Development and Characterization of Edible fruit film by using *Mangifera indica* leaf extract, having antimicrobial and prebiotic potential

Thesis submitted in partial fulfillment for the requirement of the

Degree of Master of Pharmacy

By

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CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled "**Development and Characterization of Edible fruit film by using *Mangifera indica* leaf extract, having antimicrobial and prebiotic potential** " submitted to Jadavpur University, Kolkata, for the partial fulfillment of the **Master Degree in Pharmacy**, is a faithful record of bonafide and original research work carried out by **Mr. Khubaib Akhtar Khan** bearing Class Roll no. 002111402029 and Registration No. 163671 of 2023-2024 under my supervision and guidance.

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Declaration of the Originality and Compliance of Academic Ethics

I, Khubaib Akhtar Khan, a student of M.Pharm, 2nd year, bearing Class Roll No: 002111402029, Registration No. 163671 of 2022-2023, studying in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata-32, hereby declare that my thesis work titled – **"Development and Characterization of Edible fruit film by using *Mangifera indica* leaf extract, having antimicrobial and prebiotic potential,"** is original and presented in accordance with academic rules and ethical conduct, and no part of this project work has been submitted for any other degree of mine. All the information and works are true to the best of my senses and knowledge.

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Dedicated to My Parents and Family Members

ACKNOWLEDGEMENT

I deem it to be a privilege to work under the guidance of Prof. (Dr.) Amalesh Samanta, Division of Microbiology & Pharmaceutical Biotechnology, Jadavpur University on the very current and innovative topic **"Development and characterization of edible fruit film by using *Mangifera indica* leaf extract, having antimicrobial and prebiotic potential."** I am blessed to work under his constructive and suggestive guidance throughout my work. His expert supervision with undue patience throughout my work at every stage helped me complete this work effortlessly.

I also express my sincere gratitude to our H.O.D., Department of Pharmaceutical Technology, Jadavpur University, Prof. (Dr.) Prof. (Dr.) Amalesh Samanta for providing me an opportunity to do my thesis work successfully.

I am grateful to All India Council for Technical Education (AICTE), Government of India for providing financial support.

I express my heartfelt gratitude to Ph.D. scholars – Sohini Chatterjee, Abhishek Mohanta, Mousumi Tudu, Suparna Gorai, Ahana Hazra; PG senior Pankaj Paul; my classmate; Ankit Tiwari, Kaushiki Priya, Md. Ahesan Ansari, Riya Hazra, PG juniors; Nahid Zaman, Sahanaz Khatun, Pijush Kanti Shit.

I also want to extend my gratitude to my friends who provided moral support throughout my thesis work, Deepa Raghuwanshi and Mahak Jat.

I will remain forever grateful to The Almighty and my parents, whose dedication and untiring efforts towards me has brought me at this stage of life.

Khubaib. Akhtar. Khan 28/08/2024

(Khubaib Akhtar Khan)

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LIST OF ABBREVIATIONS

Symbols	Abbreviations
UV	Ultra Violet
MLE	Mango leaf Extract
IFNγ	interferon gamma
FTIR	Fourier Transformed Infrared
UV-Vis	Ultra violet visible spectrometer
sIgA	Serum immunoglobulin
SEM	Scanning Electron Microscope
%T	Transmittence
gm	Gram
mg	Milligram
hrs	Hours
t	Time
λ_{max}	Absorption maxima
ml	Millilitre
GO	Gum odina
TS	Tensile Strength
WVP	Water vapour permeability

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CHAPTER 1:

Introduction

1. Introduction:

The plant-based foods especially fruits, and vegetables are essential components of human nutrition. Food delivers several vital nutrients in life and many molecules or components with bioactive properties are involved in health improvement and disease protection. Guidelines for a healthy diet recommend high intakes of plant-based foods such as ingestion at least 400 g of fruits and vegetables daily (1). Plants contain minerals that are necessary components of a healthy human diet, in addition to several primary and secondary metabolites that influence nutrition and human health (2). Secondary metabolites are not crucial for the general development or functioning of plants, but these compounds present biological activity that makes them very useful as ingredients to formulate traditional and modern medicines. Fruits and vegetables are rich sources of micronutrients (magnesium, calcium, and potassium) and bioactive components (including non-nutrients) consisting of phytochemicals such as polyphenols, dietary fiber, carotenoids, and vitamins. It has been established that more than 3000 different phytochemical compounds are present in fruits, though many more remain unidentified (3). Enormously advantageous attributes of whole fruits have been associated with bioactive non-nutritional chemical components commonly known as phytochemicals. It has been suggested that whole foods and fruits may have 3000–20,000 individual phytochemicals with potentially bioactive properties (4). The wide variety of secondary metabolites present in plant sources includes antioxidant components, and vitamins, which have been confirmed to have many beneficial effects on human health, for instance antioxidative, anti-inflammatory, and cardio protective properties, in addition to preventing obesity and regulating diabetes 2 (5). Fruit is the fleshy part of plants that is sweet or sour and edible in its raw state. It provides ample sources of sugars, vitamins, and bioactive ingredients such as phenols and fiber that have been reported for their biological activity which is related to their ability to attenuate the progression of certain degenerative disorders (6).

1.1 Need to Develpement of Edible film

When fruits interact with the environment, they can add or release moisture content and odor, cause rancidity or microbiological contamination, and decline in quality and age of storage. Moisture content, odor, or fat that can migrate from one component to another will reduce the product's quality and age of storage in food products with many components. Foods must be

packaged to be stored and protected from environmental factors. Food packaging needs to be biodegradable in addition to protecting and distributing the product and packing it throughout distribution.

In general, the type of packaging that is frequently used in our community is plastic. Plastic used by the community is made from petroleum and natural gas that is derived from petrochemical products and non-renewable natural resources. The raw material of plastic is made and compiled via polymerization using monomer raw material, which is composed of dial-connect into one in the form of polymer. Many kinds of food and beverage packaging from plastic e.g. polyethylene, polypropylene, polystyrene, polyamide, polysulfone, polyester, polyurethane, polycarbonate, Polyvinylchloride and polyacrylonitrile. Plastics have an advantage, the price is cheap, can be produced in large amounts, light, transparent, flexible, and selective in permeability to H_2O, CO_2 and O_2 but, plastic also has a weakness, can't stand the heat, easily torn, can be a pollution in our environment and can contaminate the packaged food.

Plastics cause environmental pollution because have non-biodegradable characteristics, plastic can contaminate packaged food because the presence of certain substances is potentially carcinogenic and can move into the packaged food. Monomer-monomer on plastics can enter into the packages food next can enter into the body of consuming. Accumulation of chemical substances in our body is insoluble in water so can't waste with urine and feces. Accumulation of chemical substances can disrupt our health and cause cancer (7). So, we have to find packaging which has character biodegradable, elastic and stands the heat. One alternative to substitute plastic is edible film. Because it is biodegradable and acts as a barrier to oxygen uptake, and vapor transfer so edible film is harmless.

Edible films are defined as thin protein, polysaccharide, and lipid-based layers created between food ingredients or on food surfaces to maintain quality, prevent spoilage, prolong the shelf life, and protect the sensory properties of food (8). Edible packaging was traditionally used to prevent the food product's appearance and nutritional quality. The edible film has the ability to barrier properties with mechanical strength (Khwaldia et al., 2004) and control the mass transfer capacity of food components and their environment. It helps to extend the food product shelf life and enhance their quality and appearance. (9) The edible coating is cast by different components such as polysaccharides, proteins, and lipids or combinations. Edible coating or film is a primary packaging made up of an edible component such as polysaccharides, lipids, and proteins. It is a thin and transparent layer of food wrap

without disturbing the original ingredients and processing methods of food products. It can improve properties such as moisture, gas barriers (oxygen and CO_2), sensory, and appearance. It protects from microbial spoilage and helps to extend the shelf life of the food products (10.). Edible coating and film can help to reduce the requirement of conventional packaging and waste. It extends the shelf life of food products and improves the efficiency of packaging materials economically. Mezemir et al. (2017) investigated the effects of bee wax and linseed oil coatings on the quality of orange juice extracts at ambient temperature. Since the 1930s, the use developed an emulsion with a combination of oil and wax with water for improving the appearance (colour, shininess), controlling the ripening process, and retard moisture of fruits and vegetables (11).

Edible film is a packaging material used as a wrap for the separation of layers of food components. It is a substitute for synthetic plastic for food packaging. It has palatability and degradability in nature. Edible films help to improve the recyclability of the packaging material as compared to non-eco-friendly packaging materials. Presently, natural polymer and food-grade additives are highly used in the food processing sector. The natural products (polysaccharides, proteins, and lipids) are blended with plasticizers (glycerol, glycol, polyol) and surfactants for the formation of the edible film. (12)

The polysaccharide components are commercially used as thickening and gelling agents in food industries. Which also have encapsulation and crystallization inhibitors property and used as stabilizers agent (Stephen and Churms, 2006). The main polysaccharide components used for prepared edible coating are the following: starch, chitosan, pullulan, alginate, carrageenan, modified cellulose, pectin, gellan gum, xanthan gum, etc. (13).

The edible coating has ability to the preservation of fruits and vegetables (Sapper and Amparo, 2018). The edible film was applied successfully on fruits and vegetables such as apples, oranges, cherries, grapefruit, cucumber, strawberry, tomato, and capsicum. The edible film and coating are a packaging technology and minimally processed for prepared ready-to-eat products and it provides functional and healthy food for consumers (14). The food and food-based edible products are perishable and semi-perishable, they are affected by environmental; impact and biological contamination. The edible film and coating help to reduce contamination and undesirable effects and improve the food product's shelf life and nutritional properties (15). Food preservation technique such as salting, acidification, and heat treatment was traditionally used for the preservation of food products, but during these techniques, there were unacceptable losses of food nutritional properties. The edible film and

coating are more effective in protecting the organoleptic and nutritional properties of the food products.

The previous research investigated, that edible coating and film play an important role in the food processing sector, it can improve the quality of the food products (16). The edible coating and film are used to control the gas exchange, and oxidation process of food products. The functional and specific properties of the edible film are responsible for decelerating organic vapour such as (solvent, aroma), water vapour, solutes such as (salts, lipids, food additives, and pigments), and gases (carbon dioxide, oxygen, nitrogen). The water barrier properties of the film are responsible for retard the surface dehydration of fruits, vegetables, meat, seafood, and other frozen food products (17). The edible coating is the barrier of gas exchanges mostly oxygen and controller of the fruit ripening and helps to minimize oxidation of oxygen-sensitive food products. Edible film and coating controllers of mass transfer between the food products and environment. It also helped to minimize radical air reactions (UV light effect) in foods. It also improved the mechanical properties of food and food products during handling and storage. The organoleptic properties such as transparency, colour, roughness, shininess, and stickiness can be improved by edible film and packaging.

1.2 Component of edible film

The edible film polymers are nontoxic, biodegradable, environmentally friendly, and simple derivatives of plants and animals. The coating and film produced from polymeric edible films are generally designed to be flexible and tough (18). The edible film-forming materials and components are such as polysaccharides, proteins, and lipid-based (Figure 1.1). These components are used to develop edible coating composition and formulations. It varies from commodity to commodity and functionality can be better expressed by using different ratios and combinations with additives and plasticizers.

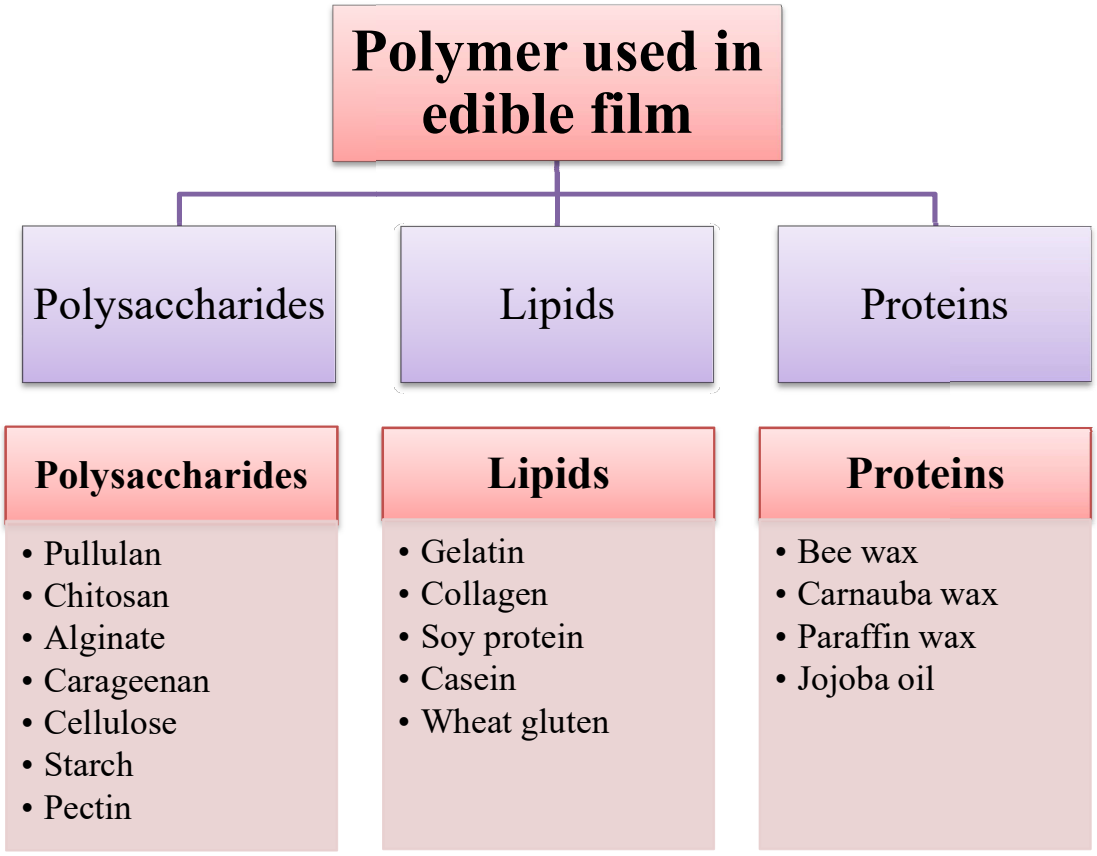


Fig 1.1 Polymer used in edible film

1.2.1 Polysaccharide-based film forming material and component

Polysaccharide-based film is widely used for edible film formation in agro-food and pharmaceutical industries. The Polysaccharide-based film is made up of different polysaccharide components such as starch, chitosan, cellulose ethers, alginate, carrageenan, and pectin. These components are produced from plant sources in the ecosystem. The polymer chains of the polysaccharide-based components formed edible film and coatings. Polysaccharides are commercially used in food industries as thickening and gelling agents, crystallization inhibitors, stabilizers, and encapsulating agents (19). The main polysaccharide components used for prepared edible coating are followings: chitosan, pullulan, starch, alginate, carrageenan, modified cellulose, pectin, gellan gum, xanthan gum, etc. (20).

