract film's tensile strength (98.0 MPa) was higher than that of the starch biofilm (88.2 MPa), proving that the papaya extract biofilm is a stronger material relative to its thickness. Thus, the introduction of cellulose in biofilms can raise their tensile strength.



Figure 3.4.1: Vertical hanging test setup — biofilm strip (1 cm × 5 cm) clamped to stand with weights.



Figure 3.4.2: Vertical hanging test — biofilm strip at breaking point under applied mass.

3.5 Oil Resistance (Grease Permeability)

Film squares (2 cm × 2 cm) were placed on clean white filter paper, and 0.5 ml of edible oil or liquid paraffin was applied to the centre. The setup was left undisturbed for 24 hours at room temperature, after which the underlying filter paper was examined for oil stains or penetration. After 24 hours, oil penetration marks were observed only on the filter paper with the papaya leaf extract. The absence of stains on the underlying filter paper in both the α -cellulose and starch biofilms indicates that these materials have a dense, non-porous structure that successfully prevents the migration of edible oil or liquid paraffin. Although the papaya leaf extract film failed to act as a complete barrier, it can be used in oil blotting skincare and for items that need to breathe to prevent moisture buildup.



Figure 3.5.1: Filter paper appearance at 0 hours — no oil penetration observed.

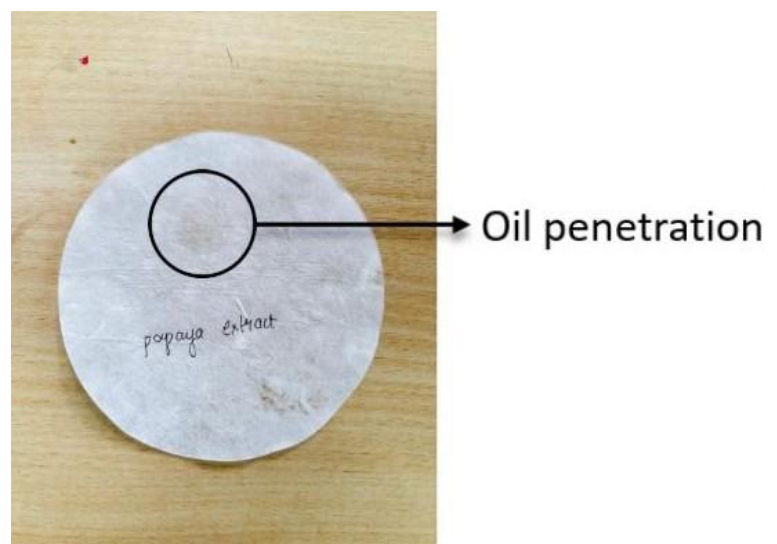


Figure 3.5.2: Filter paper after 24 hours — oil penetration visible only beneath the papaya leaf extract film; α -cellulose and starch films show no staining.

3.6 Opacity and UV-Shielding

Film squares (approximately 5 cm × 5 cm) were placed directly over a lux meter sensor. The light intensity of a fixed source (L_0) was measured, followed by the light intensity through the film (L_i). Opacity (O) was calculated as $O = (1 - L_i/L_0) \times 100$.

Biofilm Sample	Incident Light (lx)	Transmitted Light (lx)	Opacity (%)
α -Cellulose Biofilm	362	51	85.91
Starch Biofilm	362	95	73.75
Papaya Leaf Extract	362	22	93.92

Table 2: Opacity and UV-Shielding Properties of Biofilm Samples

The papaya leaf extract film exhibited the highest opacity (93.92%), making it the most suitable candidate for shielding light-sensitive products like Phytonadione (Vitamin K). The starch film showed the least opacity (73.75%), making it least suitable for products of medical grade that require UV shielding. The opacity of the α -cellulose biofilm (85.91%) makes it suitable for outer packaging that protects tablets like Furosemide or Hydrochlorothiazide that are sensitive but not instantly destroyed by ambient light.

3.7 Surface Morphology (Microscopy)

Thin sections (5 mm × 5 mm) were placed on clean glass slides without a coverslip and examined under a compound microscope at 10x and 20x magnification of the objective lens. The distribution of fibers and any microscopic cracks or air bubbles in the matrix were analyzed.

