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Development of Biodegradable Starch-Based Edible Films Enhanced with Periwinkle Extracts for Sustainable Food Packaging

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Abstract
The increasing demand for sustainable and biodegradable packaging materials has driven the development of edible films with antimicrobial properties. This study explores the potential of potato starch-based edible films incorporated with periwinkle (Catharanthus roseus) extract as a natural antimicrobial agent. The antimicrobial activity of the periwinkle extract was evaluated using the Agar Well Diffusion Method against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. The results indicated a concentration-dependent inhibitory effect, with the highest inhibition observed at 100 µg/mL. Further identification of these compounds was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), which provided insights into the specific antimicrobial components present in periwinkle extract. The optimal antimicrobial concentration was then incorporated into potato starch-based films, which were characterized for physicochemical properties (moisture content, solubility), mechanical properties (tensile strength, flexibility), and antimicrobial efficacy in food packaging applications. The results demonstrated that the developed films exhibited promising antimicrobial activity and desirable mechanical properties, making them a viable alternative to conventional plastic packaging. This study highlights the potential of periwinkle extract as a natural antimicrobial additive in biodegradable edible films, contributing to sustainable food packaging solutions.

Keywords: *Antimicrobial Activity; Bioactive Compounds; Biodegradable Packaging; Periwinkle Extract; Sustainable Edible Films*

1. Introduction
The increasing global concern over plastic pollution has intensified the search for sustainable alternatives to conventional food packaging. One promising solution is the development of biodegradable edible films, which are thin layers of biopolymer material that serve as a protective barrier between food and external contaminants while being safe for consumption or natural degradation [1]. These films are typically composed of natural polymers such as polysaccharides, proteins, and lipids, which provide mechanical stability and protective properties [2]. Among the various biopolymers used for edible film production, potato starch has emerged as a highly promising candidate due to its renewable nature, biodegradability, and excellent film-forming ability [3]. However, to enhance the functional properties of edible films, researchers are incorporating bioactive plant extracts with antimicrobial and antioxidant properties [4]. One such plant with significant antimicrobial potential is periwinkle (Catharanthus roseus), a well-known medicinal plant recognized for its bioactive compounds that exhibit antimicrobial activity [5]. By integrating periwinkle extract into potato starch-based edible films, the resulting packaging material can actively inhibit the growth of foodborne pathogens, thereby extending food shelf life and improving food safety. This study aims to develop a sustainable edible film composed of potato starch, glycerol, water, and periwinkle extract, with a primary focus on its antimicrobial efficacy against

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three major foodborne pathogens responsible for contamination and food spoilage. Previous studies have demonstrated the broad-spectrum antimicrobial activity of periwinkle extract due to its rich phytochemical composition, including alkaloids such as vincristine and vinblastine, flavonoids, saponins, and terpenoids. These bioactive compounds disrupt bacterial cell structures and inhibit essential metabolic processes, making them effective against common foodborne pathogens [6]. The antimicrobial properties of Catharanthus roseus have been reported against bacteria commonly associated with food contamination, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. To extract the bioactive antimicrobial components, the Soxhlet extraction method was employed using methanol as the solvent, followed by various chromatographic techniques to isolate the specific compounds responsible for antimicrobial properties. The antimicrobial activity of Catharanthus roseus extract was evaluated using the agar well diffusion method at different concentrations (25, 50, 75, and 100 µg/mL). The results were compared against negative controls (solvent used) and positive controls (ampicillin) to assess the efficacy of the extract. These antimicrobial components were further incorporated into potato starch-based edible films to enhance their stability and antibacterial properties. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify the specific bioactive compounds responsible for antimicrobial activity. Various tests were conducted to evaluate the physicomechanical and antimicrobial efficacy of the developed edible films, ensuring their potential as an eco-friendly and functional alternative to conventional plastic-based food packaging.This research explores the potential of periwinkle extract-enhanced starch-based edible films as an innovative approach to sustainable food packaging, with an emphasis on antimicrobial efficacy, biodegradability and food safety enhancement.