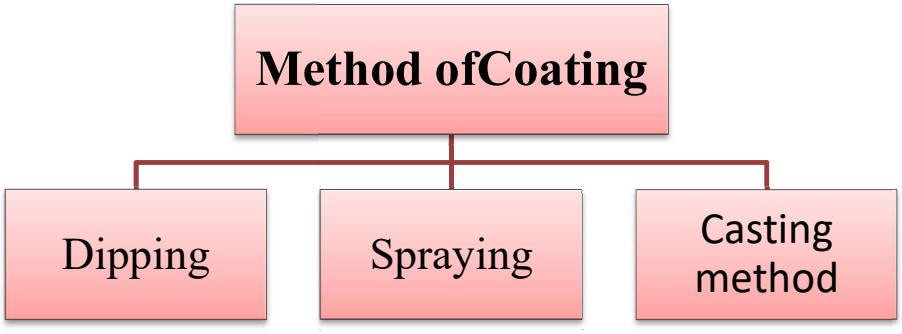
1.2.2. Lipids

These coatings show a good barrier property against water vapor due to their hydrophobic characteristics. Lipid materials do not stay in properly fixed positions by themselves as they are unable to polymerize (21). They are used to give brightness to the surfaces of fruits and vegetables (Işık et al., 2013). Animal and vegetable oils (coconut, peanut, palm, cacao, butter, fatty acids, and mono-, di- triglycerides), waxes (candelilla, carnauba, beeswax, jojoba and paraffin), natural resins (gum, guarana and olibanum), basic fatty acids and their extracts (mint, camphor, essential oils of citrus fruits), emulsifiers and surface active agents (lecithin, fatty acids) are included in this group (22). Other than the basic 3 groups, composite or mixed films formed by different formulations of polysaccharides, proteins, and lipids can be created (Işık et al., 2015) Thus, the quality is improved by combining different film components in one single coating (23).

1.2.3. Protein

Proteins with a structure providing a number of functional properties have the potential to form bonds in different positions and to be able to make numerous bonds. As a result, the mechanical properties of protein-based films are better than polysaccharide and lipid-based films. Besides being used as a film for the product (24), they also increase the nutritional value of the food they are coated with (Bourtoom, 2009). Due to its hydrophilic nature, the water barrier and mechanical properties of protein films are weaker than synthetic polymer films (Bourtoom, 2009; Duran, 2013). In this group; animal-origin casein, whey protein concentrate and isolate, gelatin, egg albumin, and vegetative corn, soy, wheat, cotton seed, rice, and peanut are included (25).

1.3 Method of Coating



1.3.1 Dipping

The method of dipping is the most widely used film-forming method (26). It is carried out by dipping and taking the foods off the coating solution. The dipping process is carried out in three steps immersion of food in the coating solution, draining the food taken off from the solution, and drying the coating (27). The coating solution forms a thin film layer on the food surface. This method is the best for foods with rough and uneven surfaces. It is also a method that allows the recovery of excessive coating material (28).

1.3.2 Spraying

The method of spraying is used when a thin layer of coating is required and only one surface is desired to be coated. It is a more controllable coating application method than pan or fluidized bed coating. However, spray coating requires that the lower surface of the product be covered with a separate process after coating and drying of the upper surface. The product must be rotated to cover the lower part of the food during coating application. Spray coating is preferred for foods with large surface areas. The spray nozzle plays a critical role in the coating process for design data such as flow rate, droplet size, spray distance and angle, and overlap speed. The coating fluid pressure, fluid viscosity, temperature, surface tension, and nozzle shape or design all affect spraying efficiency (29).

Fig 1.2 Diagrammatic representation of coating methods

1.3.4 Casting method

The casting method is the most widely used film coating method in laboratory and pilot scale. A polymer and a solvent are used to create a coating solution. Film is prepared from biopolymers in three steps dissolving the biopolymer in a suitable solvent, pouring the solution into a container, and drying the casting solution. The casting method was developed more than a hundred years ago. By using this method, solutions are spread on flattened plates such as acrylic, silicone, or Teflon plates, followed by a drying process under ambient or controlled conditions. Hot air oven, microwave dryer, and vacuum drying methods are used to easily evaporate the solvent and peel off the film (30).

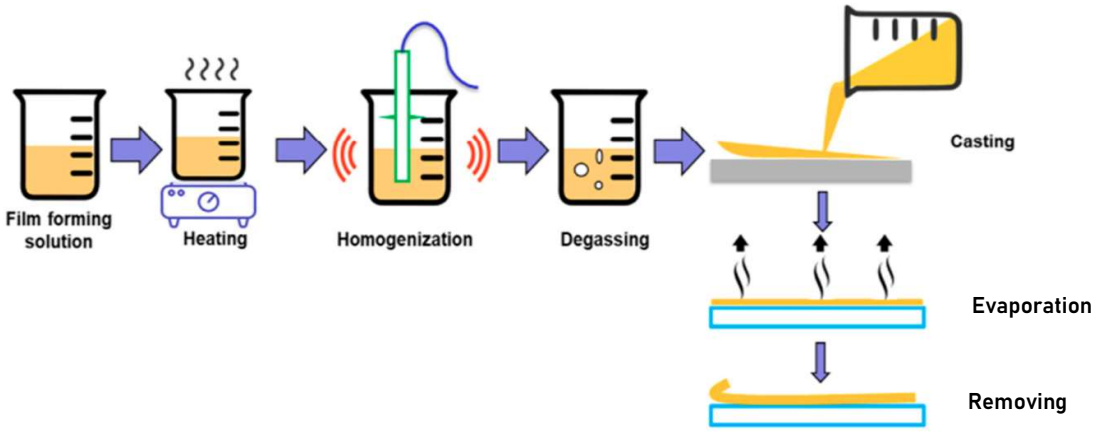


Fig 1.3 Schematic representation of the edible film formulation

CHAPTER 2:

Literature review

K.Rambabu et al., 2019, research that the development, characterization, and application of chitosan films enhanced for antioxidant activity by mango leaf extract (MLE) incorporation. The study found that increasing MLE concentration resulted in films with increased thickness and decreased moisture content. MLE inclusion reduced hydrophilicity and water vapor penetrability, and MLE films exhibited better tensile strength and reduced elongation ratio than pure chitosan films. Microscopic studies revealed the smooth, compact, and dense nature of MLE-chitosan films, favoring low oxygen transport rates. The results suggest MLE MLE-impregnated chitosan films as a promising alternative for active packaging films for food preservation. These biodegradable active film materials are primarily obtained from by-products and waste streams of food industries.

Thiet Anh Dinh et al, 2023, prepared environmentally friendly and antibacterial edible films using a blend of chitosan, guava leaf extract, and glycerol. The research evaluated the antioxidant, antibacterial, and physical properties of the films using various techniques. Results showed that increasing GLE concentration improved the antioxidant properties and biodegradation of the films. The study aimed to increase the production of such films.

Gisella M. Rodríguez et al. 2020, developed edible films with antioxidant activity for food preservation using papaya. The films were characterized by antioxidant activity, physicochemical properties, mechanical properties, bromatology, and sensory acceptance. The dehydrator was chosen for further film-forming procedures due to its reasonable drying time and preservation of natural properties. Both films incorporated with ascorbic acid and a mixture of moringa and ascorbic acid had the highest antioxidant activity. The incorporation of both bioactive compounds influenced the shelf-life stability of minimally processed pears, with ascorbic acid enhancing sensory acceptance.

Masha Nouraddini et. al, 2018, focuses on the preparation of biodegradable and edible films using eggplant flour (EF) and corn starch (CS). The films were evaluated for mechanical, barrier, physical, and biodegradability properties. The results showed that pure starch films had higher tensile strength, elongation at break, Young's modulus, thickness, and density. The substitution of CS by EF increased solubility, water vapor permeability, moisture content, and swelling index. EF is a promising material for edible and biodegradable films with adequate physical properties for food applications.

Ruchir Priyadarshi et. al, 2018, prepared Chitosan films with a crosslinker and plasticizer for food packaging using solvent casting. The cross-linker was citric acid, enhancing stability, and the plasticizer was glycerol, providing flexibility. The modified films showed improved water resistance, transparency, moisture barrier, and elongation percentage value. However, they decreased tensile strength and Young's modulus. The films also showed better thermal and antioxidant properties and enhanced green chili shelf life compared to neat chitosan films.

Tan Phat Chau et.al, 2024, studied and evaluated the antimicrobial activity of *Lannea coromandelica* bark's extract with methanol against various microbes and bacteria, including fungi, bacteria, and fungi. The extract showed dose-dependent activity against these microbes, with Penicillium showing the most antifungal activity. The extract was found to be biocompatible, with average light transmittance and few vital functional groups responsible for the interaction. The coated Citrus maxima fruit quality characteristics, including total soluble solids, weight loss, pH of fruit pulp juice, and decay percentage, were significantly better than uncoated fruit. The study concluded that the extract's biocompatibility and antimicrobial properties make it a promising solution for fruit preservation.

Alqasim Abdullahi Mustapha et.al., 2014, studied and analyzed the bioactive compounds in palm wine and ethanol extracts of Mangifera indica leaves, revealing alkaloids, carbohydrates, anthranol glycosides, triterpenes, phenol, flavonoids, and amino acids. The palm wine extract showed the highest antibacterial activity against *Shigella flexneri*, *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus spp.* The MIC of palm wine and ethanol extract was 12.5 mg ml-1 for all tested organisms. *Shigella flexneri* was the most susceptible organism to the palm wine extract, while *Pseudomonas aeruginosa* was the least susceptible.

R. K. DHALL et. al., 2013, Elaborate that Edible coatings are environmentally friendly technologies used to control moisture transfer, gas exchange, and oxidation processes on products. They can enhance safety, nutritional attributes, and sensory attributes by incorporating active ingredients into the polymer matrix. However, their success depends on controlling internal gas composition and monitoring quality parameters like color change, firmness loss, and weight loss. Recent advances in edible coatings include antimicrobials,

texture enhancers, and nutraceuticals while considering sensory implications, regulatory status, and future trends.

Rajat Suhag et.al, 2020, Described that Food manufacturers face challenges in preserving food quality during storage, leading to waste. Edible packaging offers a potential solution by delaying microbial spoilage and providing moisture and gas barrier properties. The edible packaging market is expected to reach $1097 million by 2023, growing at a 6.81% CAGR from 2017 to 2023. Edible packaging can be applied in two forms: edible coatings applied directly on food products or preformed films wrapped around food products. Methods for film formation and coating deposition include casting and dipping, with extrusion and spraying being preferred on a commercial scale. Further research is needed to develop efficient and cost-effective edible packaging methods.

R. K. DHALL et al.(2013), reported that Edible coatings control moisture, gas exchange, and oxidation processes on products, enhancing safety, nutrition, and sensory attributes. Success depends on gas composition and quality parameters. Advances include antimicrobials, texture enhancers, and nutraceuticals.

CHAPTER 3:

Aims and objectives

Aim

The aim of this project is to develop and characterize an edible fruit film incorporating *Mangifera indica* leaf extract, focusing on its antimicrobial properties and potential prebiotic benefits, with the goal of creating a functional and sustainable food packaging solution.

Objectives

- **Formulation of Edible Films:** To optimize the formulation of edible fruit films using different ratios of gum odina (GO) and pectin, combined with varying concentrations of Mangifera indica leaf extract (MLE).
- **Characterization of Physicochemical Properties:**To evaluate the tensile strength, moisture content, water vapor permeability, thickness, water absorption, and contact angle of the developed edible films.
- **Assessment of Antimicrobial Activity:** To investigate the antimicrobial efficacy of the edible films against common foodborne pathogens, including *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus.*
- **Biodegradability Evaluation:** To assess the biodegradability of the formulated edible films under controlled conditions.
- **Exploration of Prebiotic Potential:** To discuss the potential prebiotic properties of the edible films based on the known prebiotic effects of gum odina, and propose future studies to confirm this functionality.
- **Sustainability and Practical Application:** To evaluate the overall potential of the edible films as sustainable alternatives to conventional food packaging materials, considering their environmental impact and functional properties.

