Moroccan Journal of Chemistry
ISSN : 2351-812X

Mor. J. Chem., 2023, Volume 11, Issue 4, Page 1100-1115
https://revues.imist.ma/index.php/morjchem
https://doi.org/10.48317/IMIST.PRSM/morjchem-v11i04.40210

Development of biopolymeric film using natural polymer extracted from *Muntingia calabura* leaves

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Received *18 May 2023,*
Revised *04 Aug 2023,*
Accepted *06 Aug 2023*

Citation *Nivetha A., Christina Ruby Stella P., Irudaya Irin Sceelva P., Keerthana D., (2023) Development of Biopolymeric Film Using Natural Polymer Extracted From Muntingia Calabura* Leaves*, Mor. J. Chem.,11(4), 1100-1115*

Abstract: Over the last decade, there has been an increase in research on innovative biobased products like food packaging, mostly due to concern about worldwide environmental plastic pollution. The use of plant-based natural polymer in the preparation of durable biopolymers and functional polymeric materials offers a viable alternative to synthetic polymers. Their popularity has grown in part due to the environmental degradation created by improper disposal of plastic packaging. Adequate hydroxyl moiety, quick accessibility, environmentally-safe, and reaction affinity with other substances make cellulose a basic factor for polymeric synthesis and for preparing antimicrobial food packaging for the shelf life extension of perishable foods. This work aims to prepare a film-forming polymeric solution with leaves of *Muntingia calabura* (MC) as an active ingredient for the formation of the *Muntingia calabura* cellulose-based biopolymeric film (MCCF). Cellulose, extracted from the leaves of MC and the leaf extract were used for the preparation of the biopolymeric film. The properties of cellulose-based film were studied. The XRD and FTIR spectroscopy recognized the interaction of the cellulose/Poly vinyl alcohol blend; the morphology of the film was studied by SEM, where we can understand the surface of the film and texture of the extracted cellulose. The prepared MCCF shows promising antimicrobial activity against bacteria and fungi present in the oral environment. Hence this film could be suitable for food packaging and wound healing applications. The results of the present investigation suggest that the biosynthesized polymeric film from *Muntingia calabura* leaves has a good potential to be a remarkable technological innovation to prevent microbiological contamination to ensure food safety and preservation.

Keywords: Hydroxyl moiety, *Muntingia calabura,* Shelf-life

1. Introduction

Muntingia calabura (MC) extract is employed in biomedical applications such as anticancer, antibacterial, antiviral, and antiseptic. The biological functions of MC have been thoroughly investigated, and the majority of studies have demonstrated its diverse range of pharmacological effects, including anti-inflammatory, anti-diabetic, antibacterial, antioxidant, and antiviral properties (SA Sari *et al.,* 2020). The evergreen MC plant has irregularly shaped irregularly toothed, alternating, oblong leaves that are long, sharp at the apex, and oblique at the base. The MC leaves

are bright green in colour and have tiny hairs on the top of it (Panneerselvam *et al.,* 2020). Over the last decades, some plants had the efficacy to treat many diseases caused by specific microorganisms. Now we are using those treatments as antimicrobials. MC is one of the plants with great antimicrobial characteristics. The chosen research plant has a long history of usage in traditional folk medicine among the peoples of Peru, Cambodia, Columbia, Vietnam, and the Philippines, and scientific studies on the leaves, in particular, have revealed numerous anti-microbial, anti-inflammatory, and antioxidant capabilities (Gurning *et al.,* 2020).

Figure 1. *Muntingia calabura* leaf part was used for the extraction of cellulose

Phytochemical screening is the scientific process of evaluating, and identifying different varieties of phytoconstituents existing in different parts of the plants which are the base for the development of drugs, the major components might be taken for various research and study. Phytochemical screening not only exposes the composition of plant extracts and which one pervades over others, but it also assists in the search for bioactive agents that can be used in the development of valuable medications (Reddy *et al.,* 2020). Several flavonoids and phenolic chemicals isolated from MC leaves have previously been demonstrated to inhibit the majority of microorganisms found in the oral environment. Plants have been utilized to cure common infectious diseases from ancient times, and some of these natural remedies are still used in the routine treatment of different diseases (Ansori *et al.,* 2019). There are some researches carried out that analyze the potential antimicrobial crude drugs extracted from MC leaves as well as a source for natural compounds identified, that act as new anti-infection agents. Despite the fact that many researchers have studied MC, none of them have worked on cellulose extraction from MC for eco-friendly packaging material and skin tissue healing. Eco-friendly food and other packaging materials have recently been developed in response to rising concerns about the disposal of plastic bags. The research on biodegradable films has expanded to lessen the negative environmental effects of synthetic packaging materials (Rodsamran *et al.,* 2019; Tabaght *et al.,* 2023). Mostly synthetic materials derived from non-renewable sources are used for the preparation of food packaging material. However, the use of synthetic materials for food packaging is regarded environmentally unfriendly due to the development of massive volumes of non-biodegradable solid waste. The usage of packing, on the other hand, is crucial. Aside from its primary purpose of enclosing food, it also reduces the direct interaction between food products and the environment. This can improve the maintenance of food quality. Products and by-products obtained from agriculture or agro-industries are renewable resources that are used in the recent research for preparing the biopolymeric film. Biopolymer-based films are often highly sensitive to environmental conditions and have limited mechanical resistance. To improve these qualities, the films are mixed with appropriate synthetic polymers (Ju *et al.,* 2019).