The α -cellulose biofilm showed uniform fiber distribution, a dense and stable matrix, minimal defects (cracks or bubbles), and an opaque-translucent appearance. The cornstarch biofilm showed non-uniform distribution, presence of cracks and voids, less compact structure, low structural stability, and a translucent appearance. The papaya leaf extract biofilm showed higher heterogeneity with clustered regions, presence of voids and minor cracks, less compact structure compared to the α -cellulose film, and an opaque-translucent appearance. These features explain its higher swelling ratio ($Q \approx 1.29$), as the loose structure and voids allow more water absorption but may reduce mechanical strength.

The α -cellulose biofilm has higher structural stability compared to the starch biofilm and papaya leaf extract biofilm. The papaya leaf extract biofilm has a moderately uniform structure, while the starch biofilm has the least structural stability.

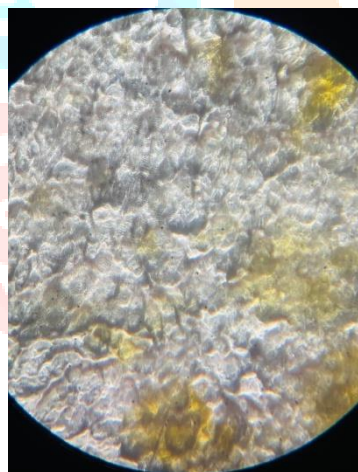


Figure 3.7.1: α -Cellulose biofilm under 10× magnification — uniform fiber distribution and dense matrix.

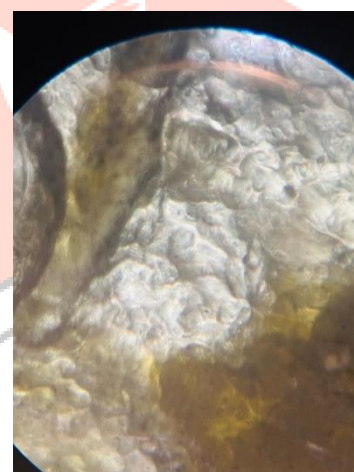


Figure 3.7.2: α -Cellulose biofilm under 20× magnification — minimal cracks or voids, opaque-translucent appearance.

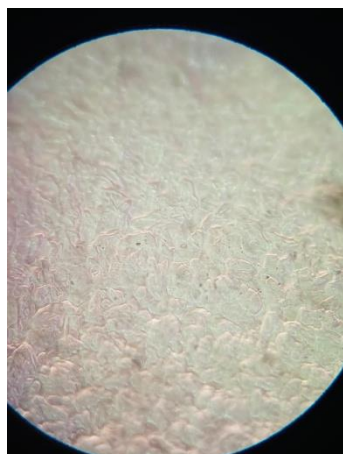


Figure 3.7.3: Starch biofilm under 10× magnification — non-uniform distribution with visible cracks and voids.

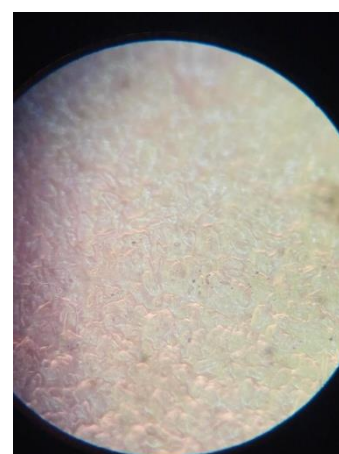


Figure 3.7.4: Starch biofilm under 20× magnification — less compact structure and low structural stability.

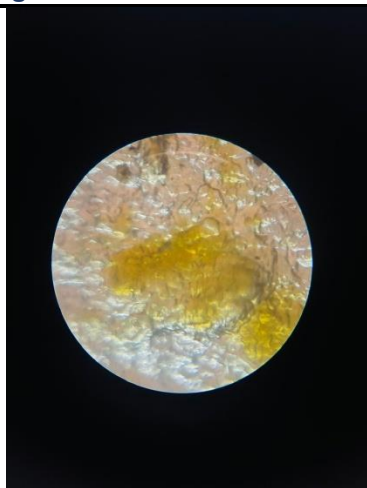


Figure 3.7.5: Papaya leaf extract biofilm under 10× magnification — heterogeneous surface with clustered regions.

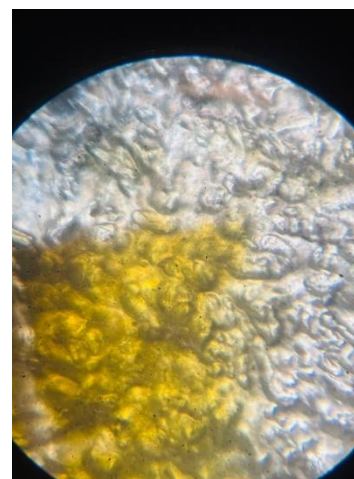


Figure 3.7.6: Papaya leaf extract biofilm under 20× magnification — voids and minor cracks consistent with higher swelling ratio.