CHAPTER 4:

Materials and methods

4.1 Polymer used

4.1.1 GUM ODINA

Gum Odina is a plant-derived gummy polysaccharide with nontoxic, easily available, and low cost. Gum Odina has already been employed in pharmaceutical excipients in various dosage forms such as emulsions and tablets. This chapter includes a comprehensive and useful discussion of Gum Odina as a pharmaceutical excipient. In addition, the article also briefly discusses the source and properties of Gum Odina (31).

Gum Odina is extracted from the bark exudates of Odina wodier Roxb. (belongs to the family of Anacardiaceae). The tree of Odina wodier Roxb. is larger and locally acknowledged as the Jingan or Kamlai tree. These trees are very frequently found in India, Myanmar, Sri Lanka, China, Malaysia, Cambodia, and the Philippine Islands. Gum Odina and different parts of Odina wordier Roxb. plant are traditionally utilized in Indian folk medicine and Ayurvedic medicine. The leaves of Odina wodier Roxb. have been used in the treatment of Elephantiasis of the legs. The juice of green branches of Odina wordier Roxb. plants is employed in case of coma or insensibility produced by narcotics as an emetic. It was reported that poor villagers used dried and powdered bark as toothpowder. Bark extracts of Odina wodier Roxb. have been found very constructive in vaginal difficulty, curing ulcers, diseases of the heart, and so on (32).

4.1.2 Gum Odina Used as a Prebiotics

Mitra et. al 2016, Study and evaluates the ability of gum Odina, a prebiotic derived from Odina wodier bark, to improve intestinal flora. According to the in vitro prebiotic activity assay, gum odina boosts probiotic growth by releasing lactic and acetic acids. Studies conducted in vivo demonstrated that natural gum removes enteric infections and promotes *Lactobacillus sp.* Additionally, it improves the immune system by raising IFNγ and sIgA levels in response to infection. This suggests that gum odina is a potential prebiotic that enhances gut ecology in addition to providing nutrition.

4.2 Pectin

Pectin is a white, amorphous, and colloidal carbohydrate of high molecular weight occurring in ripe fruits, especially in apples, currants, etc., and used in fruit jellies, pharmaceuticals, and cosmetics for its thickening and emulsifying properties and ability to solidify to gel. All these properties and applications have put pectin in the market of biopolymers with great potential and possibilities for future developments.

Pectic substances are present in the primary cell walls and middle lamellae of many plants and fruits, and they are frequently associated with cellulose, hemicellulose, and lignin structures [33]. Their presence in the cell is important for some essential functions: (a) adhesion between cells; (b) mechanical strength of the cell wall; (c) ability to form stabilizing gels; and (d) they play a significant role in the growth of plant

cells (34). Pectin forms the most complex class of polysaccharides, mainly composed of high molecular weight heterogeneous groups of glycanogalacturonans and acidic structural polysaccharides with diverse structures. Pectin backbone consists of (1→4)-α-D-galacturonic acid molecules linked to a small number of rhamnose residues in the main chain and arabinose, galactose, and xylose in the side chains (35). Several authors stated that pectin polysaccharides can be classified into three types.

Homogalacturonan (HG) is a linear polymer formed by D-galacturonic acid and it can be classified into three different families depending on the acetylation or methylation reactions suffered during polymerization: (i) pectin with more than 75% of methylated carboxyl groups; (ii) pectinic acid with less than 75% of methylation; and, finally, (iii) pectic acid or polygalacturonic acid without methyl-esterified carboxyl groups [37].

Rhamnogalacturonan I (RGI) is composed of the repeating disaccharide rhamnose–galacturonic acid groups acetylated and linked to side chains of neutral sugars, such as galactose, arabinose, and xylose.

Rhamnogalacturonan II (RGII) is also formed by homogalacturonan chains, but with complex side groups attached to 12 different types of glycosyl residues, such as rare sugar species (2-O-methyl xylose, 2-O-methyl fucose, aceric acid, 2-keto-3-deoxy-D-lyxo heptulosaric acid, and 2-keto-3-deoxyD-manno octulosonic acid). RGI and RGII are called hairy regions in the pectin structure whereas HG is the smooth part of the molecule (37, 38).

4.2.1 Applications in Food Industry of Pectin

In general terms, pectins are used in the food industry as stabilizers, thickening and gelling agents, crystallization inhibitors, and encapsulating agents. But, in all cases, galacturonic residues should be previously modified by the addition of methyl groups to yield methoxides. This chemical modification is essential to improve pectins' physical properties, which are dependent on their molecular masses and, primarily, on the degree of methyl-esterification (DM), calculated as moles of methanol per 100 moles of galacturonic acid (39). In this context, two different DM degrees can be achieved resulting in different applications (40). Pectins with high DM (HM pectins) containing 50% or higher of galacturonic residues. They are used as gelling components in heat-resistant bakery jams, fruit preservatives and juices, confectionaries jellies, milky products, glazing cakes, and soft drinks. HM pectins form gels by hydrophobic interactions under acidic conditions in aqueous media and high sugar content.

Pectins with low DM (LM pectins) are obtained by de-esterification of HM pectins under controlled pH, temperature, and time. LM pectins show DM degrees between 20% and 30% (41). They are used to prepare gels at low pH values in the presence of divalent calcium. Applications in the food industry include jams and jellies with low-sugar content, dairy desserts, ice cream with fruit gels, thickening agents of syrups for fruit and vegetable canning, and food coatings.

In this context, edible coatings obtained from pectin and derivatives (pectate and amidated pectin) have been recently proposed in food-related applications because of their excellent barrier to oxygen, aroma preservation, barrier to oil, and good mechanical properties, but they are not effective against moisture transfer through films by their hydrophilic nature (42). They are currently used in fresh and minimally processed fruits and vegetables, such as avocado, apple, apricot, chestnuts, berries, guava, melon, papaya, peach, walnuts, carrot, and tomato (43). Depending on the product, three different coating methods can be used (44).

4.3 *Mangifera indica* Leaf

Mango (*Mangifera indica* Leaf.), a predominant floral member of the Anacardiaceae family is available in N1000 varieties around the world (45). The various parts of the mango tree possess a wide range of nutrients such as fibers, minerals, starch, lipids, antioxidants, etc. Although the mango fruit is considered as the major economic product of the tree, most of the other components, especially the leaves are discarded as waste by the mango industry.

Studies have indicated that mango leaves possess more antioxidants than the kernel, peels, and bark parts of the tree (or) fruit (46). Mango leaf extract (MLE) is rich in gallic acid, mangiferin, glucosides, and other phenolic compounds which provide excellent antioxidant capacity to MLE. Recently, mango kernel extract was incorporated into soy protein isolate and fish gelatin to develop food packaging films with enhanced strength, thickness, and antioxidant activities (47). Gelatin films blended with mango peel extract were reported to have better free radical scavenging activity (48). To the best of our knowledge, the utilization of mango leaves as a potential natural additive to enhance the antioxidant activity of the biopolymer films has not much explored.

Mango leaf extract (MLE) is increasingly recognized for its antimicrobial properties, making it a valuable addition to edible film formulations. The antimicrobial activity of MLE is primarily attributed to the presence of bioactive compounds like phenolics, flavonoids, and tannins. These compounds can inhibit the growth of a wide range of bacteria, including common foodborne pathogens such as *Escherichia coli, Staphylococcus aureus*, and *Klebsiella pneumoniae*. The antimicrobial activity of MLE in edible films is largely due to the disruption of microbial cell membranes. Phenolic compounds in the extract can interfere with the integrity of bacterial cell walls, leading to cell lysis and death. Additionally, flavonoids can inhibit bacterial enzymes and nucleic acid synthesis, further impairing bacterial growth (49).

When incorporated into edible films, MLE not only enhances the antimicrobial efficacy of the film but also contributes to its overall functionality, including potential antioxidant properties. The combination of these properties makes MLE-infused edible films a promising solution for extending the shelf life of food products by reducing microbial contamination (50).

4.4 MATERIALS

Pectin (degree of esterification 65-70%), was purchased from HiMedia Laboratories Pvt. Ltd. Maharatra, India. Calcium chloride, DPPH (2, 2-diphenyl-1-picrylhydrazyl), and Glycerol were purchased from Merck Specialities Private Limited, Mumbai. Fresh prepared deionized water was used for solution preparation and dilution. All the chemicals were of analytic grade and were used directly in the experiments without any additional treatment.

4.5 METHODS

4.5.1 Collection of Gum Odina

The exudate crude Gum Odina (GO) oozes during the months of August-October from the stems of the tropical deciduous plant Odina wodier Roxb., family Anacardiaceae, and then dried into translucent, tear-shaped flakes on contact with air (Figure 4.1). The gum was collected from Garhbeta (Geographically, Garhbeta is at 22° 51' 47.2824" North and 37° 21' 13.5900" East), Paschim Medinipur District, West Bengal, India; and stored in a sterile container in desiccated condition for further studies. For authentication, the plant specimen was identified by R. Gogoi (Scientist- D). Central National Herbarium, Botanical Survey of India; Ministry of Environment, Forest and Climate Change: Howrah, West Bengal, India. For future reference, a voucher specimen was deposited in the Department of Pharmaceutical Technology, Jadavpur University Kolkata, India.

4.5.2 Purification of Gum Odina

The chemical purification improves crude gum polysaccharides' unacceptable color and flavor by minimizing impurities content. Several techniques were reported for the purification of crude plant gums. The purification of GO was performed according to the previously described procedure (Mitra et al., 2016) with slight modifications. Briefly. crude gum (GO, 60 g) was soaked in deionized water (500 mL) overnight for complete swelling followed by vigorous stirring using a mechanical stirrer (REMI Lab, India) for 8 hours at room temperature. The brown homogenized viscous solution was then kept at room temperature for 24 hours to separate any undissolved materials and passed through a double-folded muslin cloth. Then the solution was centrifuged (Thermo Scientific Heraeus Biofuge Stratos Centrifuge, Osterode, Germany) at 2500 rpm for 30 minutes. The clear supernatant was then gradually poured into cold acidified ethanol (pH 4-5, acetic acid, x5 volume) to obtain a white precipitate. The precipitate was allowed to settle overnight at 4 °C and collected by centrifugation (10000 rpm). The precipitate was re-dissolved in water (100 mL) and re-precipitated as described above. Finally, the precipitate was washed twice with absolute ethanol and dry ether (1:1, 2×20 mL) and collected by centrifugation (10000 rpm). The

purified gum odina dried overnight at room temperature was powdered, and stored in a desiccator for further use. A pictorial illustration of the purification of GO is presented in Figure 4.1.

Fig 4.1 Gum odina purification

4.5.3 Extraction *Mangifera indica* Leaf

Fresh leaves of *Mangifera indica* were collected from near the campus of Jadavpur University in the month of February 2024, and then the freshly collected leaves were spread to dry under shade at normal room temperature for seven days. After drying, the leaves were cut into finer pieces using a motor pestle and soaked in ethanol 2:1 ratio. The mixture was kept as such in a dark condition for 24 hours. The soaked sample was then filtered using Whatman no.1 filter paper, the filtrate was evaporated to dryness using a water bath maintained at 40 ^{0}C and the dried substance was stored in airtight bottles until required (51).

4.5.4 Fabrication of films

The films were developed by following method. At first, aqueous slurry of Gum Odina and pectin were prepared separately. Polymeric mixtures were prepared by varying amount of Gum Odina and pectin (1:1, 1:2, 2:1) and well mixed with aqueous solution on magnetic stirrer (REMI Lab Stirrer, India) at 500 rpm up to 4 hour. The gum odina pectin solution was

then given glycerol as a plasticizer and stirring was continued for 30 min. After that, different ratio of mango leaf extract (MLE) were added to the mixed solution and homogenized for 45 min. Then, the mixture was placed into the ultrasonic bath to remove the air bubbles. After being poured into petridish plates, the film- forming solutions were dried for 24 h at 45^0 C. Then the films were peeled from the petridishes and stored at a temperature of 25 °C.