Because of their biological origin and effectiveness, plant-derived bioactive chemicals are being regarded as attractive elements for the development of biodegradable and bioactive films. The utilization of renewable, sustainable, and environmentally friendly materials has gained popularity due to increasing global awareness (Moshi *et al.,* 2020). Since biopolymers disintegrate quickly in the environment and resemble the characteristics of ordinary polymers, they can be employed as a remedy to the issues caused by plastics. The usage of natural polymers in the medicinal and industrial applications has significantly increased during the last several years. Finding new prospective resources is therefore crucial for the long-term sustainability of raw materials in industries. As a result, researchers from all over the world seem to become interested primarily in using bio-based composites due to its numerous benefits, notably their high mechanical strength, biodegradability, non-toxicity, low cost, and availability (Rodsamran *et al.,* 2019: Ju *et al.,* 2019). The plant cell wall is made up of bundles of cellulose chains that are arranged in microfibrils. This arrangement shows that cellulose is a biomaterial with outstanding mechanical qualities and great strength, in addition to assisting in the stability of plant structures. The core support for trees and other plants, cellulose is the most abundant polymer on Earth in terms of quantities. Wood, which contains 40–50% cellulose by weight, is the main source of industrial cellulose (Moshi *et al.,* 2020). By using chemical processes, such as sulphate pulping, which involves treating wood chips with sodium hydroxide and sodium sulphide solution while under high pressure and heat, lignin and hemicellulose are removed from the wood in order to isolate the cellulose. Cellulose fibers which are extracted from wood pulp have chain lengths of 500-2000 glucose molecule. An insoluble, densely packed crystalline substance is produced by the regularity of the cellulose chain and the potent hydrogen bonding between the hydroxyl groups of adjacent chains (Ravindran *et al.,* 2020). These potent intermolecular interactions prevent cellulose from having plastic characteristics, and instead cause thermal disintegration to take place before the material softens during heating. The strong intermolecular interactions can be reduced, resulting in solubility and enhanced softness of the polymer, by replacing the hydroxyl groups with bigger groups, such as nitrate or acetate (Ravindran L *et al.,* 2019; Prado *et al.,* 2019). There has been a rise in interest for using natural additions with antioxidant qualities derived from plant extracts as replacements as a means of reducing the use of added chemicals in the food sector. However, the majority of the time, the direct addition of natural chemicals may have a negative impact on the flavour of food products. The inclusion of these natural antioxidants into films seems to be a better procedure to achieve slow release of the additives into the food throughout its shelf life (Tesfay *et al.,* 2017). Packaging is thought to be a significant way to improve the shelf life of food products while retaining food quality. Active packaging is a technological marvel that extends the shelf life of food products by protecting them from microbiological decomposition and chemical deterioration through the addition of appropriate components to the packaging material (Kanatt *et al.,* 2020). The use of plant extract as an antibacterial agent in food wrapping is advancing technology, and one of the hot subjects in this sector is food-borne disease protection. Antimicrobial packaging films have found successful in killing or controlling the growth of food-borne pathogens. Natural polymers have recently been utilised in the manufacture of antimicrobial packaging films. However, the efficacy of polymer films in food packing is limited. Given the toxic effects of plant extract to a broad range of microorganism the combination of polymer and plant extract may be a preferable solution for biodegradable food packaging applications (Liu *et al.,* 2021). Certain biopolymer packaging may have strong barrier features, antibacterial and antioxidant properties depending on the composition. Thus, polysaccharides and proteins can be mixed to generate a variety of composite films with

superior mechanical and biological properties, making them appropriate for food packing (Elidrissi *et al.,* 2012). Biodegradable packaging stuffs has gained a lot of attention due to the hazards of using conventional oil-based polymers. However, the prime aspects of biodegradable polymers are not similar to those of conventional polymers. Most recent researches have concentrated on exploring different bio-polymers that, in addition to having beneficial qualities, also have the other characteristics required for active food packaging (Iniguez-Moreno *et al.,* 2021). Even though there has been a increasing interest in the researches in this field, accessibility of antimicrobials and new polymeric materials, appropriate testing procedures hinder the developments. As previously stated, MC has numerous beneficial effects in medicine and other fields. Hence this study sought to prepare a natural polymeric based film using plant extract.