2. Method

The materials used for the fabrication of edible films and the extraction of antimicrobial compounds included potato starch, water, glycerol, periwinkle (*Catharanthus roseus*) extract and various solvents. Potato starch (1 g) was selected as the primary biopolymer due to its biodegradability, film-forming ability, and suitability for food applications. Water (10 mL) served as the solvent, ensuring the uniform dispersion of starch and other additives during film preparation. Glycerol (3 mL) was incorporated as a plasticizer to enhance the flexibility and mechanical strength of the film, preventing brittleness. Periwinkle extract was added as an antimicrobial agent, as it contains bioactive alkaloids and flavonoids known for their antibacterial properties.The extraction of bioactive compounds from *Catharanthus roseus* leaves was carried out using the Soxhlet extraction method, followed by purification and identification through chromatography and GC-MS analysis. To extract and purify these bioactive compounds from periwinkle leaves, methanol, ethyl alcohol, and chloroform were employed as solvents, aiding in the dissolution of plant metabolites for further chromatographic analysis.

2.1.Plant Sample Preparation

Fresh leaves, stem and roots were collected, thoroughly washed, and air-dried at room temperature to remove moisture. Once dried, the leaves were ground into a fine powder using a mechanical grinder to facilitate the extraction process.

2.2.Soxhlet Extraction

Figure 1 Powdered periwinkle sample

Soxhlet extraction is a widely used technique for extracting bioactive compounds from plant materials using a continuous solvent circulation process [7].In this method, 100g of dried and powdered plant material (e.g., *Catharanthus roseus* leaves) is placed

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in a porous thimble within the Soxhlet apparatus where methanol is used as athe organic solvent for continuous extraction at 60°C for 6 hours. The obtained extract was filtered using Whatman No.1 filter paper..The collected extract is then concentrated by evaporating leaving behind the crude bioactive compounds for further purification and analysis.

Figure 2 Soxhlet Extraction

2.3. Separation of Components Using Chromatography

The extracted bioactive compounds from *Catharanthus roseus* were further purified and analyzed using chromatographic techniques to isolate the key antimicrobial components. These techniques included Column Chromatography and Thin-Layer Chromatography (TLC) to separate and identify the bioactive fractions efficiently.

2.3.1. Column Chromatography

The column was packed with silica gel, which served as the stationary phase, while a solvent system of ethyl alcohol and chloroform acted as the mobile phase. The crude extract was carefully loaded onto the top of the column, and the solvent was gradually passed through, allowing different compounds to elute at varying rates based on their polarity and interaction with the stationary phase. The collected fractions were then analyzed for their antimicrobial activity to identify the most potent bioactive components.

2.3.2. Thin-Layer Chromatography (TLC)

A TLC plate coated with silica gel was spotted with small amounts of the collected fractions from column chromatography as the solvent ascended the plate by capillary action, the different compounds migrated at different rates, forming distinct spots. The separated compounds were visualized under UV light. The Retention Factor (Rf values) of the spots were calculated to determine the number and identity of components present in the extract. This process ensured that only the most bioactive fractions were selected for further characterization using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the antimicrobial compounds responsible for enhancing the edible film's protective properties.