3.8 Swelling Ratio

Film squares (4 cm × 4 cm) were weighed (W_d), immersed in distilled water for exactly 5 minutes, blotted to remove excess surface water, and reweighed (W_s). The swelling ratio (Q) was calculated as $Q = (W_s - W_d) / W_d$.

Biofilm Sample	Dry Weight W_d (g)	Swollen Weight W_s (g)	Swelling Ratio (Q)
α -Cellulose Biofilm	0.45	0.90	1.00
Starch Biofilm	0.60	1.20	1.00
Papaya Leaf Extract	0.51	1.17	1.29

Table 3: Swelling Ratio of Biofilm Samples

Property	α -Cellulose Biofilm	Papaya Leaf Extract	Starch Biofilm
Swelling Ratio (Q)	1.00	1.29	1.00
Water Absorption	Moderate	High	Moderate
Structural Stability	High	Medium (may soften)	Medium
Risk of Over-Swelling	Low	High	Low
Strength After Soaking	Good	Reduced	Fair

Table 4: Comparison of Biofilms Based on Swelling Ratio

The α -cellulose biofilm and starch biofilm both exhibited a swelling ratio of 1.00, indicating moderate and controlled water absorption. The papaya leaf extract biofilm showed the highest swelling ratio (1.29), suggesting higher water absorption due to its loose structure and voids. If high absorption is needed, the papaya leaf extract biofilm is best suited (e.g., biomedical materials). If strength and stability are important, the papaya leaf α -cellulose biofilm is best suited (e.g., packaging materials and protective films). If a moderate, low-cost option is needed, the starch biofilm is suitable.

IV. KEY FINDINGS

α -Cellulose Biofilm: It achieved the highest tensile strength (137.2 MPa) and the most uniform morphology. It demonstrated moderate UV shielding (85.91% opacity). Its highest tensile strength ensures that packaging will not break during transportation. A swelling ratio of 1.0 indicates that it can absorb a specific amount of wound exudate without losing its shape. The excellent oil resistance and dense morphology mean it acts as a high-quality barrier against contaminants, oils, and potentially airborne pathogens. It is suitable for wrapping IV bags or blister packs for drugs like Furosemide or Hydrochlorothiazide.

Papaya Leaf Extract Biofilm: It reached near-total opacity (93.92%), which is superior for blocking harmful rays that degrade chemicals. It demonstrated moderate tensile strength (98.0 MPa). With the highest swelling ratio, this film is superior for weeping wounds, as it can absorb more exudate than starch or cellulose, keeping the wound bed at an optimal moisture level for healing. Papaya leaves contain papain and chymopapain, enzymes known to help in debridement—the cleaning of dead tissue from a wound. It is a medical-grade candidate for secondary packaging of highly photosensitive injectables.

Starch Biofilm (Control): The starch biofilm served as the control and demonstrated the least performance across most parameters including tensile strength (88.2 MPa), opacity (73.75%), and antimicrobial inhibition. However, it remains a moderate, low-cost option for general applications.

V. CONCLUSION

Across the eight distinct tests performed on the three samples of biofilms, both the α -cellulose biofilm and papaya leaf extract biofilm showcased improved properties in comparison with the starch biofilm. This proves that integration of cellulose and plant pigments in biofilm could improve the quality of bioplastic in various ways. It can make the bioplastic stronger, increase its tensile strength, and add UV-shielding properties, making the bioplastic a good packaging material for dry materials.

Also, the specific use of papaya leaves has made the bioplastic further resistant to microbial growth due to its antimicrobial property. Further clinical tests and lab tests will help us determine that the bioplastics could be used in the medical field with certain improvements. Medical consumables made from this extract (like disposable tray liners, medicine cups, or protective sheets) would drastically reduce the carbon footprint of clinics compared to traditional PVC or PE plastics.

The study demonstrates the feasibility of valorizing agricultural leaf waste into sustainable, eco-friendly packaging and functional biomaterial alternatives to conventional petroleum-based plastics. Future work should focus on optimizing the cellulose-to-starch ratio, improving gelatin incorporation, and conducting advanced characterizations such as FTIR, XRD, and TGA analyses for potential industrial scale-up.

VI. ACKNOWLEDGMENT

The author acknowledges the support and guidance received during the course of this research work.

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