2. Methodology

2.1 Collection and Processing of Plant Samples

The plant samples of *Muntingia calabura* were gathered from south of Pudukkottai district, Tamilnadu. The MC leaves were sorted to separate dead and decaying leaves. The separated fresh leaves were washed and dried under shade. Then the plant leaves were pulverized and stored in airtight containers

2.2 Preparation of plant extract

The dried *Muntingia calabura* leaves were weighed into a Soxhlet extractor thimble and the extraction set up was made with condenser. 200ml of absolute ethanol was used as a solvent. A heating mantle was used to reflux the blend for 8 hours. Then the extract was collected and bring into room temperature. Then, filtered through a cone of filter paper (Whatman no. 1) and stored in the refrigerator for further use (Desrini *et al.,* 2018; MosaChristas *et al.,* 2022).

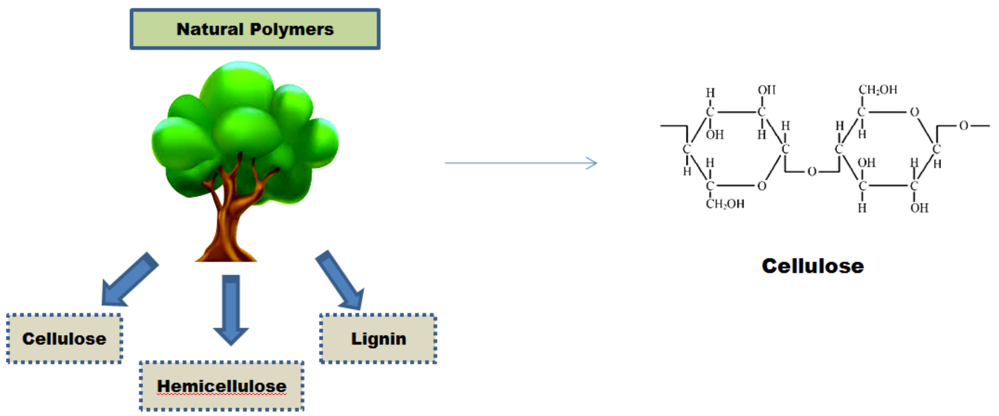


Figure 2. Natural polymers and chemical structure of cellulose

2.3 Extraction of cellulose from plant sample

The powdered leaves were processed with NaOH at 120°C for 2 hours before being bleached with $NaClO_2$ and 125°C for 4 hours (Vijay *et al.,* 2021). The leaves to solvent ratio were 1:20 (g/ml). The leave sample was washed with double distilled water after each step. Acid hydrolysis was performed using HCl solution at 40°C. To remove the excess hydrochloric acid, the hydrolyzed cellulose was centrifuged five times at 6,000 rpm for 5 min. The mixture was then dialyzed using distilled water until it attained a constant pH. The resulting suspension was filtered and kept for drying (Raghunathan *et al.,* 2022; Asrofi *et al.,* 2017).

2.4 Preparation of MCCF

0.2g of PVA was prepared by stirring and appropriate quantities of D.D water at 60° C for 6 hours, subsequently cooling to room temperature, and then the plant extract along with extracted cellulose was added to it (Abdulkadir *et al.,* 2022). The suspension was stirred for 6 hours to let the polymer penetrate into the cellulose network. Finally, films were cast onto a glass plate with controlled leveling, and dried at room temperature for 7 days. After drying, the MCCF was peeled out from the glass plate and used for further analysis (Ibrahim *et al.,* 2010; Ramesh *et al.,* 2019).

2.5 Phytochemical analysis

Qualitative phytochemical screening involves only the presence and absence of different plant secondary constituents. To achieve this goal, modified biochemical assays were developed (Akhtar *et al.,* 2018; Mahmood *et al.,* 2019). The phyto constituents found in the specified plant extract were analysed using conventional chemical techniques (Lokpur *et al.,* 2020; Haddou *et al.* 2022). The plant extract screened for the presence of flavonoids, alkaloids, terpenoids, phenol, tannins, saponins, carbohydrates, and glycosides by using the standard procedures (Bonomo *et al.,* 2020).

2.6 Determination of Ash and Moisture content of extracted cellulose

2.6.1. Moisture content

0.2 g of extracted cellulose were placed in a crucible and maintained at 100±2° C for 3 hours in an oven (Shanmugasundaram *et al.,* 2018). The resultant cellulose was stored in a desiccator until it reached room temperature before being weighed. The moisture content of the extracted cellulose was analysed by the equation (Jabli *et al.,* 2018).

$$\text{Moisture content of the cellulose (\%)} = \frac{(m-m')}{m} \times 100 \qquad \textbf{Eqn. 1}$$

Where, m – weight of the sample before dying m'- weight of the sample after dying

2.6.2. Ash content

The evaluation of ash content in the extracted cellulose was carried out by maintaining cellulose at 105°C for 3 hours (Ebrahimi *et al.,* 2019). Then it was sieved with a colander. The resultant substance was treated under muffle furnace at 500°C for an hour. The ash was then cooled in a desiccator and weighed (Lee *et al.,* 2019).

$$\text{Ash content of the cellulose (\%)} = \frac{(y-y')}{y} \times 100 \qquad \textbf{Eqn. 2}$$

Where, y – weight of the sample before dying y'- weight of the sample after dying