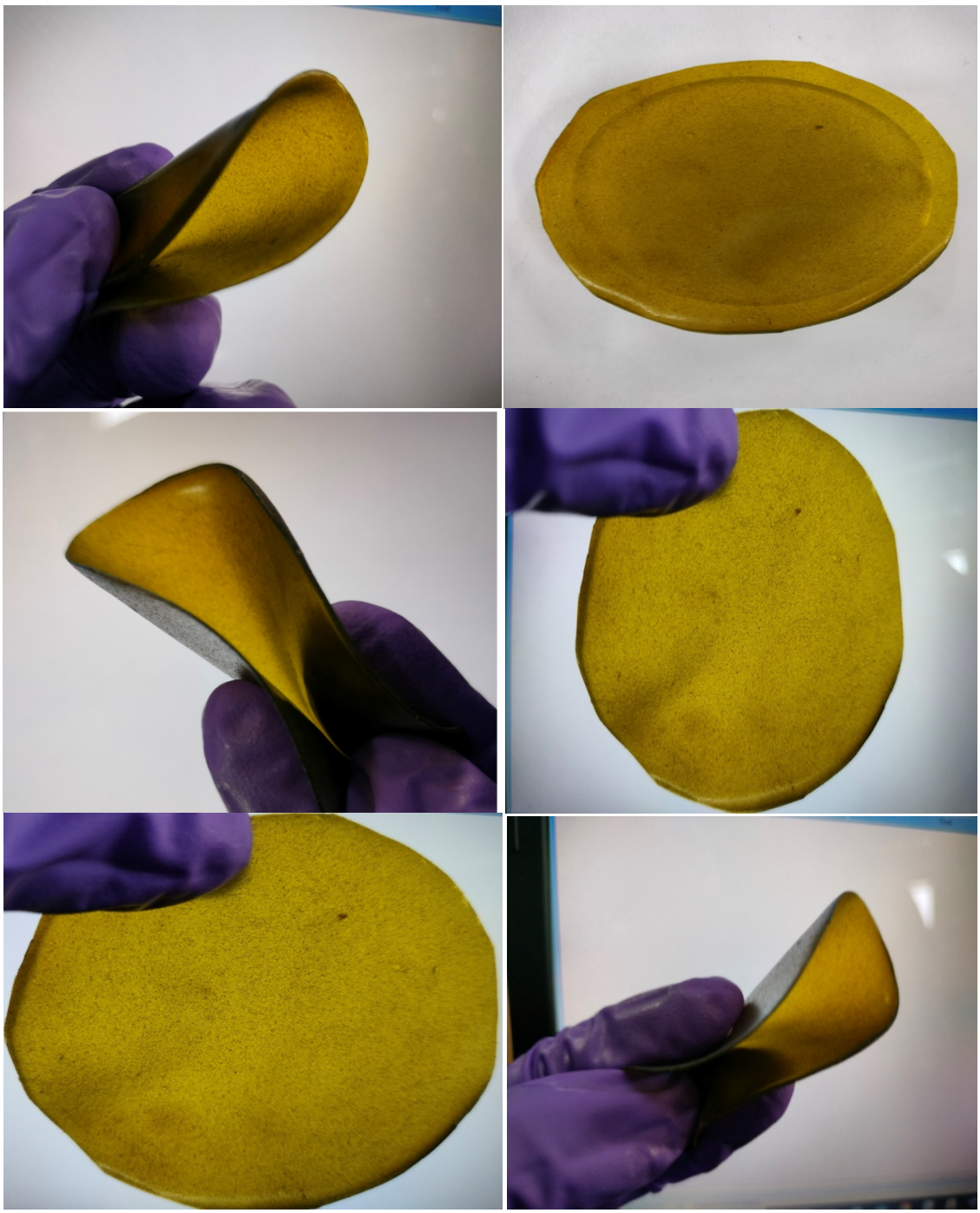


Fig 4.2 Physical appearance of the film samples

4.6 OPTIMIZATION OF FORMULATED FILM

4.6.1 Film Thickness

The thickness of the films (mm) was measured using a Workzone (India) micrometer with a resolution of 0.001 mm in the range of 0–25 mm was used to measure the thickness of the films. Each film sample was measured randomly at five different angles and the mean value was reported (53).

4.6.2 Moisture Content

The moisture content of a film can be defined as the amount of moisture taken up by the dry film from the environment till the equilibrium is established between the moisture content of the surrounding air and that of the films. To determine the moisture content, the films were cut into 2 cm × 2 cm square pieces and weighed. This initial weight of films containing moisture was denoted as Wm. The films were then kept in a BOD incubator shaker at 45℃ for 24 hours and kept in a desiccators for three consecutive days to lose the maximum moisture. The final weight (Wd) was measured in a triplicate manner, and their mean value was taken (54). The moisture content was calculated using the following equation:

$$\text{Moisture content } \% = \frac{\text{Wm} - \text{WD}}{\text{Wm}} \times 100$$

4.6.3 Solubility

The solubility of the films is determined in terms of the percentage of the film dry matter that is soluble in water. The solubility of the films was determined following earlier reported methods with modification (Hafsa et al., 2016; Ren, Yan, Zhou, Tong, & Su, 2017). Briefly, the films were cut into 2 cm × 2 cm square pieces and kept in a vacuum desiccator to remove all moisture content from the films. The films were weighed at regular intervals till a constant weight was obtained which corresponds to completely dried films, and this weight was denoted as the initial dry weight. The films were dipped in 50 ml deionized water in a glass beaker and then removed from the beakers and dried at 56 °C till constant weight. This weight was denoted as the final dry weight.

The solubility percentage was calculated using the following equation:

$$\text{Water solubility } \% = \frac{\text{Initial Dry Weight} - \text{Final Dry Weight}}{\text{Initial Dry Weight}} \times 100$$

4.6.4 Water vapour permeability test

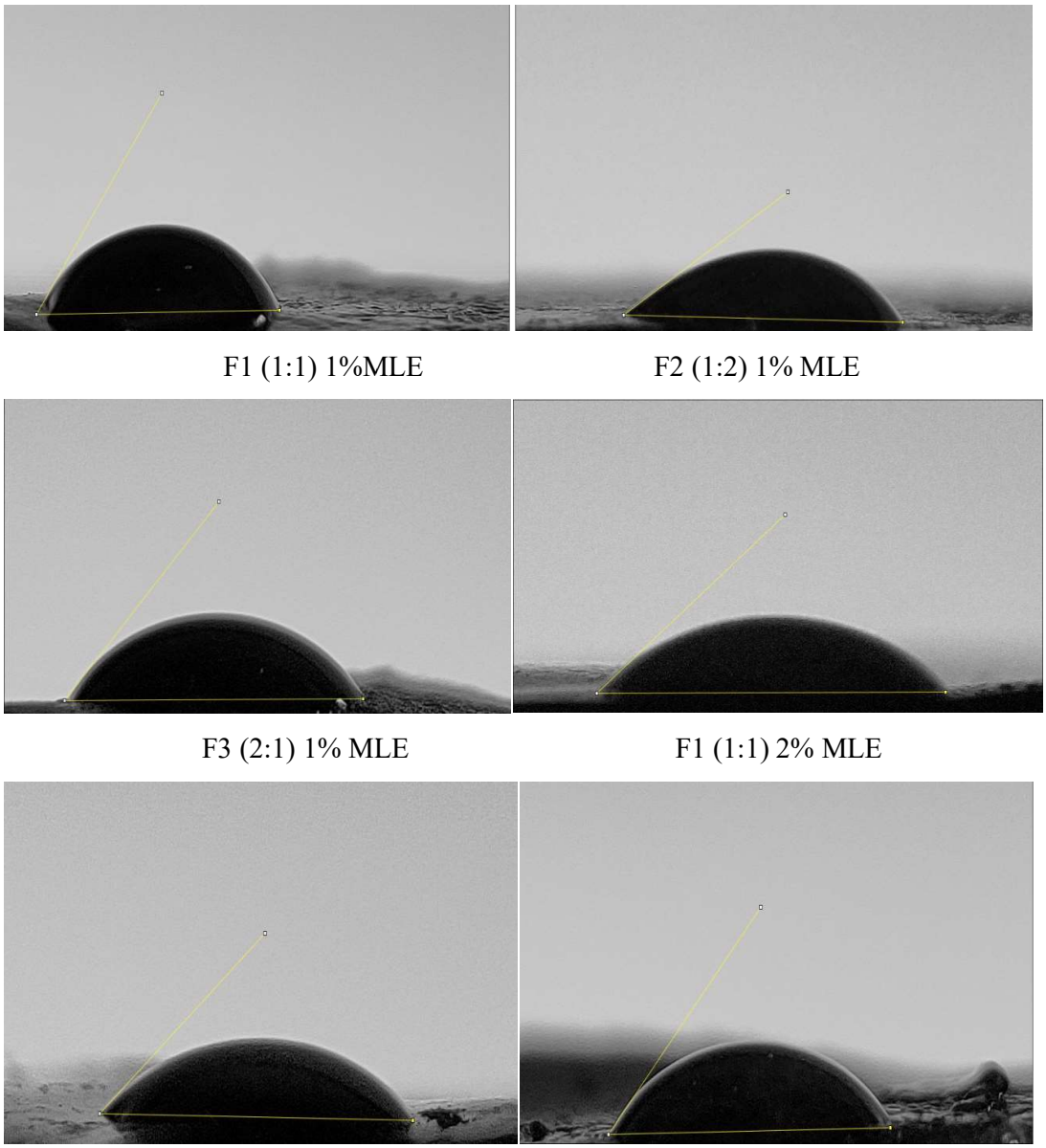
The water vapor transmission rate of prepared hydrogel films was determined by using the desiccant method. The circular pieces of hydrogel films (diameter: 1 cm) were cut and fixed on the mouth of the vials (diameter: 1 cm) containing anhydrous $CaCl_2$. The vials were filled with anhydrous $CaCl_2$ in such a way that the distance between the surface of $CaCl_2$ and the lower side of the film was about 10 mm. The vial without film was used as a reference. These vials were placed in desiccators containing a saturated solution of NaCl. The vials were weighed on analytical balance at 0, 6, 24, 36, 48 and 72 h (55). The water vapor transmission rate (WVTR) through the hydrogel films was determined using the formula given below:

$$\text{WVTR} = \frac{[(\Delta W/\Delta t) \times 24\,]}{A}$$

Where, $(\Delta W/\Delta t)$ is the slope obtained from the plot of 'w' versus 't', 'W' is the weight gain (g) along the specified period, 't' (h), and 'A' is the effective transfer area (m^2).

4.6.5 Contact angle

Contact angle plays a vital role in examining the hydrophilic nature of biopolymeric gum odina pectin hydrogel film. The contact angle was measured by the following method. Firstly hydrogel film was placed on the surface of the slide and the whole setup was kept leveled so that it would not wear or lean to one side. 15 micro litter deionized water was carefully dropped over the film and the images were captured using the lens. Finally, the contact angle was measured with the help of software. Total experiments were done in triplicate for better evaluation.



F1 (1:1) 1%MLE F2 (1:2) 1% MLE

F3 (2:1) 1% MLE F1 (1:1) 2% MLE

F3 (1:2) 2% MLE F3 (2:1) 2% MLE

Fig 4.3 Contact angle study

4.7 CHARACTERIZATION OF OPTIMIZED FORMULATION

4.7.1 Tensile strength

The tensile strength of the hydrogel films was determined using the stretch test. An assembly designed in the laboratory was used to perform this test. The film specimens (6.5 cm^2) were

positioned between two mounting clamps. An empty pan was attached to the lower clamp. The weights were added to the pan to exert a downward force on the film. The addition of the weights was continued until the film breaks. The final weight required to break the film was recorded (56). The study was conducted in triplicate. The tensile strength was calculated by using the following equation. Tensile strength kg/cm^2

$$\text{Tensile strength kg/cm}^2 = \frac{\text{Load at break (kg)}}{\text{Cross sectional area (cm2)}}$$

4.7.2 Antioxidant assay

The antioxidant activity of the optimized formulation was determined by its scavenging ability on the stable radical DPPH (2, 2-diphenyl-1-picrilhydrazil). The film was prepared by adding 500 mg of film pieces to 15 ml of methanol and ultrasonicating the mixture for 2 hours, followed by centrifugation at 5000 rpm for 20 minutes to collect the supernatant. A separate solution of DPPH was prepared by the following method: dissolve DPPH in methanol to obtain a 0.1 MM solution. This can be done by dissolving 0.0039 grams of DPPH in 100 ml of methanol. To protect the solution from light, wrap the container in aluminum foil or store it in a dark place. To determine the antioxidant activity of the extract film, it was incubated with a methanolic solution of DPPH for 8 hours in a dark place at room temperature. After that, 200 µl of sample solution was withdrawn, and absorbance was measured using a microtitreplate reader (Spectra Max M5, Molecular Devices, CA) at 517 nm AS. (57). The absorbance of the control experiment was measured as Ac. The free radical scavenging activity (%) was calculated according to the following equation:

$$\text{The percentage of free radical scavenging activity (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}}$$

4.7.3 Opacity measurement

According to Nouraddini, Esmaiili, and Mohtarami (2018), the films were cut into rectangle pieces (1 cm× 4 cm) and placed directly in a quartz spectrophotometer cell. An empty test cell was used as the reference. The absorbance was measured at 600 nm using a UV–Vis spectrophotometer and the opacity of the film was calculated using equation.

$$O = \frac{Abs600}{T}$$

Where O is the opacity, Abs600 is the absorbance at 600 nm and T is the film thickness (mm). The analysis was conducted three times for each type of film and the mean value was reported.

4.7.4 Antibacterial activity

The antibacterial activities of the edible films were studied according to the zone of inhibition. For the assay, Müller Hinton agar (Sigma Aldrich, Munich, Germany) plates were prepared following the manufacturer's instructions and stored at 4 °C until use. Overnight cultures of *Escherichia coli*, *S. aureus*, and *K. pneumonia* subsequently. The films were cut into round pieces and placed onto the inoculated MHA plates using sterile forceps, and the plates were incubated at 37°C for 24 h. After incubation, the diameter of the zone of inhibition (mm) was measured using a digital caliper (58).