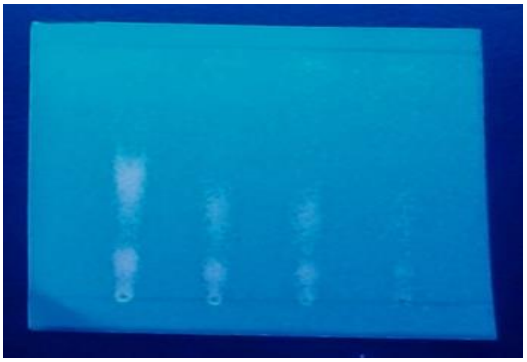

Figure 3 Identification of components

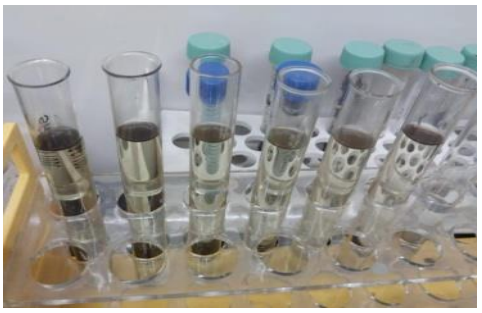

Figure 4 Column Chromatography Fractions

2.4. Antimicrobial Activity Testing Using Well Diffusion Method

The antimicrobial activity of the prepared sample was evaluated using the agar well diffusion method to determine its inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This method was performed at different concentrations of the test sample (25, 50, 75, and 100 µg/mL) to assess its antimicrobial efficacy and establish the optimal dosage for incorporation into the edible films. Ampicillin was used as a positive control, while a negative control containing the

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solvent used for sample preparation was included to ensure accurate interpretation of the results.

2.4.1. Media Preparation and Bacterial Culture Growth

Muller Hinton Agar (MHA) was prepared by dissolving 21 g of MHA in 1000 mL of distilled water, with a proportionate calculation for smaller volumes. Agar (3 g) was added to solidify the media, which was then sterilized and poured into sterile petri plates. Nutrient broth was prepared using 13 g per 1000 mL of distilled water, and bacterial cultures were grown in this medium at 37°C for 24 hours to ensure optimal bacterial proliferation.

2.4.2. 2.4.2 Inoculation and Well Diffusion Method for Antimicrobial Testing

After bacterial incubation, 100 µL of the bacterial suspension was evenly spread onto MHA plates using a sterile cotton swab to ensure uniform distribution. Wells of 6 mm diameter were created in the agar using a sterile well borer, ensuring adequate spacing between wells to prevent overlapping of inhibition zones. The test sample was loaded into the wells at different concentrations (25, 50, 75, and 100 µg/mL), while 50 µL of 10 µg/mL Ampicillin was used as a positive control, and 50 µL of the solvent served as a negative control. The plates were incubated at 37°C for 24 hours in an inverted position to prevent condensation interference.

2.4.3. Measurement and Analysis of Antimicrobial Activity

Following incubation, the plates were examined for zones of inhibition—clear areas around the wells indicating bacterial growth inhibition. The diameter of each inhibition zone was measured using a ruler or digital caliper to quantify the antimicrobial effectiveness of the test sample. These results were further analyzed to identify the optimal concentration of periwinkle extract for incorporation into starch-based edible films and the bioactive compounds responsible for antimicrobial effects are determined by GCMS.

2.5. Preparation of Starch Based Edible Films

After identifying the presence of antimicrobial compounds in prepared sample, the preparation of biodegradable starch-based edible films involved a series of steps, including film solution preparation, film casting, and drying, to ensure the formation of a flexible, uniform, and antimicrobial film suitable for food packaging applications. Initially, 1 g of potato starch was accurately weighed and dispersed in 10 mL of distilled water, with continuous stirring at room temperature to prevent clumping.

The mixture was then heated to 75–80°C until the starch was fully gelatinized, forming a viscous film-forming solution. To enhance film flexibility and reduce brittleness, 3 mL of glycerol was added as a plasticizer, ensuring its uniform incorporation into the starch matrix through continuous stirring.For antimicrobial functionality, different concentrations of *Catharanthus roseus* (periwinkle) extract were prepared and gradually introduced into the gelatinized starch solution while stirring. This process ensured a homogeneous distribution of bioactive compounds, with additional stirring for 20 minutes to achieve uniform dispersion. The prepared film-forming solution was then carefully poured into sterile petri dishes and spread evenly to maintain consistent thickness and prevent bubble formation. The drying process was carried out in a sun dried environment at 35–37°C for 24–48 hours, ensuring complete moisture removal while preventing cracks in the final film.