2.7 Volatile mass fraction

Around 1cm diameter of a dried piece of MCCF was weighed and placed in an oven at 100°C for 10 hours. The dried mass of the MCCF was cooled and weighed. The weights of the sample were utilized for the calculation of volatile mass fraction. The volatile mass fraction of the prepared film was calculated using the equation below:

$$\text{Volatile mass fraction (\%)} = \frac{(w-w')}{w} \times 100 \qquad \textbf{Eqn. 3}$$

where, w – weight of the sample before dying w'- weight of the sample after dying

2.8 Biodegradability

The biodegradability test was performed by the soil burial method. The soil was collected near the roots of plants which are rich in nitrogenous bacteria and stored in a container (Abdollahi *et al.,* 2019). The sample piece of the MCCF was buried in the soil at two different depths, 2cm (F1), and 3cm (F2). The sample was weighed before and after burial. The biodegradability of the film was measured by the following equation (Anggraeni *et al.,* 2021; Priyadarshi *et al.,* 2020; Tabaght *et al.,* 2019).

$$\text{Weight loss (\%)} = \frac{(w_a - w_b)}{w_a} \times 100 \qquad \textbf{Eqn. 4}$$

Where, w_a - initial weight of the sample w_b - final weight of the sample

2.9 Water Solubility

This test was performed to determine how quickly the MCCF dissolves in water. The small piece of film was trimmed and weighed accurately. Then it was stirred with water. The stirring was set at 150 rpm for 5 hours. After 5 hours of stirring, it was filtered and dried at 100°C. The dried mass was weighed and accounted for the estimation.

$$\text{Water Solubility (\%)} = \frac{(S_i - S_f)}{S_i} \times 100 \qquad \textbf{Eqn. 5}$$

Where, S_i & S_f – initial and final weight of the sample

2.10 Swelling ratio

To measure the swelling degree, 0.05g of measured sample was dried at 50°C in a vacuum oven for 6 h and the weight of the dried sample was determined (W_d). The dried samples were soaked in distilled water (with three different pH value), maintained and incubated at 37 °C, then weighed (W_s) at specific time intervals. The swelling ability of biopolymeric film was determined using the following Equation (Ezati *et al.,* 2020).

$$\text{Swelling degree (\%)} = \frac{(w_s - w_d)}{w_d} \qquad \textbf{Eqn. 6}$$

2.11 Characterisation of Biopolymeric film

2.11.1. Fourier-transform infrared spectroscopy

FTIR analysis of the biopolymeric film was examined through the potassium bromide (KBr) pellet (FTIR grade) method in 400 – 4000 cm^{-1} and spectrum was documented using Jasco FTIR-6300 Fourier transform infrared spectrometer loaded with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm.

2.11.2. Morphological analysis

Morphological investigation of the biopolymeric film was performed using the Scanning Electron Microscopy (SEM) machine model JEOL JEM-7500F (Japan, Tokyo) operated at a 2kV accelerating voltage.

2.11.3. X-ray diffraction

The crystalline nature of biopolymeric film was assessed by X-ray diffraction pattern analysis at ambient temperature using CuKα radiation throughout 2theta range of 10 to 90° with a scanning speed of 1.2min.

2.12 Antimicrobial activity

Antimicrobial activity against *Bacillus subtilis, E.coli, and Candida albicans* were examined adopting a well diffusion method. The efficacy of the sample was determined by the clear zone of inhibition in the cultures. Strains used for the activity were derived from Eumic analytical Lab, Trichy. The nutrient agar medium was developed and placed into sterilized Petri plates and rests it at room temperature. A well cutter was used to create wells in the Petri plates. The dried MCCF was dissolved and the suspension was added to each well aseptically. This procedure was repeated for every Petri plates then they were incubated at 37˚C for a day. Antimicrobial activity was assessed from the zone of inhibition at the end of the incubation time.

3. Results and Discussion

3.1 Phytochemical analysis:

MC leaves were evaluated qualitatively for the presence and absence of different secondary metabolites by applying devised biochemical tests flavonoids, alkaloids, terpenoids, phenol, tannins, saponins, carbohydrates, and glycosides. The results are shown in the **Figure 3** and **Table 1**.

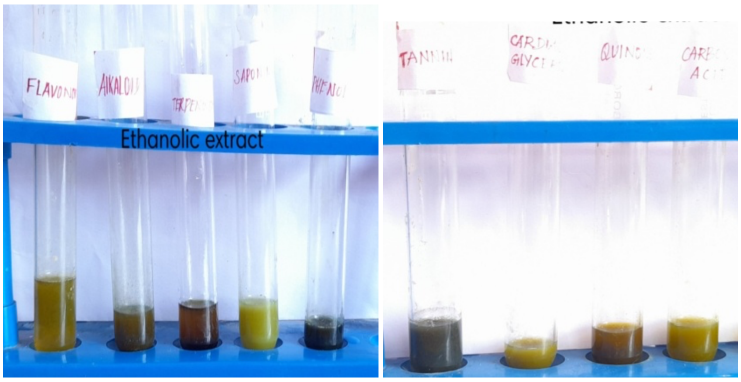


Figure 3. Results of Phytochemical screening of ethanolic extract of MC leaves.