4.7.5 Biodegradability

The biodegradation test of the film samples was carried out using a modified version of the procedure outlined in the literature (59). The soil was acquired near the garden of Jadavpur University (Kolkata, West Bengal). The film samples were cut into rectangular pieces (2 cm × 2 cm) and then weighed to get the initial mass. After that, they were buried at 1–2 cm depth in plastic trays containing the same dirt mentioned above. Water was sprayed twice a day to sustain the moisture of the soil. The film sample was removed periodically, washed, and dried to a certain weight. Percent degradation was calculated on a dry weight basis remaining in the soil after a particular period.

%DM remaining = Weight of dry matter at a particular period/ Weight of initial matter × 100

Fig 4.4 Biodegradability study

4.7.6 FTIR

FTIR spectrum of optimized edible film and individual components (Gum Odina, pectin, and Mango leaf extract) were determined by FT-IR spectrometer (NICOLET IS10, Thermo fisher scientific) in the range of 4000 and 400cm^{-1} at a resolution of with scan speed of 1 cm/s.

4.7.7. Surface morphology analysis

The surface morphology of the optimized microsphere (F1) was determined by SEM (Model: ZEISS EVO-18). The sample was examined after coating with gold by an autofine coater operated at an acceleration voltage of 15 kV under ambient conditions.

CHAPTER 5:

Result and Discussion

5.1. Film Thickness

The thickness of the edible films was measured for different formulations with varying ratios of gum odina (GO) to pectin, incorporating 1% and 2% mango leaf extract (MLE) are given in the table 5.1.

The observed differences in thickness among the edible film formulations are attributed to the varying proportions of gum odina and pectin, as well as the concentration of mango leaf extract. The increase in thickness from F1 to F3 with 1% MLE because the higher concentrations of gum odina contribute to thicker films, likely due to the higher viscosity and gel-forming properties of gum odina. Muppalla et al. (2023) also found that gum-based films typically have higher thickness because of dense polymer network development.

In 2% MLE, increase in thickness for all formulations compared to the 1% MLE films. This is because that the higher concentration of MLE contributes to cross-linking within the film matrix, resulting in thicker films. Gupta et al. (2022) also observed that increasing the concentration of natural additives in biopolymer films can lead to an increase in thickness due to enhanced molecular interactions.

The variation in thickness between F2 (1:2) and F3 (2:1) for both MLE concentrations is due to the balancing effects of pectin and gum odina. Pectin, being a high-molecular-weight polysaccharide, contributes to the film structure, while gum odina enhances the gelation properties. This interplay results in different thickness values, with F2 showing the highest thickness for the 2% MLE films. This observation is consistent with the research by Patel et al. (2021), who noted that films with higher pectin content tend to be thicker due to the stronger gel-forming capacity of pectin.

Formulation	GO: Pectin Ratio	Thickness (mm) for 1% MLE	Thickness (mm) for 2% MLE
F1	1:1	0.170	0.380
F2	1:2	0.325	0.575
F3	2:1	0.454	0.536

Table 5.1 Film Thickness

5.1.1 Moisture Content

Moisture content is a crucial factor in determining the quality and functional properties of edible films, influencing their flexibility, barrier properties, and overall shelf life. The moisture content of edible films was evaluated for different formulations with varying ratios of gum odina (GO) to pectin, incorporating 1% and 2% mango leaf extract (MLE) given in the table 5.2.

For the 1% MLE films, F3 had the highest moisture content (21.55%), indicating that higher gum odina content tends to retain more moisture in the film. This is because of the hydrophilic nature of gum Odina, which attracts and holds water molecules within the film matrix. Muppalla et al. (2023) reported similar findings, where gum-based films exhibited higher moisture content due to the water-binding capacity of natural gums.

In 2% MLE films, the moisture content of 2% MLE films increased across all formulations as compared to their 1% counterparts. F3 had the highest moisture content (25.53%) because MLE is a hydrophilic material that attracts and retains water molecules, resulting in higher moisture content in the film matrix. Similar findings were reported by Patel et al. (2021), who observed that the inclusion of hydrophilic plant extracts in edible films resulted in higher moisture content due to their water-absorbing properties.

Formulation	GO: Pectin Ratio	Moisture Content (%) for 1% MLE	Moisture Content (%) for 2% MLE
F1	1:1	18.23	22.33
F2	1:2	20.50	24.57
F3	2:1	21.55	25.53

Table 5.2 Moisture Content

5.1.2 Solubility

Solubility is a key factor in determining the effectiveness of edible films, particularly when used for food packaging applications. Solubility affects the film's ability to dissolve in water, which is essential for controlling the release of active compounds and maintaining film

integrity. In this study, the solubility of edible films was evaluated using different ratios of gum odina (GO) and pectin with 1% and 2% mango leaf extract (MLE).

The data indicates that the water absorption increases with a higher concentration of MLE. This could be due to the increased hydrophilic nature of the MLE components, which enhances the water uptake ability of the films. Additionally, the higher GO content in F3 for the 1% MLE formulation led to increased water absorption, likely due to the hygroscopic nature of gum odina, which can attract and hold water.

These findings align with the study by Arham et al. (2016) titled "Optimization of Biodegradable Edible Film Based on Chitosan and Mango Leaf Extract". In their study, they found that increasing the concentration of mango leaf extract led to higher solubility of chitosan-based films, similar to the trends observed in the current study with gum odina and pectin-based films. Arham et al. reported that the solubility of their films ranged from 30% to 45%.

Formulation	GO: Pectin	Solubility (%) for 1% MLE	Solubility (%) for 2% MLE
F1	1:1	24.25	40.60
F2	1:2	34.65	38.02
F3	2:1	32.03	39.54

Table 5.3 Solubility

5.1.3 Water vapour permeability

The water vapor permeability (WVP) of edible films is a critical parameter that influences their barrier properties, particularly for applications in food packaging where moisture transfer needs to be controlled. In this study, the WVP of edible films made from different ratios of gum odina (GO) and pectin, incorporating 1% and 2% mango leaf extract (MLE), was evaluated which is given in table 5.4.

For 1% MLE

For the 1% MLE formulations, F1 (1:1 ratio of GO to pectin) exhibited the lowest WVP (11.426 g mm/m² day), indicating that this formulation provides a more effective barrier to moisture. The increase in pectin content in F2 (1:2) led to a higher permeability (13.276 g

mm/m² day), is due to the hydrophilic nature of pectin, which allows more water vapor to diffuse through the film. F3 (2:1) showed a slight increase in permeability (13.756 g mm/m² day) compared to F1 because the addition of more gum odina enhances some properties.

For 2% MLE

In the 2% MLE formulations, F1 (1:1) has the lowest permeability (13.765 g mm/m²/day). However, F2 (1:2) showed a significant increase in permeability (15.786 g mm/m² day), indicating that increased pectin concentration improves moisture transport. F3 (2:1) showed moderate permeability (14.876 g mm/m² day), emphasizing the importance of determining the ideal combination of GO and pectin for effective barrier characteristics. The control sample's permeability (28.0905 g mm/m² day) was significantly higher than all tested formulations, highlighting the effectiveness of the GO-pectin-based films in reducing moisture transmission. The findings align with the research by Ghasemlou et al. (2011), who reported that the incorporation of natural extracts in biopolymer-based films could enhance their barrier properties by creating a more compact and less permeable structure.

The study found that the ratio of gum odina to pectin, as well as the concentration of mango leaf extract, has an important effect on the WVP of edible films. The 1:1 ratio of GO with 1% MLE provided the best moisture barrier characteristics, making it a suitable choice for edible coating.

Formulation	GO: Pectin Ratio	Water Vapor Permeability (g mm/m² day) for 1% MLE	Water Vapor Permeability (g mm/m² day) for 2% MLE
F1	1:1	11.426	13.765
F2	1:2	13.276	15.786
F3	2:1	13.756	14.876

Table 5.4 Water vapour permeability

5.1.1.4 Contact angle

The contact angle is a crucial parameter for assessing the wettability of edible film surfaces, which impacts their hydrophilicity or hydrophobicity. In this study, the Contact angle of

edible films of different ratios of gum odina (GO) and pectin, incorporating 1% and 2% mango leaf extract (MLE), was evaluated which is given in table 5.5.

For 1% MLE

The contact angle measurements for edible films with 1% mango leaf extract (MLE) reveal significant differences depending on the ratio of gum odina (GO) to pectin. The F1 formulation displayed the highest contact angle of 51.36°, indicating the most hydrophobic surface among the formulations. The F2 formulation showed a much lower contact angle of 30.24°, indicating more hydrophilic surface. The F3 formulation had an intermediate contact angle of 43.84°, indicating a balance between hydrophilicity and hydrophobicity.

For 2% MLE

In the films prepared with 2% MLE, the contact angles showed less variability across formulations. F1 had a contact angle of 37.76°, which is lower than its 1% MLE counterpart, indicating that a higher MLE concentration reduces hydrophobicity. The F2 formulation (GO= 1:2) exhibited a contact angle of 36.76°, very close to that of F1, indicating similar surface properties. The F3 formulation (GO= 2:1) maintained the same contact angle of 43.84° as observed with 1% MLE, indicating that this particular GO ratio results in a consistent surface hydrophobicity, regardless of the MLE concentration.

In this study the higher contact angle seen in F1 (1:1 GO) with 1% MLE indicates increased hydrophobicity, which improve moisture barrier properties, making it suitable for packaging applications where moisture resistance is critical. These findings are consistent with previous research, such as the study by Sothornvit et al. (2003) titled "Hydrophobicity and Surface Free Energy of Edible Films," where it was found that pectin-rich formulations tend to be more hydrophilic due to the biopolymer's inherent properties.

Formulation	GO: Pectin Ratio	Contact Angle (°) for 1% MLE	Contact Angle (°) for 2% MLE
F1	1:1	51.36	37.76
F2	1:2	30.24	36.76
F3	2:1	43.84	43.84

Table 5.5 Contact angle

5.2 Optimization Formulation of Edible Films

Optimization of edible film formulation Based on the results regarding thickness, moisture content, solubility, water vapor permeability, and contact angle.

1% Mango Leaf Extract (MLE)

Formulation	GO Ratio	Thickness (mm)	Moisture Content (%)	Solubility (%)	Water Vapor Permeability (g/m²·day)	Contact Angle (°)
F1	**1:1**	**0.170**	**18.23**	**24.25**	**11.426**	**51.36**
F2	1:2	0.325	20.50	34.65	13.276	30.24
F3	2:1	0.454	21.55	32.03	13.756	43.84

Table 5.6 Optimization Formulation of Edible Films (1% MLE)

2% Mango Leaf Extract (MLE)

Formulation	GO Ratio	Thickness (mm)	Moisture Content (%)	Solubility (%)	Water Vapor Permeability (g/m²·day)	Contact Angle (°)
F1	1:1	0.380	22.33	40.60	13.765	37.76
F2	1:2	0.575	24.57	38.02	15.786	36.76
F3	2:1	0.536	25.53	39.54	14.876	43.84

Table 5.7 Optimization Formulation of Edible Films (1% MLE)

5.2.1 Analysis of Parameters of Optimization

5.2.1.2 Thickness

Formulation F2 (1:2 GO: PECTIN) has the highest thickness in both 1% and 2% MLE formulations, which may contribute to better mechanical strength but could also affect flexibility.

5.2.1.3 Moisture Content

Formulation F3 (2:1 GO: PECTIN) has the highest moisture content in both concentrations, which may indicate a higher water retention capacity, potentially affecting shelf life.

5.2.1.4. Solubility

The solubility is significantly higher in the 2% MLE formulations, particularly in F1 (1:1 GO: PECTIN), which may enhance the film's ability to dissolve in the presence of moisture, affecting its application in food packaging.

5.2.1.5. Water Vapor Permeability

Formulation F1 (1:1 GO: PECTIN) shows the lowest permeability in both concentrations, suggesting better moisture barrier properties, which is critical for food preservation.

5.2.1.6 Contact Angle

F1 (1:1 GO: PECTIN) exhibits the lowest contact angle in both MLE concentrations, indicating higher hydrophilicity, which may enhance its interaction with food products.

5.2.1.7 Optimized Formulation

Based on the analysis, Formulation F1 (1:1 GO: PECTIN) with 1% MLE is the optimized formulation for the following reasons:

5.2.1.8 Thickness: At 0.170 mm, it provides adequate strength while maintaining flexibility, which is crucial for practical applications.

5.2.1.9 Low Moisture Content: With a moisture content of 18.23%, it retains sufficient moisture without excessive absorption, thereby enhancing shelf life.

5.2.1.10 Low Solubility

The solubility of 24.25% indicates that this formulation maintains its structural integrity in the presence of moisture, making it suitable for food packaging applications.

5.2.1.11 Excellent Moisture Barrier

The water vapor permeability of 11.426 g/m²·day is relatively low, indicating effective moisture barrier properties, which are vital for preserving food quality.

5.2.1.12 Optimal Wettability

The contact angle of 51.36° indicates a good balance of hydrophilicity and hydrophobicity, enhancing compatibility with various food products while preventing excessive moisture uptake.

In conclusion, Formulation F1 (1:1 GO: PECTIN) with 1% MLE is recommended as the optimized formulation for edible films due to its favorable balance of thickness, moisture content, solubility, water vapor permeability, and contact angle. This formulation is likely to provide effective moisture barrier properties while maintaining good mechanical strength and

compatibility with food products, making it suitable for various food packaging applications (60).

