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DEVELOPMENT OF BIOFILM USING NATURAL PLANT SOURCES AUGMENTED WITH *PUNICA GRANATUM* PEEL EXTRACT

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Abstract

This abstract explores the potential of biofilms as innovative components of food packaging materials. Biofilms, comprised of beneficial microorganisms such as *lactic acid* bacteria, can be engineered to form bio-based coatings on packaging surfaces. These biofilms exhibit antimicrobial properties, effectively inhibiting the growth of pathogenic bacteria and extending the shelf life of packaged food products. Moreover, they create a protective barrier that reduces the risk of physical and chemical damage to the food. This abstract serves as a concise overview of the promising concept of using biofilms in food packaging materials, highlighting their sustainable, antimicrobial, and protective attributes. Here is the solution to combine the plant source and organic matter in the field of bioplastic which is a part of bio-textiles. The product of biofilm is developed with the *Punica granatum* peel extract augmented with the base ingredients to develop the biofilm is *Zea may* (Corn) Starch, Coconut oil, *Punica Granatum* peel essence, Sodium alginate, Glycerol, and Gelatine. Testing biofilms for use in food packaging involves various evaluations to ensure the safety, quality, and efficacy of the biofilm material. Standard testing was carried out as thickness, GSM, FTIR, and Microbial degradation. Further research and development in this area could revolutionize food preservation, reduce food waste, and promote an eco-friendly food packaging industry.

Keywords: Eco-friendly, Biofilm, *Zea may* starch, Antimicrobial, Biodegradable.

1. INTRODUCTION

The majority of people are aware of the environmental issues that plastic can bring about. Plastic can take millennia to break down. It may discharge dangerous poisons into the land and water during that period. Although a lot of individuals make an effort to limit their use of plastic, it nevertheless plays a big role in our daily lives. It's found in construction materials as well as packaging.

Food packaging plays a crucial role in preserving the quality and safety of food products. However, traditional packaging materials often contribute to environmental degradation and have limited capabilities in preventing spoilage and contamination. In response to these challenges, the integration of biofilms into food packaging materials is emerging as a promising and sustainable solution. Biofilms are structured communities of microorganisms that adhere to surfaces, and they offer several advantages for food packaging applications.

The use of biofilms in food packaging aligns with the global drive toward sustainability, as they are biodegradable, renewable, and can be produced from agricultural waste or byproducts. Additionally, their presence on packaging materials can reduce the need for synthetic preservatives and additives, contributing to cleaner and more natural food products. Furthermore, biofilm-coated packaging can offer improved barrier properties, enhancing the product's resistance to moisture, oxygen, and UV radiation. While biofilms in food packaging hold tremendous potential, there are challenges and considerations to be addressed, including the control of biofilm growth, potential allergenicity, and regulatory compliance.

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Zea may (Corn) Starch is a cheap, plentiful, and renewable natural biopolymer that is crucial for human nutrition. In addition, its main constituents include amylose and amylopectin. Over the past few years, starch has found widespread use in both the culinary and non-food industries.

*Cocos nucifera (*Coconut) oil is a plant scourse widly used in traditional medicines. Medium-chain fatty acids, such as lauric acid, decanoic acid, and octanoic acid, along with their corresponding medium-chain triglycerides, are abundant in coconut oil. It has antimicrobial and antioxidant qualities in addition to practical qualities like easy digestion and absorption. There are several varieties of coconut oil, such as virgin coconut oil (VCO), copra oil (CO), and coconut testa oil (CTO). Because VCO is not subjected to chemical or thermal processing, like CO and CTO are, bioactive substances including sterols, polyphenols, and vitamin E are preserved.[2]

*Punica Granatum (*pomegranate) **is** a good source of bioactive ingredients. The required fruit's biological activity is collected from pomegranate peel extract, which contains minerals, hydrolyzable tannins (gallic acid), flavonoids (anthocyanins), and phenolic acids. Because of their well-established chemical properties and ethno-medical relevance, the macromolecules present in pomegranate peel and peel extract have been suggested as alternatives to manufactured nutraceuticals, food additives, and chemo-preventive agents.[3]

Sodium Alginate or Alginate hydrogels, composed of L-guluronic acid and D-mannuronic acid residues, have been widely used in wound healing and drug delivery due to their mucoadhesive properties and biodegradability. Sodium alginate, a specific type of alginate, is derived from marine brown algae and is environmentally friendly, non-toxic, and biodegradable11. It forms hydrogels through the addition of Ca^{2+} ions and is resistant to the acidic environment of the gastrointestinal tract. In recent years, polyethylene glycerol, poly(lactic-co-glycolic) acid, polycaprolactone, polyvinyl alcohol, and chitosan are frequently used in biomedical applications due to their potent biodegradation features.[4]