Table 1. Phytochemical analysis of ethanolic extract of MC leaves.

Components	Results	Activity
Flavonoid	++	Antimicrobial
		Antidiarrhoeal
Alkaloid	++	Antidiarrhoeal
		Antihelmintic
		Antimicrobial
Tripenoid	+++	Antimicrobial
		Antitumor
		Anti-inflammatory
Saponin	–	Anticancer
		Antihelmintic
Phenol	+++	Antiseptic
		Disinfectant
Tannin	++	Antioxidant
		Antimicrobial
Cardiac glycoside	++	Treating heart failure
Quinone	++	Antioxidant
Carboxylic acid	++	Antibiotic

The presence of nutritious and nontoxic phytoconstituents greatly facilitates some bioactive therapeutic qualities. This could be the most important aspect in healing and combating many health problems. The researchers demonstrated the biological activities of these phytochemicals, and current research revealed their diverse qualities for a wide range of industrial uses (Mahmood *et al.,* 2019). The active components in the plant extract support the antimicrobial activity that we were looking for in this study. This promising phytoconstituent finding indicates that the MC ethanolic extract would be a better component for making an efficient antimicrobial biopolymeric film. The usage of plant extracts as an additive for manufacturing eco-friendly food packaging material has recently expanded due to the primary benefit of not altering the uniqueness of food products (Lokpur *et al.,* 2020). As a result, because it does not alter the qualities of food stuff, it is regarded as the best additive for manufacturing packaging materials. Furthermore, the researchers demonstrate the antimicrobial activity of flavonoids and phenols found in the plant extract, giving the film added value and making it an ideal antimicrobial packaging material. This will considerably aid in extending the time that food products can be stored fresh. Hence, this phytochemical screening and its positive results highly support the preparation of antimicrobial packaging materials by incorporating plant extract as a natural additive (Bonomo *et al.,* 2020).

3.2 Determination of Ash and Moisture content of extracted cellulose

The moisture and ash content of extracted cellulose determined were found to be 1.35% and 1.05%, respectively. The moisture content of the extracted cellulose was shown to be directly connected to other film characteristics such as solubility. The inclusion of hydrophilic molecules in the cellulose increases the soluble nature of the film, whereas hydrophobic compounds decrease the level of solubility (Abdulkadir *et al.,* 2022). The calculation shows that the proportion of moisture content is quite low, and that the film's solubility is also low. This will extend the packing material's shelf life. The MCCF's solubility follows the same pattern as expected.

According to the literature, the crystalline form of the cellulose does not absorb water, which increases the life of the film. This is further supported by the prepared film's mild crystalline and amorphous characteristics. Some purification methods for extracted cellulose, such as bleaching, alkali treatment, and chlorination, may eliminate undesired impurities such as lignin, hemicellulose, and other colouring agents. However, some inorganic substances, such as aluminium, calcium, and magnesium, will remain in the extracted cellulose. These unwanted contaminants are regarded as part of the ash content, which impedes the proper conversion of film into packaging material. The extracted cellulose with the least amount of ash satisfied these criteria (Raghunatham *et al.,* 2022).

Table 2. Properties of MCCF

Sample	Ash Content (%)	Moisture content (%)	Volatile mass fraction (%)	Solubility (%)	Biodegradability (%)
Film sample	1.35	1.05	2.51	95	55

3.3 Volatile mass fraction

Volatile mass fraction denotes to the percentage of film mass reduction in the initial weight of the film as given in **Table 2**. The volatile mass content of the biolpolymeric film was 2.51%. According to the literature, the volatile mass content of the films is comparatively lower than that of other polymer-based films due to the strong interaction between both the plant extract and film matrix, which reduced the free volume in the film matrix, causing a reduction in the film's ability to hold

and capture moisture (Abdulkadir *et al.,* 2022; Prado *et al.,* 2019). These arguments strongly support the use of the film as a food packaging material.

3.4 Biodegradability

The weight loss of buried samples indicated the process of biodegradation of the MCCF by the micro-organisms. According to the weight loss calculation, MCCF samples buried at two different depths degrade at a similar pace.

Table 3. Initial and final weight of the film in soil burial method.