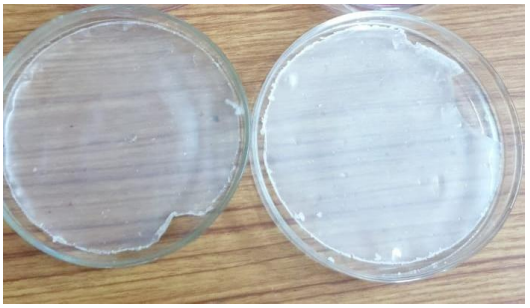

Figure 5 Casting of Film

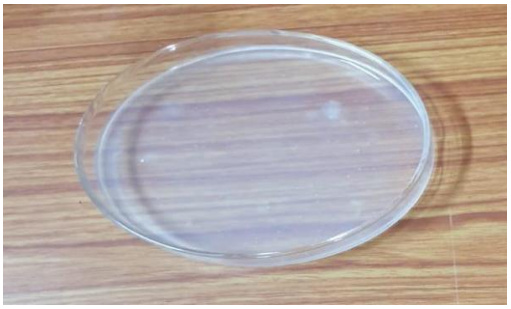

Figure 6 Drying of Film

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2.6. Film Characterization

To assess the functionality and effectiveness of the developed starch-based edible films, various characterization techniques were employed. Physicochemical properties, including moisture content, solubility, and water vapor permeability, were analyzed to determine the film's stability and barrier performance. Mechanical properties, such as tensile strength and elongation at break, were evaluated to ensure the film's flexibility and durability.

3. Results and Discussion (12 Pt)

3.1. Result Interpretation of Antimicrobial Activity from Agar Well Diffusion Assay

The antimicrobial activity of the test sample was evaluated using the agar well diffusion assay against *Escherichia coli*, *Staphylococcus* sp., and *Pseudomonas* sp. at different concentrations (25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL). The formation of clear inhibition zones around the wells indicated antimicrobial activity, with the zone diameter increasing proportionally with sample concentration. The negative control exhibited no inhibition, confirming that the solvent alone did not contribute to antimicrobial activity. For *E. coli*, inhibition zones were observed at all tested concentrations, with the largest inhibition zone recorded at 100 µg/mL, indicating a strong antimicrobial effect. A similar trend was observed for *Staphylococcus* sp., where inhibition zones increased in size with increasing concentration, suggesting effective antimicrobial activity against Gram-positive bacteria. Additionally, *Pseudomonas* sp., which is known for its intrinsic resistance to many antimicrobial agents, exhibited inhibition zones at all concentrations, demonstrating the test sample's efficacy against this strain. These findings suggest that the test sample exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial effect was dose-dependent, with the highest concentration (100 µg/mL) producing the most significant inhibition. The absence of inhibition in the negative control further confirmed that the observed effects were due to the active compounds in the test sample rather than the solvent. These results indicate the potential of the test sample as an effective antimicrobial agent for further investigation.

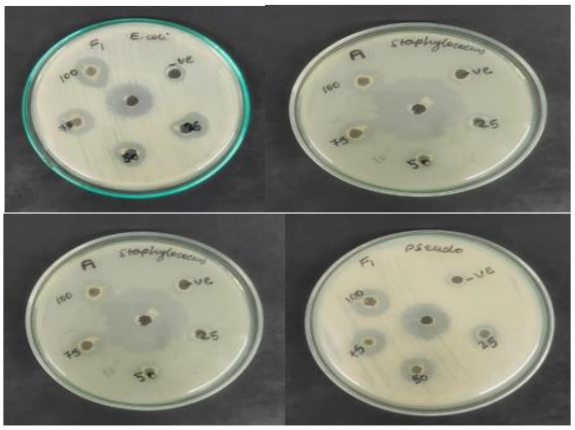

Figure 7 Inhibition Zones

The above images Figure 7 shows clear inhibition zones at all tested concentrations and confirms that the sample has great antimicrobial effect against *Escherichia coli*, *Staphylococcus* sp., and *Pseudomonas* sp at highest concentration of 100 µg/mL.