Glycerol is a useful byproduct of the hydrolysis reaction, saponification, and transesterification processes used to produce biodiesel is glycerol. Due to contaminants such as water, soaps, salts, esters produced during the reaction, and leftover catalysts, the glycerol obtained has a low purity. A useful byproduct of the hydrolysis reaction, saponification, and transesterification processes used to produce biodiesel is glycerol. Due to contaminants such as water, soaps, salts, esters produced during the reaction, and leftover catalysts, the glycerol obtained has a low purity.[5]

Gelatine is a naturally occurring polymer derived from the hydrolytic breakdown of collagen protein. Its unique amino acid composition confers many health advantages. Gelatin is typically found in pills, granules, or powders, though it occasionally needs to be dissolved in water before usage. Researchers have been experimenting a lot with gelatin as a scaffold for tissue engineering and as a matrix for three-dimensional cell culture. The protein concentration of gelatin is high. Because it can gel, gelatin plays a crucial role in the food industry and in modern cuisine. Gelatin is a naturally occurring polymer derived from the hydrolytic breakdown of collagen protein. Its unique amino acid composition confers many health advantages.[6]

Testing biofilms for food packaging is a critical step in ensuring their safety, effectiveness, and sustainability. The specific tests conducted may vary depending on the biofilm's composition, intended use, and regulatory requirements.

Fourier-transform infrared spectroscopy (FTIR) is a powerful analytical technique used for testing and characterizing biomaterials. FTIR provides information about the chemical composition of materials by measuring the absorption of infrared radiation. Several standards are established for FTIR testing to ensure consistent and accurate results across different laboratories and applications.

Physical testing standards typically refer to established criteria or benchmarks that define the parameters, protocols, and expectations for evaluating various aspects of physical performance or characteristics. These standards are often developed and maintained by relevant organizations, regulatory bodies, or professional associations. The purpose of physical testing standards can vary depending on the context, such as fitness assessments, sports competitions, workplace evaluations, or medical examinations.

GSM testing standards, generally relate to testing and quality standards associated with Global System for Mobile Communications (GSM) technology. GSM is a widely used standard for mobile communication, particularly for voice and data services. Various organizations and standardization bodies have established specifications and guidelines to ensure interoperability, reliability, and performance in GSM networks and devices.

Microbial Degradation testing standards are used to assess the performance and durability of materials, products, or systems over time or under specific environmental conditions. These standards help ensure that products can withstand long-term

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use and exposure to various factors without significant deterioration. Different industries and applications may have specific degradation testing standards tailored to their needs.

2. MATERIALS AND METHODS

2.1 SELECTION AND COLLECTION OF PLANT SOURCES

2.1.1. *Zea may* (Corn) starch

Cornstarch is primarily used as a thickening agent. It's made up of a long chain of starch molecules that will unravel and swell when heated in the presence of moisture. This swelling, or gelatinization, is what causes thickening. The biofilms formed with improved tensile strength are biodegradable. The developed biofilms can be used as a packaging material for carrying household goods. Corn starch for this study was procured from Makkal ponmani hypermarket located in Coimbatore. it is commonly available in any market sector.

Fig. 1. *Zea may* (corn) starch

2.1.2. *Cocos Nucifera (*Coconut) oil

The medium-chain fatty acids in coconut oil have antimicrobial properties that can help protect against harmful microorganisms. The coconut oil reduced C. albicans fungus biofilm by 65.48% but a low eradication level was observed in the case of bacterial biofilms. The dehydrating mechanism of action of sulfonated phenolics turned out to be ineffective against streptococcal biofilm which in turn was effectively eradicated by silver nanoparticles. Coconut oil for this study was procured by natural extraction from pure dried copras which is commonly available in organic farms. The extraction process is discussed in detail below.

Fig.2. *Cocos nucifera* (coconut) oil

2.1.3. *Punica granatum* (pomegranate)

Pomegranate peel, which is frequently seen as agricultural waste, serves as a great source of antioxidants and a variety of phytochemicals. Gallo tannins, ellagic acid, punicalagin, punicalagin, and gallic acid are some of the major phytochemicals present in pomegranate peel. Methanolic extract of pomegranate was shown to inhibit the formation of biofilms by Staphylococcus aureus, methicillin-resistant S. aureus, Escherichia coli, and Candida albicans. All pomegranate peel extracts demonstrated selective antimicrobial activity against all pathogenic bacteria without affecting beneficial ones. Pomegranate for this study was procured from fresh juice shoppers. The extracted peel of pomegranate is dried in shadow dry.