Sample name	Weight of sample (Before burial) (mg)	Weight of sample (After burial) (mg)	Percentage of weight loss (%)
F1	0.253	0.107	57.7
F2	0.240	0.119	50.4

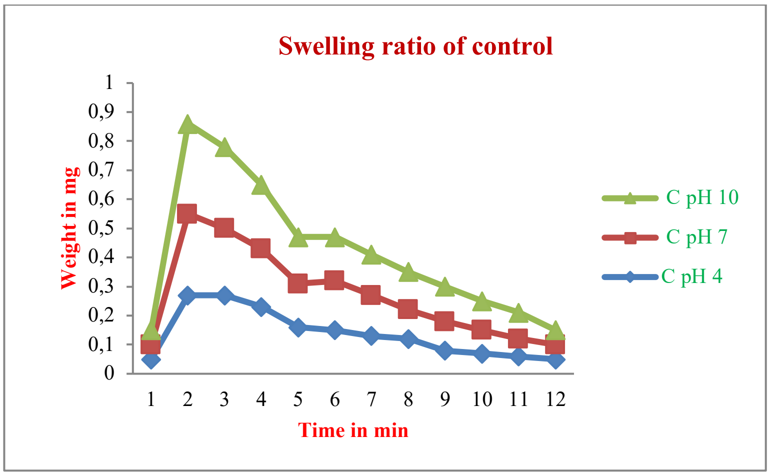
Accordingly the biopolymer prepared from plant cellulose is biodegradable as seen by the visual modifications after the tests were conducted in the actual environment. **Table 3** shows that the depth of the sample dumped in the soil level has no effect on the degradation mechanism (Liu *et al.,* 2021). The better biodegradability values of the both samples reveal that the prepared biopolymeric film would be suitable for packaging applications and can be easily disposed of. This will also result in a large reduction in municipal waste.

3.5 Water solubility

The initial and final weight of the film was measured and their percentage of solubility was calculated. After the continuous stirring the weight of the soluble mass was 0.005 and percentage of solubility is 95%. When the biopolymer's solubility exceeds 60%, it can be used for pharmaceutical drug encapsulation. When the biopolymer's solubility falls below 50%, it can be used as a food packaging material. The less soluble biopolymers can be used as disposal bags like shopping bags. Well-dissolving biopolymers are appropriate to be used as packaging material for soaps and detergents.

3.6 Swelling ratio

The swelling capacity of the film in three different pH buffers was monitored. All three samples behave partially similar with slight variation. Whenever a water content particular to each polymer is achieved, polymer mobility raises dramatically, a phenomenon known as polymer chain relaxation. This is most likely due to the plant extract being hydrophilic and forming hydrogen bonds (Moshi *et al.,* 2020). As a result, the MCCF is easily impacted by water with a pH of 7. All of the film samples were found capable of absorbing water after 5 minutes from being immersed. Following that, the film samples get saturated with water and after that no more penetration of water occurs. The film samples treated with pH 4 and pH 7 showed the lowest water absorption as compared to film sample treated with pH 10. When the film matrix begins to absorb water molecules from its surroundings, it begins to inflate. When the film sample combines with the polar ends, the film swells more, which is considered as a drawback of employing such material as a food packaging material (Rodsamran *et al.,* 2019). The conservation of the film in its original shape is a benefit, as it becomes more stable and suitable for all types of food products.



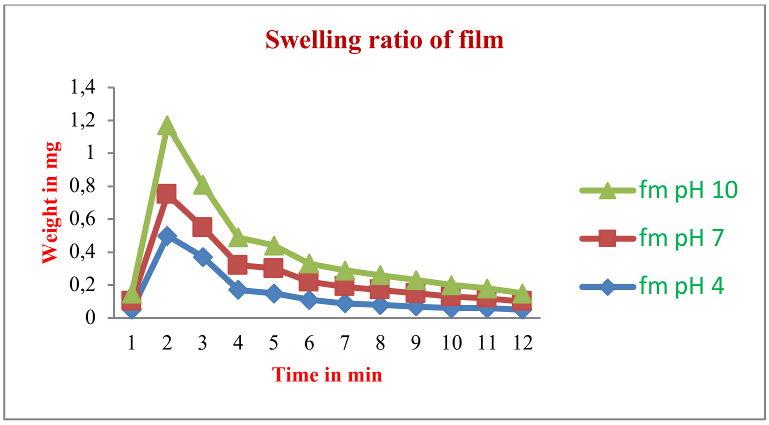


Figure 4. Swelling ratio of control and film (MCCF) with different pH

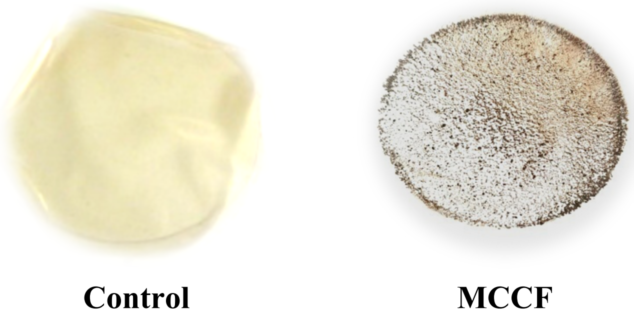


Figure 5. Images of biopolymeric film

3.7 Fourier-transform infrared spectroscopy

FTIR is a widely used method for determining the structure of a chemical molecule by confirming the presence of a functional group in an unknown material. In general, FTIR spectroscopy was examined to identify and confirm complex formation, and it was even utilised to validate compound interactions (Kanmani *et al.,* 2014). The peaks at 3424cm^{-1} and 2923cm^{-1} confirmed the functional groups of cellulose's -OH and -CH groups. Bending vibrations of the cellulose -CH group and C-O group were found at 1449 cm^{-1} and 1233 cm^{-1}, respectively. When a polymeric film is subjected to FTIR, the spectrum reflects the functional group of the extracted biopolymer and the matching specific rotation around carbon atoms (Badita *et al.,* 2020).