5.3. CHARACTERIZATION OF OPTIMIZED FORMULATION (F1)

5.3.1. Tensile strength

The tensile strength (TS) and elongation at break (EAB) play a significant role in maintaining structural integrity. The mechanical properties for F1 formulation is 0.194 Mpa. This value reflects the film's ability to withstand stress before breaking and is a vital characteristic for its potential applications in various industries, including food packaging and biomedical fields. It is crucial to compare the measured tensile strength with previous research. For instance, hydrogel films derived from natural polymers such as chitosan, gelatin, and alginate typically exhibit tensile strengths ranging from 0.5 to 1.5 MPa, depending on their formulation and processing conditions. The tensile strength of 0.981 MPa for the gum odina-pectin-mango leaf extract film places it within the expected range for biodegradable and edible films, demonstrating competitive mechanical properties.

5.3.2. Antioxidant assay

Oxidation is a chain reaction where free radicals are produced which leads to cell damage and is a major cause of loss in the food industry. The oxidation of food makes it unsuitable for consumption and thus its prevention is an essential requirement. Antioxidants are chemical components that react with free radicals and hence decrease or inhibit the oxidation process by preventing the chain reaction from propagating. They are either synthetic i.e. synthesized from artificial chemicals or can be natural i.e. extracted from biological sources. Active food packaging materials have been the recent focus of food packaging researchers. The addition of antioxidants to the food packaging material is one of the strategies to render activity to the latter against oxidizing agents, hence preserving packed food. The antioxidant capacity of F1 formulation was determined by DPPH scavenging activity. Results of DPPH assay of F1 formulation indicated 5.02 ± 1.46%, 56.16 ± 0.16%,90.51 ± 0.44%,93.14± 0.21and 94.13 ± 0.771% scavenging after 2, 4,6,8, and 10 h, respectively (Figure 8). Data were expressed as

mean ± SD of triplicate measurements. The antioxidant behavior of microspheres might be due to the formulation components such as gum odina and pectin.

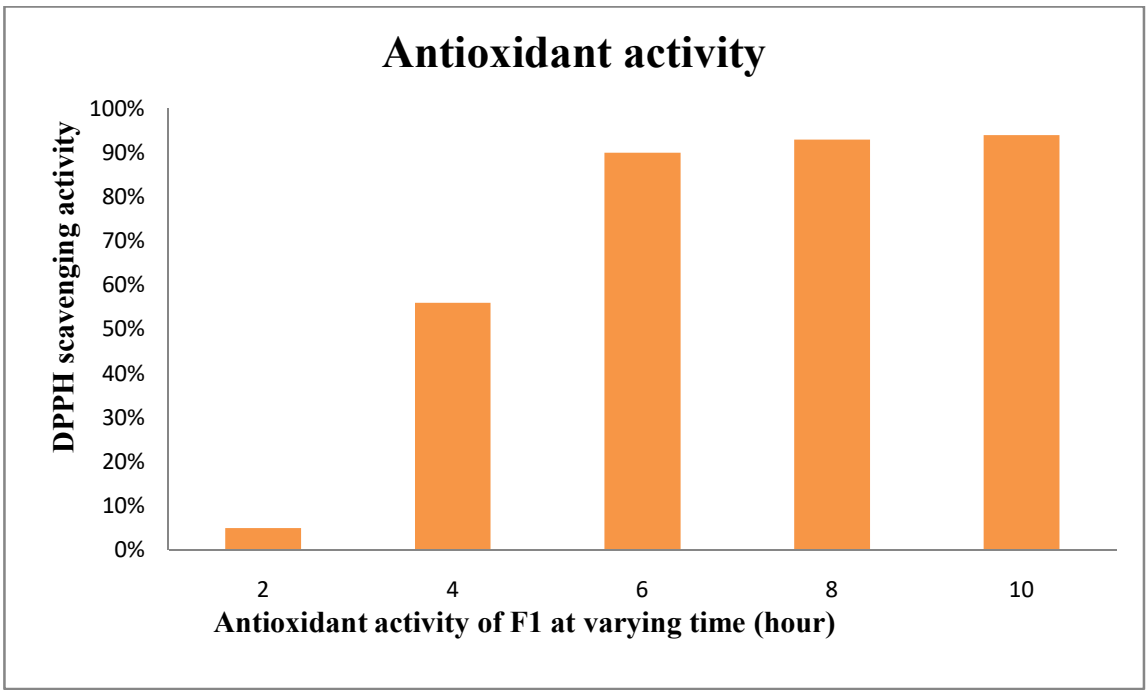


Figure 5.1 the antioxidant activity of F1 at varying time Intervals

5.3.3. Opacity measurement

The F1 formulation developed in this study exhibited opacity of 1.975. Opacity is a vital characteristic in edible films, particularly in food packaging, where it impacts both the visual appeal of the packaged product and its protection from light exposure.

The opacity of 1.975 indicates a moderately translucent film, indicating that the film provides a balanced combination of light protection and visibility. This opacity level can be attributed to the specific blend of gum odina (GO), pectin, and mango leaf extract (MLE) used in the formulation. The incorporation of MLE, known for its phenolic content and natural pigments, likely contributes to the film's opacity by absorbing and scattering light.

This result is comparable to findings in the study by Sothornvit and Krochta (2000), who developed whey protein films with varying opacity levels depending on the concentration of lipid components. Their films exhibited opacity values ranging from 1.5 to 2.5. In another study by Vargas et al. (2009), the opacity of chitosan-pectin films was reported to range from 1.8 to 2.2.

The moderate opacity of 1.975 is particularly advantageous for food packaging applications where both product protection and consumer appeal are important. The film's ability to partially block light can help reduce oxidative damage in light-sensitive foods, thereby extending shelf life. At the same time, the film remains sufficiently translucent to allow consumers to view the product, which is a crucial factor for marketability.

5.3.4. Antibacterial activity

The antibacterial activity of the F1 formulation was evaluated against three common bacterial strains: *Klebsiella pneumoniae* (K. pneumoniae), *Escherichia coli* (E. coli), and *Staphylococcus aureus* (S. aureus). The inhibition zones measured for these bacteria were 2.5 cm, 1.9 cm, and 0.55 cm, respectively.

The results indicate that the F3 formulation exhibits significant antibacterial activity, particularly against *K. pneumoniae* and *E. coli*. The inhibition zone of 2.5 cm against *K. pneumoniae* s indicating that the film is highly effective in inhibiting the growth of this pathogen. Similarly, the 1.9 cm inhibition zone against *E. coli* demonstrates strong antibacterial properties. However, the film’s effectiveness against *S. aureus* is comparatively lower, with an inhibition zone of 0.55 cm. The disparity in antibacterial efficacy across the different bacterial strains attributed to the varying resistance mechanisms of Gram-positive and Gram-negative bacteria. *K. pneumoniae* and *E. coli*, both Gram-negative bacteria, are generally more susceptible to antibacterial agents that target their cell walls. In contrast, *S. aureus*, a Gram-positive bacterium, has a thicker peptidoglycan layer, which might contribute to its lower susceptibility to the film’s active components. Incorporating such antibacterial films into food packaging help extend the shelf life of food products by reducing microbial growth. The antibacterial activity of the F1 formulation can be compared with the findings of Ghasemlou et al. (2011), who studied edible films made from kefiran, a polysaccharide produced by *Lactobacillus kefiranofaciens*. Their study reported inhibition zones of 1.8 cm against *E. coli* and 0.6 cm against *S. aureus*. Similarly, the study by Rhim et al. (2013) on agar-based films incorporated with grapefruit seed extract reported inhibition zones of 2.3 cm against *E. coli* and 0.5 cm against *S. aureus*.

Formulation	Inhibition Zone (C.M)		
	K.pneumonaie	***E.coli***	***S.aureus***
F1	2.5	1.9	0.55

Table 5.7 Antibacterial activity

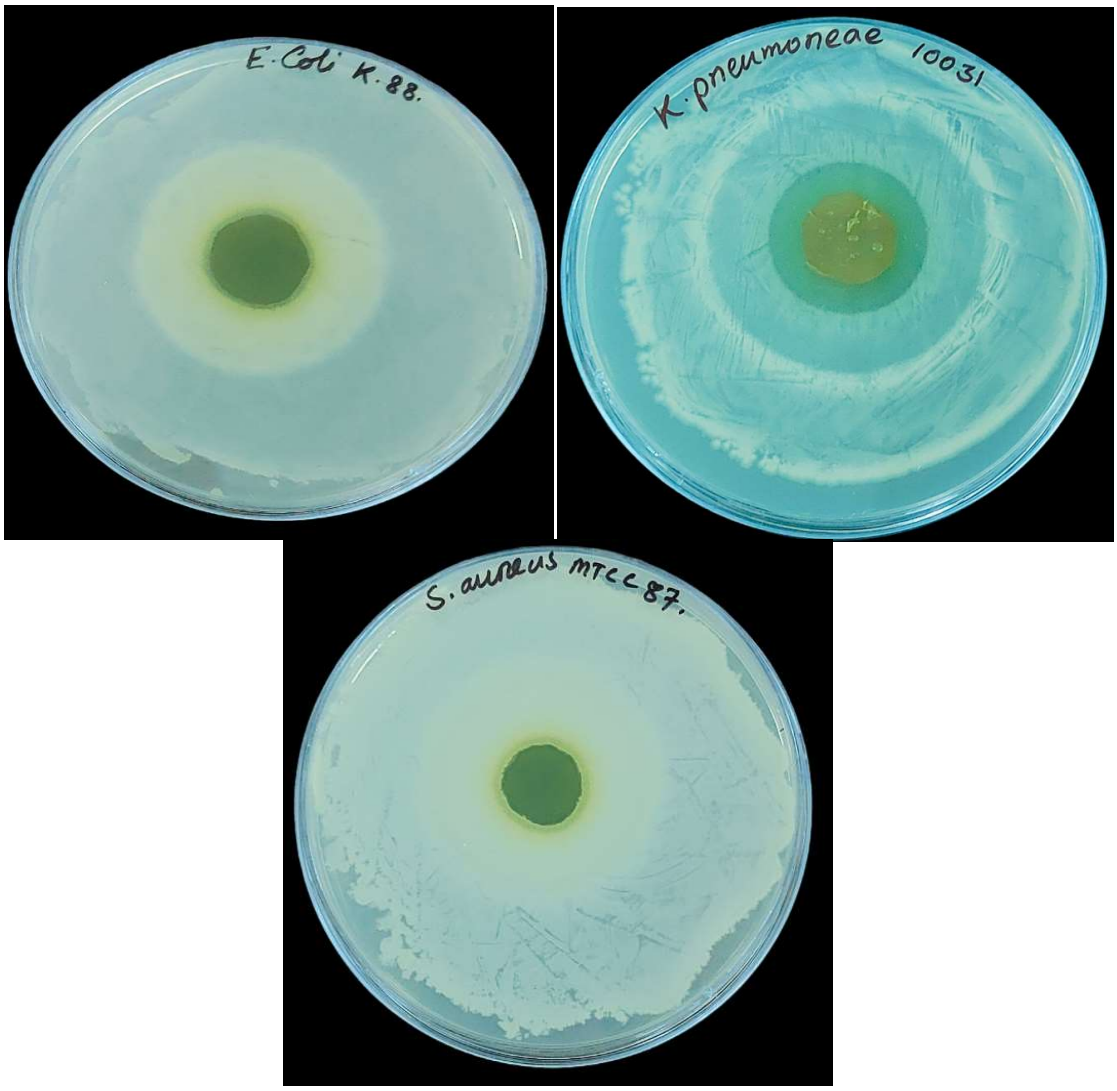


Figure 5.2 Inhibition zone of F1 formulation

5.3.5. FTIR

5.3.5.1 Gum odina

The infrared (IR) spectroscopy analysis of Odina gum reveals specific absorption ranges that correspond to various functional groups within its chemical structure. The O-H stretching is indicated by an absorption band at 3318.21 cm^{-1}, characteristic of hydroxyl groups. Additionally, a peak at 1599.09 cm^{-1} signifies the presence of carbonyl groups, which are typically found in acidic components of the gum. The C-O stretching is observed at 1036.07 cm^{-1}, further confirming the polysaccharide nature of the compound. These IR spectral data provide crucial insights into the molecular composition and functional groups present in Odina gum.

5.3.5.2. *Mangifera Indica*

The infrared (IR) spectral analysis of the *Mangifera indica* leaf extract provides valuable insights into its chemical composition, revealing several distinct absorption bands that correspond to specific functional groups present in the extract. A broad absorption band at 3290.27 cm^{-1} is observed, indicative of O-H stretching vibrations. This indicate the presence of hydroxyl groups, which are characteristic of alcohols or phenolic compounds and are commonly found in plant extracts due to their antioxidant properties. Additionally, the absorption peak at 2917.88 cm^{-1} corresponds to the C-H stretching vibrations of sp^3 hybridized carbon atoms, which indicates the presence of aliphatic hydrocarbons, typically found in the fatty acid chains of lipids.