Fig.3. *Punica granatum* (pomegranate) peel extract

2.1.4. Sodium alginate

Alginate serves to protect the bacteria from adversity in its surroundings and also enhances adhesion to solid surfaces. Transcription of the alginate biosynthetic genes is induced upon attachment to the substratum and this leads to increased alginate production. Sodium alginate for this study was procured from located in tiruppur. it is commonly available in laboratory or other chemical deparments.

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Fig.4. Sodium alginate

2.1.5. Glycerol

The impact of glycerol on biofilm formation is regulatory, not solely metabolic, because it is required for expression of numerous biofilm-associated genes. Restoration of expression of three of these genes that specify cell surface adhesins enables the glycerol-synthetic mutant to create a biofilm. glycerol metabolism promotes biofilm formation by both a chronic CF isolate (FRD1) and a wound isolate (PAO1) of P. aeruginosa. Glycerol for this study was procured from located in tiruppur. it is commonly available in laboratory or other chemical deparments.

Fig.4. Glycerol

2.1.6. Gelatine

Gelatin-based films are regarded as promising alternatives to non-environmentally friendly plastic films for food packaging. Nevertheless, although they have great biodegradability, their weak mechanical properties and high solubility limit their applications. . Sodium alginate for this study was procured from located in tiruppur. it is commonly available in laboratory or other chemical deparments.

Fig.6. Gelatine

2.2. EXTRACTION AND PREPARATION OF PLANT SOURCE

2.2.1. preparation of Corn Starch paste

Cornstarch, also known as cornflour, has been studied in the context of biofilms, particularly as a component in the development of biodegradable and edible films for various applications. Here's how cornstarch is used in biofilms:

Measure the Cornstarch: Determine the amount of cornstarch you need for your specific purpose. The ratio of cornstarch to water can vary depending on the desired consistency of the solution. A typical ratio is about 1 to 2 tablespoons of cornstarch for every cup of water. Adjust the amount based on your needs.

Cold Water: Use cold or room temperature water. Cold water helps prevent clumping when mixing with cornstarch.

Mix Cornstarch and Water: In a mixing bowl, add the measured cornstarch. Gradually add a small amount of cold water (about 1/4 cup) to the cornstarch. Stir or whisk well to create a smooth paste. Make sure the cornstarch is fully dissolved in a small amount of water before adding more.

Dilute the Paste: Gradually add the remaining cold water while stirring continuously. Continue to mix until the cornstarch paste is fully diluted in the water and the solution is smooth. If you notice any lumps or clumps, continue to stir until they are completely dissolved.

Desired Consistency: Adjust the amount of water or cornstarch to achieve your desired consistency. If you need a thicker solution, add more cornstarch. For a thinner solution, add more water.

Use or Store: Use the cornstarch solution as needed for your intended application. If you have a leftover solution, you can store it in a covered container in the refrigerator for a short period. Be aware that cornstarch solutions can thicken over time; you may need to stir or reheat it before using it again.

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2.2.2. Extraction of *Cocos Nucifera (*Coconut) Oil

Coconut oil is known for its potential antimicrobial properties, primarily attributed to its high content of lauric acid and capric acid, which have demonstrated antibacterial and antifungal activity. In the context of biofilms, coconut oil has been explored for its ability to inhibit biofilm formation and disrupt existing biofilms. Here are some ways in which coconut oil can be relevant to biofilms.

The extraction of coconut oil is a straightforward process that involves the removal of oil from coconut meat or copra (dried coconut kernels). There are different methods to extract coconut oil, but one of the most common and traditional methods is the expeller-pressed or cold-pressed method. Here is a simplified overview of the process:

Extraction Process: If using fresh coconut meat, start by grating or shredding the coconut to obtain small pieces. If using Copra, skip this step.

Drying (for Copra): If using Copra, ensure it is properly dried to reduce its moisture content. Drying can be done under the sun or with the use of dying equipment. Copra should have a moisture content of around 6-7% before extraction.

Feeding the Machine: The grated coconut meat or dried copra is fed into the expeller or cold-press machine. In the machine, the coconut meat/copra is subjected to mechanical pressure to release the oil.