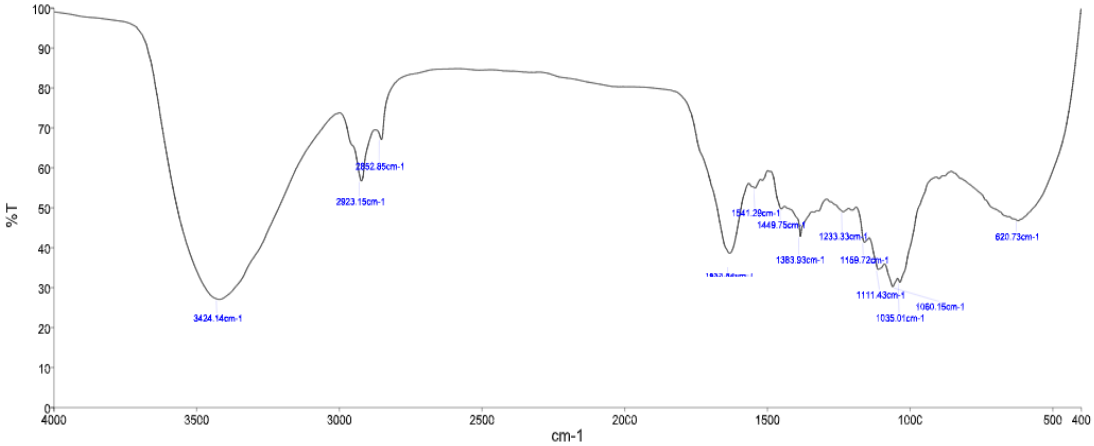
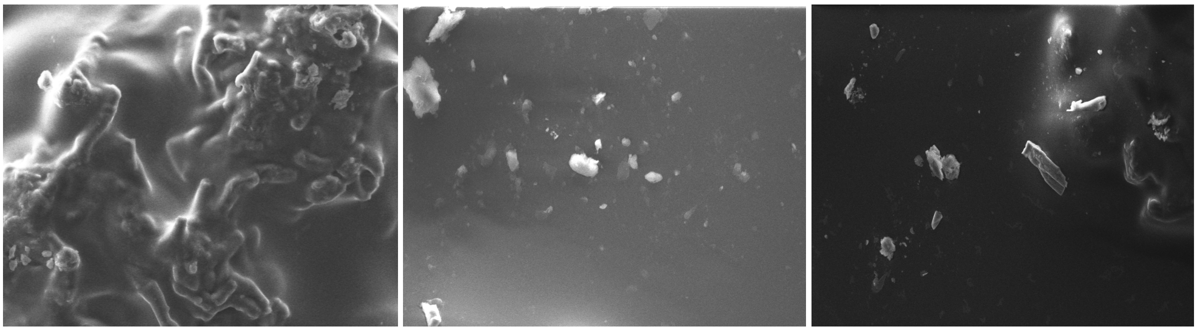


Figure 6. FTIR of cellulose based film

3.8 Scanning Electron Microscopy



(a) **(b)** **(c)**

Figure 7. SEM images of cellulose based film

The SEM analysis was carried at the increasing magnified orders. Polymer morphology is a physical phenomena that concentrates on the study of polymer structures and interactions. The significance of these phenomena is its capacity to describe the configuration of molecules on a large scale. This kind of formation can be amorphous, crystalline, or semi-crystalline. From the other spectral analysis, it is understood that the synthesized biopolymeric film would be in semi-crystalline and amorphous arrangement (Jafarzadeh *et al.,* 2017). These arrangement can be seen which consists of small crystalline portions (crystallites) surrounded by domains of amorphous phase from the 20 µm magnified image (**figure 7-b**). The fibrous appearance of extracted cellulose was seen in the (**figure 7-c**). On increasing the resolution to 1.50KX the rough surface of polymeric film and was observed and few micropores were seen which developed possibly due to interaction of –OH groups of cellulose and plasticizer (Mostafavi *et al.,* 2016 ; Ruggero *et al.,* 2020)

3.9 X-ray diffraction

The crystalline nature and the amount of crystallinity is described by the sharpness and the peak intensity value respectively. The percentage of crystalline index was determined as 54.58% which is an inherent property that governs its mechanical properties, affinity for water, and accessibility to chemical reagents (Mostafavi *et al.,* 2016). The amorphous nature of the film has an important

inference in the profile shape, which suggests a mix of a highly crystalline material, and other less crystalline material like nanocomposites. The crystalline index value obtained is virtually identical to the previous result by Kale *et al.,* who worked with commercially available cellulose. These findings support the suggestion of isolating cellulose from other plants such as sugarcane and soy hulls. The size of crystals was calculated through the resolved diffraction peak value and full width half maximum value using the Scherrer equation. The estimated crystal size was 16.92nm which indicates the presence of nanocrystals. As the size of the crystal gets reduced its effective surface area increases, ultimately increasing the solubility and bioavailability (Jabli *et al.,* 2018).