3.2. GC – MS ANALYSIS

Gas Chromatography Mass Spectroscopy was performed to analyse the compounds responsible for antimicrobial properties and listed below.

Table 1 GC-MS Analysis for Selected Bioactive Compounds

S.NO	COMPOUND NAME	NATURE OF THE COMPOUND	RETENTION TIME	PEAK AREA	POSSIBLE BIOLOGICAL ROLES
1.	1-Heptadecamine	Alkylamines	23.294	0.44%	Anti-microbial agent, Hormone precursor
2.	6-Dodoecanol acetate	Alcohols and Polyols	25.27	0.31%	Anti-microbial agent, Emollient
3.	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	Indole alkaloid derivative	44.175	0.06%	Potent Antimicrobial activity, Neurotransmitter precursor, metabolic regulation
4.	Methyl oleate	Fatty Acids and esters	30.075	0.60%	anti-bacterial, anti-inflammatory, skin permeation enhancer
5.	Hexadecanoic acid, Methyl ester	Fatty Acids and esters	27.09	1.08%	anti-microbial, anti-inflammatory effects
6.	Nonanoic acid-TMS	Organic acids	42.392	0.31%	antibacterial agent
7.	Tridecanoic acid	Fatty Acids and esters	24.165	0.20%	anti-microbial, flavoring agent
`8.	Dodecanoic acid,3-hydroxy-	Organic acids	24.385	0.23%	antibacterial, antifungal, anti-cancer property
9.	Palmitic acid, TMS derivative	Fatty Acids and esters	29.413	6.50%	antimicrobial agent, emollient
10.	Methyl palmitoleate	Fatty Acids and esters	26.177	0.33%	anti-microbial, skin protectant
11.	Octadecanoic acid	Fatty Acids and esters	31.961	4.08%	antimicrobial agent, skin conditoning
12.	n-Hexadecanoic acid	Organic acids	28.437	19.31%	Anti-microbial , Anti-inflammatory effect

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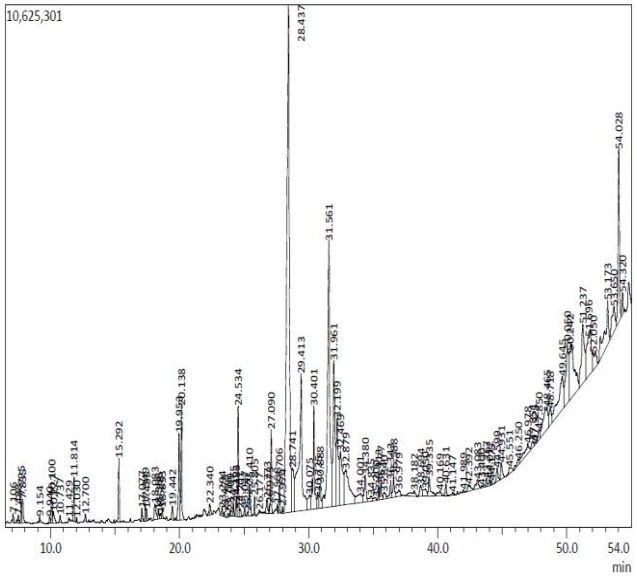