Oil Extraction: The machine's screw or expeller applies pressure to the coconut meat/copra. This pressure causes the oil to be released from the cells within the coconut. The oil is then forced out of the machine through small openings.

Collection: The extracted coconut oil is collected in clean containers. It may contain some impurities and moisture, which can be removed through settling or filtration.

Filtering (Optional): The collected oil may be filtered to remove any remaining impurities, resulting in clearer and purer coconut oil.

Storage: Store the extracted coconut oil in clean, airtight containers to preserve its quality and freshness.

2.2.3. Extraction of *Punica Granatum* Peel Extract

Punica Granatum peel extract may have some potential as a natural antimicrobial agent for use in food packaging to inhibit biofilm formation and extend the shelf life of food products. Biofilms, which are communities of microorganisms that can form on food surfaces, can lead to food spoilage and safety concerns. Extracting pomegranate peel powder involves the process of obtaining the dried and powdered form of pomegranate peel, which is often used for various culinary and medicinal purposes. Here are the steps to extract pomegranate peel powder

Prepare Pomegranate Peels: Start by collecting pomegranate peels from fresh pomegranates. Use a knife and cutting board to carefully remove the outer peels. It's a good idea to remove any residual pomegranate arils and inner membranes from the peels.

Wash and Dry the Peels: Rinse the collected peels with water to remove any dirt or residue. Then, pat them dry with a clean kitchen towel or paper towels.

Drying the Peels: Preheat your oven to a low temperature, typically around 150-170°F (65-75°C). Spread the clean and dry pomegranate peels on a baking sheet in a single layer. Place the baking sheet in the preheated oven and leave the oven door slightly ajar to allow moisture to escape. Let the peels dry in the oven for several hours, or until they are thoroughly dried and crisp. The time required may vary based on the size and thickness of the peels. Grinding the Peels: Once the peels are completely dried, remove them from the oven and let them cool.4

Use a blender or grinder to process the dried peels into a fine powder. You may need to do this in batches, depending on the quantity of peels and the capacity of your equipment.

Sifting (Optional): After grinding, you can sift the powder through a sieve or fine mesh strainer to remove any larger or coarser particles, resulting in a finer powder.

Storage: Transfer the pomegranate peel powder into an airtight container to pre

serve its freshness and flavor. Store it in a cool, dark place away from moisture and direct sunlight.

2.3. PRODUCT DEVELOPMENT

2.3.1. raw materials required

1) Pomegranate peel extract (powder) 5g
2) Coconut oil 5g
3) Gelatine 2g
4) Sodium alginate 12g
5) Glycerol 3g
6) Corn starch 2g
7) Water 200ml

2.3.2. Equipment required

1. Saucepan
2. Spoon
3. Stove
4. Tray pad

2.3.3. Preparatory Process of Biofilm

I. Fraternization of extracted and prepared plant sources

1. Take 200ml of water in a sauce pan and heat in the gas stove at low flame temperature.

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2. Then add 12g of sodium alginate and 5g of pomegranate peel extract powder, and stir the paste in a gentle process.
3. After mixing the consistency then add 2g of gelatine, 2g of corn starch, and 3g of glycerol.
4. Stir it gently until it becomes semi-solid consistency.
5. Cool it at room temperature and keep it ready for the spreading process.

Fig.7. Mixing of raw material

II. Spreading

1. Once the paste is cooled, spread it on the tray pad by applying coconut oil for non-stickiness.
2. Spread the paste evenly to the finest filmy texture.
3. Then keep it ready for the next process.

Fig.8. Spread out biofilm material

III. Drying and releasing

1. After the spreading process allow the paste to dry in sunlight.
2. Dry it well for 24 hrs.
3. After 24 hours, remove the film from the tray carefully.
4. Check for a smooth and filmy layer of biofilm.

Fig.9. Dried biofilm material

IV. Final package biofilm material

1. After the removal process cut the film material with neat finishing edges for a great look appearance.
2. Then use these films for food packages like rolls and sheet covers.

(a) (b) (c)

Fig.10. Final Product- Biofilm material.

The given image represents the different colors of biofilm material dyed with natural dyes. They are dyed with (a) yellow, (b) red and (c) green food color natural dyes.