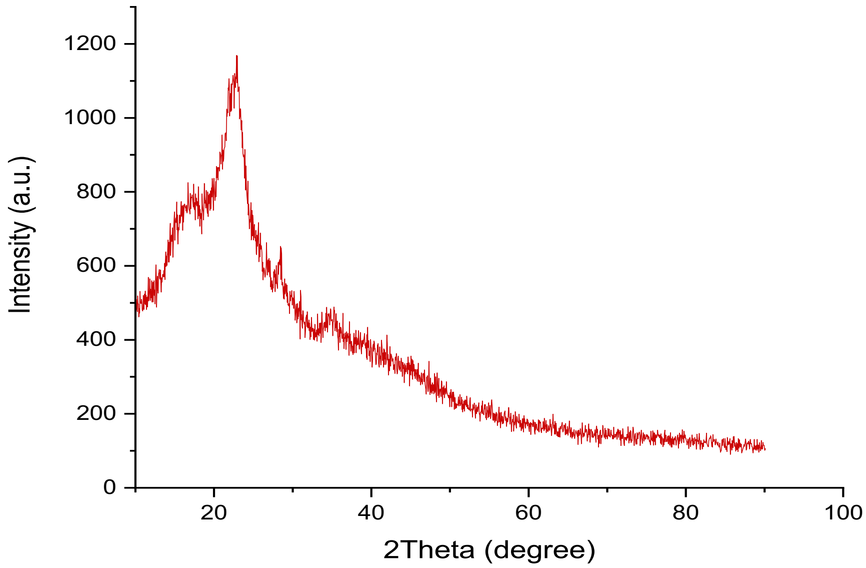


Figure 8. XRD diffractogram of biopolymeric film

3.10 Antimicrobial activity of film

The prepared cellulose-based film showed a good antimicrobial activity against all tested organism, which is likely due to the presence of plant extract. The antimicrobial activity of the control and films were tested against two bacterial strains and one fungus strain by the agar well diffusion method (Krepker *et al.,* 2017). Gentamicin was used a control and the patterns of inhibition varied with the concentration of film sample and the maximum inhibition was observed at the concentration of 100 µl.

Table 4. Zone of inhibition measurements of the biopolymeric film at different concentrations

	Zone of inhibition (mm/ml)				
SAMPLE	**25 µl**	**50 µl**	**75 µl**	**100 µl**	**Control**
Bacillus subtilis	22	25	28	32	20
E.coli	22	26	30	34	22
Candida albicans	20	23	25	28	23

The gram negative bacterial strain *E. Coil* showed maximum than the gram positive strain *Bacillus subtilis*. The promising effect of antimicrobial activity demonstrates that the incorporation of plant

extract with cellulose-plasticizer suspension is an efficient method for protecting and increasing stability and sustains the release of their bioactive compounds (Venkatesan *et al.,* 2019) (Liu *et al.,* 2017).

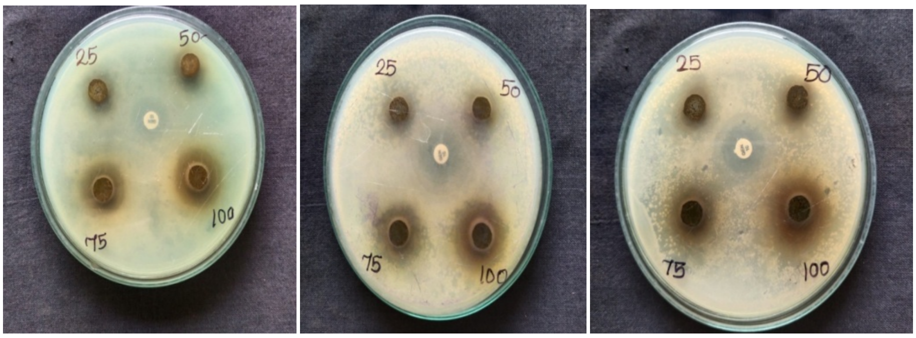


Figure 9. The diffusion assay result of biopolymeric film against bacteria and fungi (from left-right *E.Coli, Candida albicans, Bacillus subtilis*)

Conclusion

In this study plant extract incorporate in the cellulose based film was successfully prepared which could be a good alternative for the traditional food packaging and wound healing materials. The prepared biopolymeric film has better solubility and is biocompatible in nature. The film shows satisfactory results on the swelling effect with all pH ranges. The FTIR results confirmed the interaction between extracted cellulose with plasticizer. As expected the antimicrobial activity of biopolymeric film was more effective on the microbes which are present in the oral environment. This can be very beneficial in improving the shelf life of perishable foods. Hence, bioactive biopolymers incorporated with natural extracts have excessive applications for food and other packaging materials.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

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