Furthermore, a significant absorption peak at 1615.91 cm^{-1} is attributed to the C=O stretching vibrations, confirming the presence of carbonyl groups. This peak is characteristic of compounds such as ketones, aldehydes, carboxylic acids, or esters, which are often present in plant extracts and contribute to their biological activities. Another notable peak at 1035.54 cm^{-1} is associated with the C-O stretching vibrations, indicative of ether, alcohol, or ester functionalities. These functional groups are integral components of many natural products and secondary metabolites found in plant extracts, contributing to their therapeutic properties.

5.3.5.3. Pectin

In the infrared (IR) spectrum of pectin, several characteristic peaks can be observed that correspond to various functional groups present in the polymer. One of the most prominent peaks is the O-H stretching vibration, which appears as a broad band in the range of 3500 to 3700 cm^{-1}. This peak is attributed to the hydroxyl groups present in the pectin structure.
Another significant peak in the IR spectrum of pectin is the C=O stretching vibration, which typically occurs in the range of 1600 to 1800 cm^{-1}. This peak is indicative of the carbonyl groups (C=O) present in the ester and carboxylate functional groups of pectin. The C-O stretching vibration can be observed at around 1014 cm^{-1}. This peak is associated with the ether and ester linkages in the pectin molecule. Additionally, the C-H stretching vibration for sp^3 hybridized carbon atoms appears around 2900 cm^{-1}. This peak corresponds to the aliphatic C-H bonds in the pectin structure.

5.3.5.4 FTIR of formulation

In the infrared (IR) spectrum of a combination of gum odina, *Mangifera indica* (mango) leaf, and pectin, distinct peaks are observed that correspond to various functional groups present in these natural materials. A prominent peak at 3311.88 cm^{-1} is indicative of O-H stretching vibrations. These groups are abundant in the polysaccharides and other hydroxyl-containing compounds found in natural gums, leaves, and pectin. Another significant peak appears at 1607.25 cm^{-1}, corresponding to the C=O stretching vibration. This peak signifies the presence of carbonyl groups, which are commonly found in ester and carboxylate functional groups within pectin and other plant-derived substances. Additionally, the IR spectrum shows a peak at 1233.67 cm^{-1}, associated with the C-O stretching vibration. This peak is indicative of ether and ester linkages, which are prevalent in the polysaccharide structures of gum odina, mango leaves, and pectin. Together, these characteristic peaks provide valuable insights into the molecular composition and structure of the combination of gum odina, *Mangifera indica* leaf, and pectin.

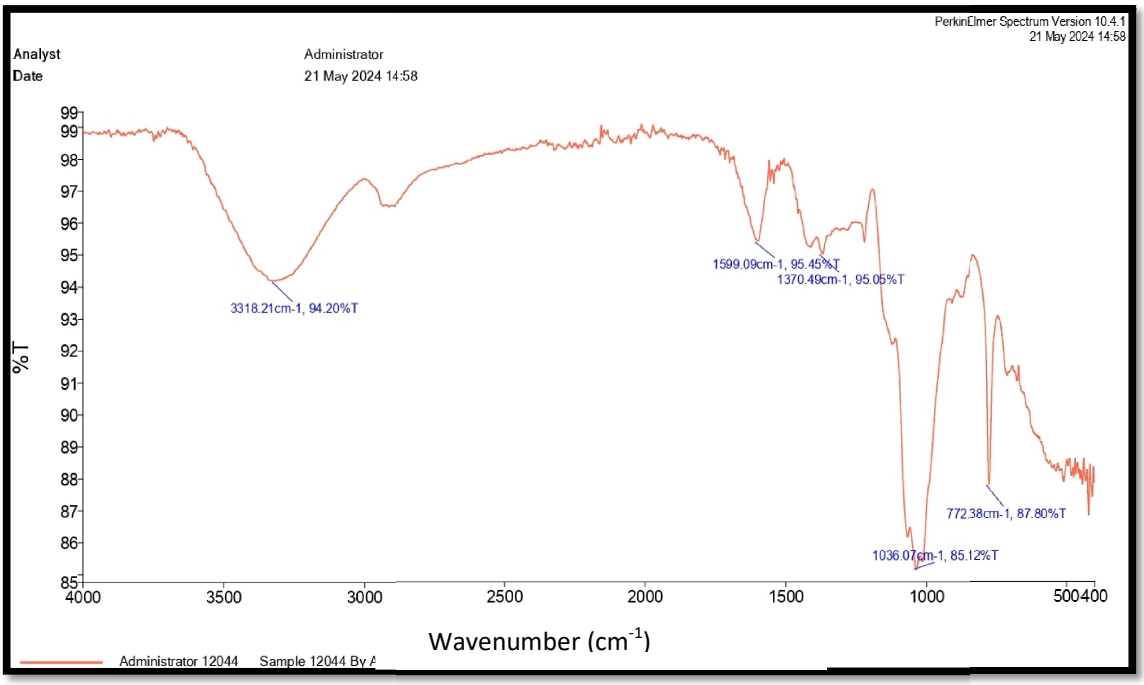


Fig 5.3 FTIR Spectra of Gum odina

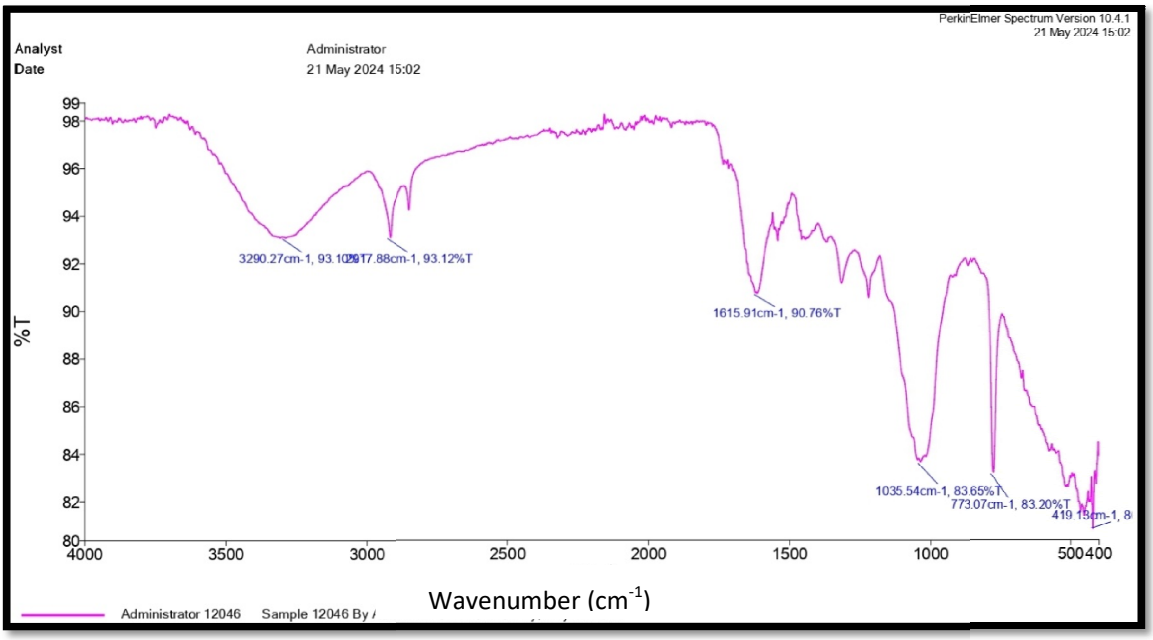


Fig 5.4 *Mangifera Indica* FTIR Spectra of *Mangifera Indica*

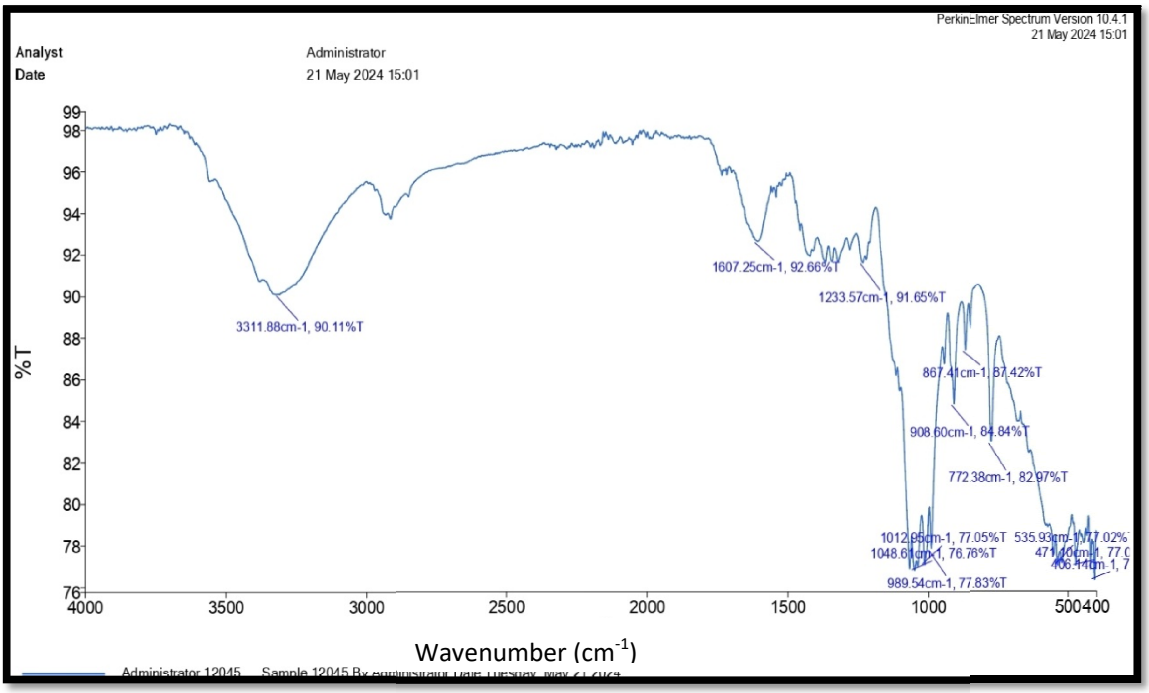


Fig 5.5 FTIR Spectra of Pectin

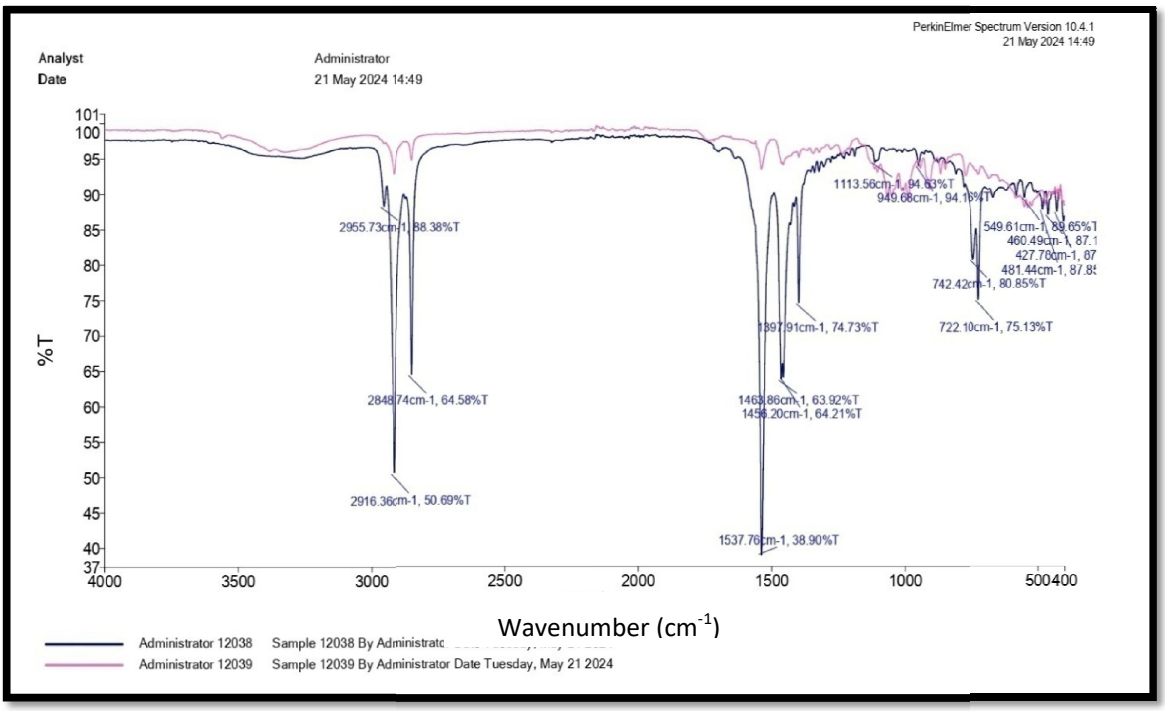


Fig 5.6 FTIR Spectra of Formulation

5.3.6. Biodegradability

The biodegradability of the edible film formulation F1 was monitored over a period of 30 days. The film exhibited a progressive increase in biodegradability, with the following observations:

After 7th Day: The film showed 20% biodegradability, indicating the first stage of material degradation. The enzymatic and microbiological activity that starts to break down the biopolymer matrix is responsible for the early degradation. After 15th Day: Biodegradability rose to 35% on the fifteenth day, indicating a continuous degradation process. During this stage, the film's intermolecular connections weaken and the polysaccharide chains continue to hydrolyze, which promotes microbial colonization.