2.4 TESTING OF DEVELOPED PRODUCT

2.4.1. Thickness Test

Measuring the thickness of a biofilm is important for various research and industrial applications, as it can provide insights into the growth and development of biofilms. There are several methods to measure the thickness of a biofilm, and the choice of method depends on the specific requirements and characteristics of the biofilm. Here are some common techniques

Fig.11. Screw gauge for Thickness test

2.4.2. GSM Test

The GSM (Gram Stain Method) is a common microbiological staining technique used to categorize bacteria based on their cell wall characteristics. It is not a method for directly testing or quantifying biofilms. However, you can use GSM in combination with other techniques to gain insights into the composition and structure of biofilms Here's how you can incorporate GSM into your analysis of the biofilm

(a) (b) (c)

Fig.12.(a) GSM cutter, (b)Cutted Biofilm sample and (c)Weighing machine

2.4.3. FTIR Test

Fourier-transform infrared (FTIR) spectroscopy is a powerful analytical technique used to identify and analyze the chemical composition of various substances, including antimicrobial compounds. It can be employed to study the

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interaction between antimicrobial agents and microbial cells, providing valuable insights into the mechanism of action. FTIR spectroscopy allows for the real-time monitoring of microbial responses to antimicrobial agents

2.4.4. Microbial Degradation Test

Testing the microbial efficacy of substances against biofilms is important in various fields, including medicine, microbiology, and environmental science. Biofilms are complex communities of microorganisms encased in a protective extracellular matrix, making them more resistant to microbial agents compared to free-floating (planktonic) bacteria. Here are some commonly used methods for conducting antimicrobial tests on biofilms:

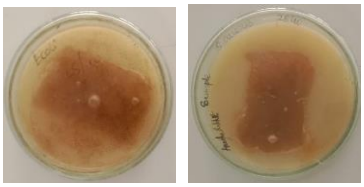

Fig.13. Microbial degradation analysis (a)*E.Coli* &, (b)*S.aureus*

3. RESULTS AND DISCUSSION

3.2. PHYSICAL TEST ANALYSIS

3.2.1. Thickness Test (ASTM D6988)

Table 1: Evaluation of biofilm thickness

S.No	Particulars	Thickness (mm)
1.	Sample 1	1.40mm
2.	Sample 2	1.37mm
3.	Sample 3	1.34mm

The given measurement is calculated by the readings of the main scale and circular scale of the screw gauge. The total reading of the main scale and circular scale is represented as the thickness of the given sample.

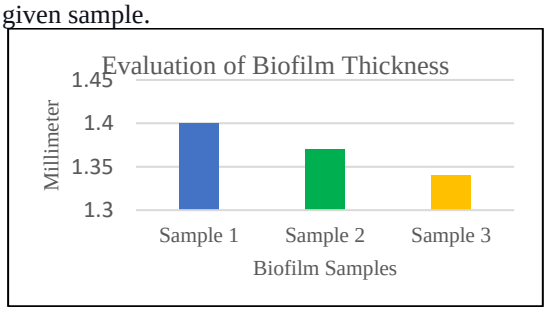

Fig.14. Evaluation of biofilm thickness

In the given table and figure the values of thickness of biofilm are found to be sample 1 = 1.40mm, sample 2 = 1.37mm, and sample 3 = 1.34mm which are evaluated in a bar graph.

3.1.2. GSM Test Analysis

The given measurement is calculated by the readings of the weighting balance machine with samples 1,2, and 3. Thus the readings are also represented in graphical representation.

Table 2: Evaluation of biofilm GSM

S.No	Particulars	Weight in GSM (gms)
1.	Sample 1	11.03g
2.	Sample 2	14.68g
3.	Sample 3	12.75g

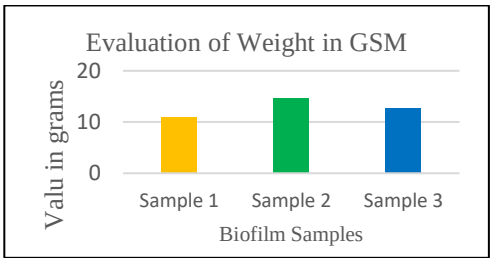

Fig.15. Evaluation of biofilm in GSM

The given table represents the weighted values of biofilm material found to be sample 1 = 11.03g, sample 2 = 14.68g, and sample 3 = 12.75g which are evaluated in a bar graph.

3.1.3. FTIR Test Analysis (ASTM D267)

The given Specimen shows 15 Peak are present. Peaks are representing the Active sites or Active components are present.