Figure 8 Chromatogram for Selected Bioactive Compounds

The GC-MS analysis of the test sample identified various bioactive compounds, including alkylamines, alcohols, fatty acids, esters, and organic acids, with potential antimicrobial, anti-inflammatory, and other biological properties. The retention times, peak areas, and possible biological roles of the detected compounds are summarized in Table 1. Among the identified compounds, *n-Hexadecanoic acid* exhibited the highest peak area (19.31%), suggesting its significant presence in the sample. This compound is known for its antimicrobial and anti-inflammatory properties, which may contribute to the overall bioactivity of the test sample. Similarly, *Palmitic acid, TMS derivative* (6.50%) and *Octadecanoic acid* (4.08%) were detected in notable quantities, both of which are recognized for their antimicrobial and skin-conditioning effects. The presence of these bioactive compounds suggests that the test sample possesses significant antimicrobial and anti-inflammatory properties. The dominance of fatty acids and their derivatives, along with the presence of indole alkaloids and organic acids, indicates the potential of this extract for pharmaceutical, cosmetic, and therapeutic applications. These findings support further investigation into the biological activity of the identified compounds and their synergistic effects in antimicrobial applications.

3.3. Film Characterization Results

Table 2 Characterization of Potato Starch-Based Edible Films with and without Periwinkle Extract

Parameter	Control Film (Without Extract)	Film with Periwinkle Extract (100 µg/mL)
Thickness (mm)	0.125 ± 0.005	0.128 ± 0.004
Moisture Content (%)	14.2 ± 0.3	12.7 ± 0.4
Water Solubility (%)	28.5 ± 1.2	24.6 ± 1.0
Tensile Strength (MPa)	15.3 ± 0.6	17.1 ± 0.5
Elongation at Break (%)	22.8 ± 1.1	19.5 ± 0.9
Transparency (Abs_{600})	1.12 ± 0.02	1.18 ± 0.03
pH	6.7 ± 0.1	6.5 ± 0.1

Conclusion

This study demonstrates the feasibility of developing biodegradable edible films from potato starch incorporated with *Catharanthus roseus* extract as an effective antimicrobial packaging material. The periwinkle extract exhibited strong, concentration-dependent antimicrobial activity against common foodborne pathogens, which was successfully retained upon integration into the starch film matrix. The films also maintained favorable physicochemical and mechanical properties, highlighting their practical applicability in real-world packaging scenarios. The use of GC-MS enabled the identification of active phytochemicals, lending scientific validation to the antimicrobial potential of the extract. Overall, the findings underscore the potential of plant-based bioactive films in addressing both environmental and food safety challenges. Future studies may focus on evaluating the films' long-term performance under storage conditions, scalability of production, and compatibility with various food types to facilitate commercial adoption.

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https://doi.org/10.47392/IRJAEH.2025.0260

References

[1]. Cahyana, V., & Verell, C. (2023). Development of biodegradable edible films as an alternative to plastic packaging. Journal of Sustainable Packaging Innovations, 15(3), 45-52.

[2]. Mohammed, S. A., & El Sakhawy, M. (2020). Biopolymer-based edible films for food packaging applications: Structure and functionality. International Journal of Food Science and Technology, 55(4), 612-620.

[3]. Development of Potato Starch Based Biodegradable Packaging Film Neha J. Hirpara, M.N. Dabhi and P.J. Rathod Processing and Food Engineering Junagadh Agricultural University, Junagadh, Gujarat 362001, India

[4]. Amjad, A., & Basit, A. (2023). Enhancing antimicrobial properties of edible films through bioactive plant extracts. Journal of Food Science and Technology, 18(2), 125-132.

[5]. Jaleel, C.A., R. Gopi and R. Paneerselvam, 2009. Alterationsin non-enzymatic antioxidant components of Catharanthus roseus exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens. Plant Omics J., 2: 30-40.

[6]. Antimicrobial Activity of Catharanthus roseus – A Detailed Study Prajakta J. Patil and Jai S. Ghosh Department of Microbiology, Shivaji University, Kolhapur 416004. MS. India

[7]. López-Bascón, M. A., & Luque de Castro, M. D. (2020). Chapter 11 - Soxhlet Extraction. In Liquid-Phase Extraction (pp. 327–354). Elsevier.