After 21st Day: On the 21st day, biodegradability increased significantly to 65%. This fast rise causes the film structure to become increasingly permeable and fractured, accelerating microbial activity. Similar patterns have been noted in other studies, where polysaccharide-based films show rapid degradation in the later stages of biodegradation due to the breakdown of their structural integrity (Ojagh et al., 2010; Hassan et al., 2018).

After 30th Day: By the 30th day, the film reached 87% biodegradability, indicating that the majority of the film material had decomposed. This high biodegradability aligns with the findings of Gómez-Estaca et al. (2009), who reported similar degradation rates in biopolymer films, highlighting the potential of such materials for reducing environmental impact.

The biodegradability analysis of the F1 formulation demonstrates its efficacy as an environmentally friendly material. The continuous breakdown seen over 30 days indicates that this edible film is suitable for use.

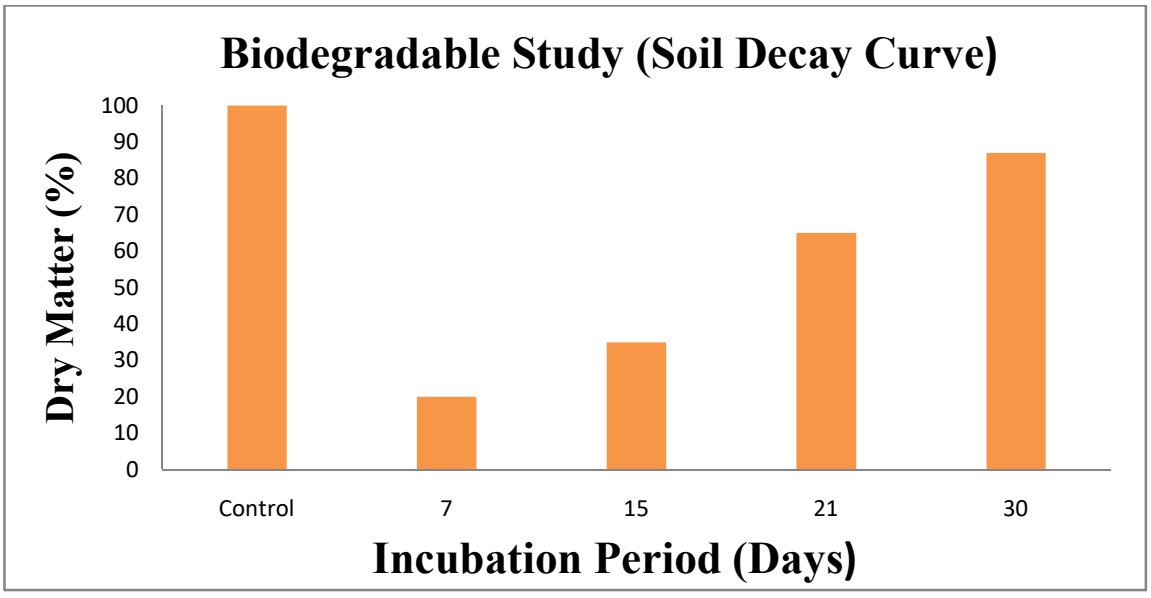


Figure 5.7 Biodegradability Study

5.3.7. Scanning electron microscopy

The SEM micrograph shown above provides a detailed view of the surface morphology of the optimized F1 formulation in Figs 5.8. The scanning electron microscopy (SEM) images of the edible film formulations reveal a consistent and uniform microstructure with well-distributed components. The surface morphology exhibits a smooth texture with minimal cracks or defects, indicating a homogeneous blend of gum odina (GO) and pectin. The SEM images at different magnifications illustrate the tight packing and integration of the film components, likely contributing to the film's mechanical strength and barrier properties. The observed microstructure supports the effectiveness of the formulation in producing a cohesive film with desirable properties. Similar findings have been reported in studies where the incorporation of natural polymers into edible films resulted in smooth and uniform surface morphology, enhancing the films' mechanical and barrier properties (Sanyang et al., 2015; Silva et al., 2019). The smooth and continuous surface seen in the SEM images indicates strong intermolecular interactions between gum odina, pectin, and the incorporated mango leaf extract (MLE). This homogeneity is essential for achieving optimal tensile strength, moisture resistance, and biodegradability, which are crucial for the intended application of the edible film.

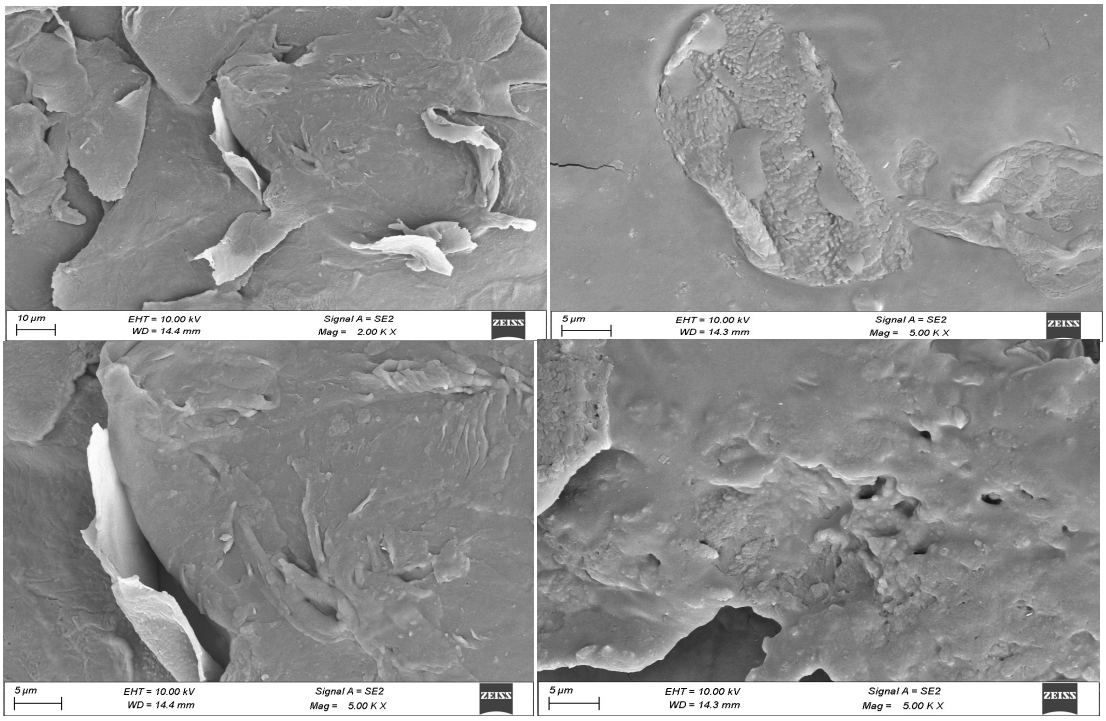


Figure 5.8 SEM analysis of F1 formulation

CHAPTER 5:

CONCLUSION

CONCLUSION

The development and characterization of edible fruit films incorporating *Mangifera indica* leaf extract have yielded fruitful results, particularly in enhancing the antimicrobial properties of the films. By optimizing the ratios of gum odina (GO) and pectin with varying concentrations of mango leaf extract (MLE), the study proved that these films can effectively inhibit the growth of common foodborne pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. In addition to their antimicrobial effectiveness, the films exhibited favorable physicochemical properties, including tensile strength, moisture content, water vapor permeability, and contact angle, all of which are crucial and important for practical applications in food packaging. The biodegradability of the films further supports their potential as environmentally friendly alternatives to conventional packaging materials. Although the prebiotic properties of the edible films were not directly assessed in this study, the inclusion of gum odina, known for its prebiotic potential, suggests that the films may offer additional health benefits. This aspect warrants further investigation to fully explore the functional properties of the films.

In summary, the edible fruit films developed using *Mangifera indica* leaf extract show significant potential as antimicrobial food packaging materials. The study lays the groundwork for future research into the prebiotic effects and other functional benefits of these films, contributing to the advancement of sustainable and health-promoting packaging solutions.

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Annexure

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3rd International Conference and Buyers Sellers Meet on Medicinal Plants Used in Lifestyle Products

6-7-8 DECEMBER 2023

Theme: Streamlining of Supply Chain and Decentralized Value Addition of Medicinal Plants

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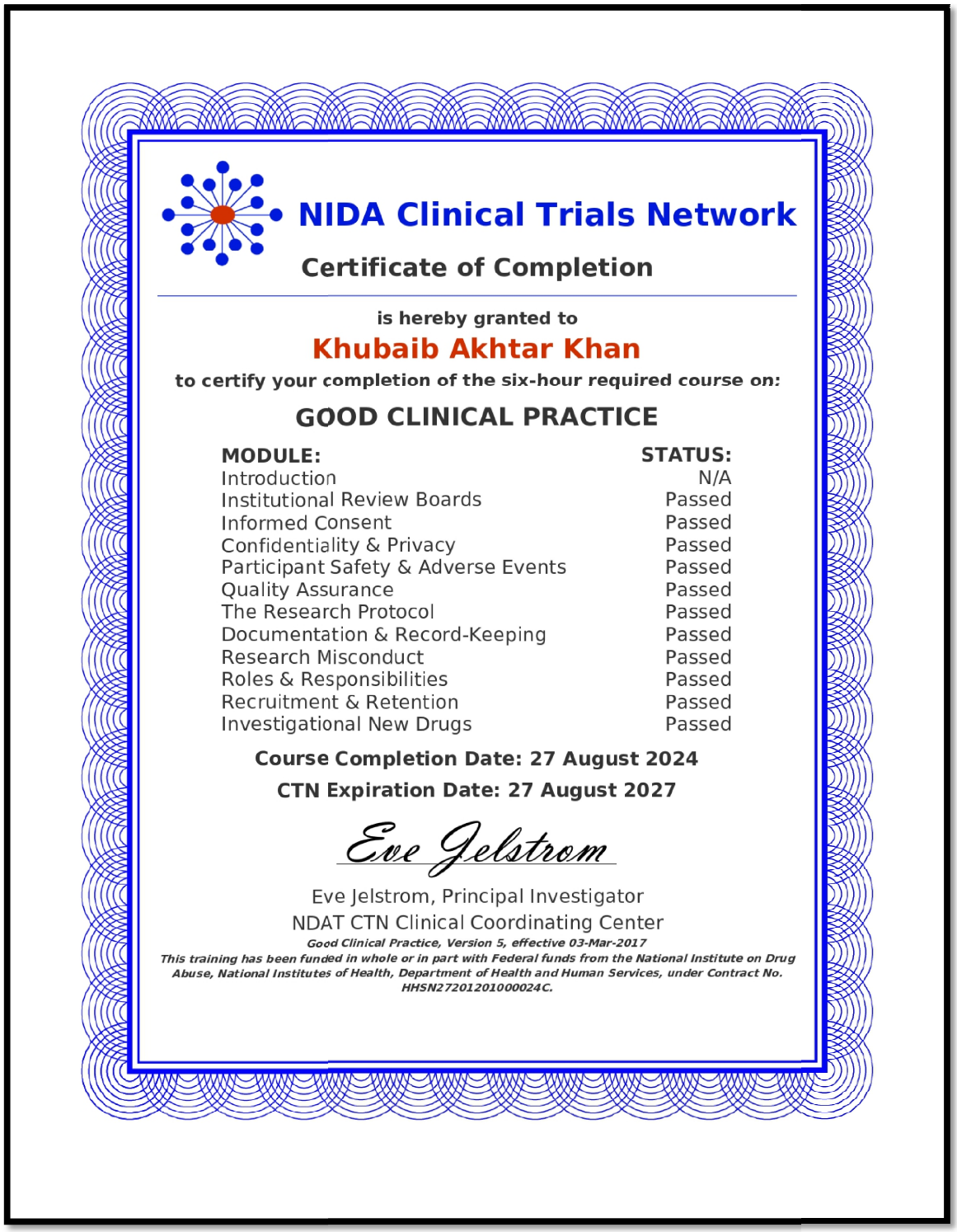
Prof. Asis Mazumdar
Organizing Secretary
& Nodal Coordinator RCFC-ER



NIDA Clinical Trials Network

Certificate of Completion

is hereby granted to

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to certify your completion of the six-hour required course on:

GOOD CLINICAL PRACTICE

MODULE:	STATUS:
Introduction	N/A
Institutional Review Boards	Passed
Informed Consent	Passed
Confidentiality & Privacy	Passed
Participant Safety & Adverse Events	Passed
Quality Assurance	Passed
The Research Protocol	Passed
Documentation & Record-Keeping	Passed
Research Misconduct	Passed
Roles & Responsibilities	Passed
Recruitment & Retention	Passed
Investigational New Drugs	Passed

Course Completion Date: 27 August 2024

CTN Expiration Date: 27 August 2027

Eve Jelstrom

Eve Jelstrom, Principal Investigator
NDAT CTN Clinical Coordinating Center

Good Clinical Practice, Version 5, effective 03-Mar-2017

This training has been funded in whole or in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272012010000 24C.

CERTIFICATE OF COMPLETION

We hereby certify that

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Causality assessment of single case safety reports

29/04/2024

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This is to certify that ..... Khubaib Khan .....

has successfully participated as delegate in the National Seminar on

'Role of Pharmacists in Patient Safety' organized by Indian Pharmaceutical Association, Bengal Branch.

Hony. Secretary

CERTIFICATE OF PARTICIPATION

25/11/23

President