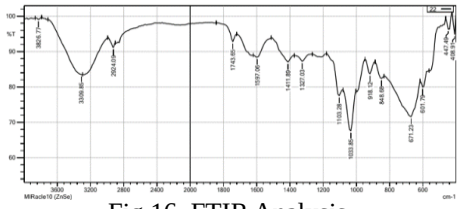

Fig.16. FTIR Analysis

Table 3: Analysis of FTIR

S.no	Peak readings	Chemical components
1	1743.65	C=O stretching - Ester
2	1411.89	- COO symmetric stretching
3	1327.03	Nitro groups
4	1103.28	C-N Stretch -Aliphatic amin
5	1033.88	C=O Stretch
6	918.12	C-H Alkenes
7	671.231	-C (Triple bond) C-H:C-H bend stretching vibration presence of alkynes
8	601.79	-C (Triple bond) C-H:C-H bend stretching vibration presence of alkynes

The above table and fig of FTIR test analysis show 8 different active peak readings in the test results. The active peaks are 1743.65 showing

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the presence of C=O stretching-Ester, 1411.89 peak showing COO stretch, 1327.03 shows Nitro groups, 1033.88 shows the highest peak ie., the presence of Aliphatic amin, 918.12, 671.231 infers C-H Alkenes, and 601.79 are to be found as fingerprint peaks in the evaluated result.

3.1.4. Microbial Degradation Test Analysis (ASTM D6691-17)

In this microbial degradation analysis, the activities of microbes like *E.Coli* and *S.aureus* have proved that the decomposition of biofilm occurs naturally. It helps nature from pollution and provides sustainability.

This growth can be concluded that the microbial activities can degrade the biofilm material after 24 hrs. we can assume that the food safety measure can be controlled by the expiration of 1 day.

This growth can be concluded that the microbial activities can degrade the biofilm material after 24 hrs. we can assume that the food safety measure can be controlled by the expiration of 1 day.

Table 4: Analysis of Biofilm Degradation

S.No.	Particulars (Analyse for microbial soil degradation at time intervals)	*E.Coli (degradation %)*	*S.aureus (degradation %)*
1	After 3 hours	5-15	5-10
2	After 6 hours	15-25	10-25
3	After 9 hours	25-40	25-35
4	After 12 hours	40-65	35-45
5	After 15 hours	65-75	45-55
6	After 18 hours	75-85	55-65
7	After 21 hours	86-90	65-85
8	After 24 hours	90-97	85-90

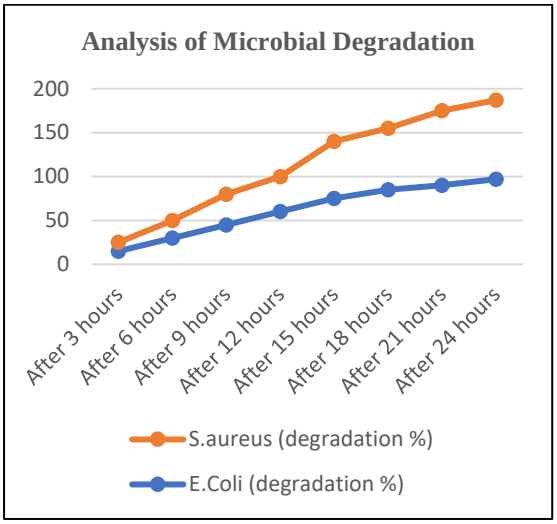

Fig 17: Analysis of Biofilm Degradation

The above table and fig of biofilm degradation show the growth of microbe E.Coli is higher than the growth of S.aureus growth. From the observation**,** the result found that the sample had an activity of *E. coli* and *S. aureus.* The result shows the given biofilm material has microbial activity for degradation

4. CONCLUSION

The food packaging biofilm sheet is prepared with the herbal finish of pomegranate peel extract and other plant sources of sodium alginate, gelatine, corn starch, coconut oil, and glycerol. This biofilm acts as an eco-friendly and sustainable product. It is tested with both physical tests and chemical tests. Also produced positive test results. It also has protective benefits.

The concluded result of the evaluated thickness results with the finest thickness of range between 1.35mm – 1.40mm. The final result of weighted GSM evaluated with biofilm material resulted in 11-15g. The FTIR test resulted in 8 highest peaks of chemical composition present in biofilm material. Finally, the microbial degradation test also resulted in per-day usage of expiration. All these results made this biomaterial with successful product.

Thus, the scope of this study aim to provide comprehensive insights into the development, efficacy safety, and sustainability of natural ingredients by traditional methods. It aims to provide solutions that enhance food safety, extend shelf life, reduce environmental impact, and improve the overall quality of food packaging. Additionally, it considers the potential of biofilm-based packaging to address the growing concerns about single-use plastics and environmental sustainability in the food industry.

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