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PREPARATION AND CHARACTERIZATION OF BIO COMPOSITE FILM DERIVED FROM MICROCRYSTALLINE CELLULOSE (MCC) AND PECTIN POLYMER

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ABSTRACT

With various loadings of bamboo leaves and glycerin, new bioactive films based on pectin and microcrystalline cellulose (MCC) were successfully created. Thermal stability was investigated, and the results revealed that it was somewhat less stable than PMCC films after being loaded with bamboo leaf and glucoraphanin. Investigated were the antibacterial and antioxidant capabilities. The results demonstrated that PMCC composite films had outstanding antibacterial activity against Escherichia coli, Candida albicans, and Staphylococcus aureus as well as good DPPH radical and hydroxyl radical scavenging activities. According to the findings, bamboo leaf and glycerin-loaded PC films have excellent antioxidant and antibacterial properties that make them acceptable for food packing and preservation.

Keywords: microcrystalline cellulose; pectin; Bamboo Leaf; antibacterial; SEM; TGA; FTIR; XRD

1. INTRODUCTION:

Materials used to package food must be nontoxic and have good mechanical, air, and moisture barrier qualities. Modern food packaging is made of petroleum-based polymers that are not biodegradable and have a negative impact on both physical safety and the marine environment**.** They lack antimicrobial characteristics as well **[1]**. Therefore, it is essential to create newly developed biodegradable food packaging materials and have strong antibacterial, antioxidant, and barrier properties to enhance food storage outcomes, lengthen shelf life, and be ecologically friendly **[2]**. Therefore, innovative films that promote eco-friendly and sustainable packaging practices are generally appealing **[3]**.

Most plants, including apple and citrus, contain pectin, a family of polysaccharides and oligosaccharides with a variety of structural variations **[4]**. Galacturonic acid is prevalent throughout its linear structure. Pectin has a molecular weight in the 50,000–150,000 Da range. Depending on how esterified and polymerized the pectin is, it can be used in a wide variety of ways **[5]**. Due because of its bioactivity, biocompatibility, biodegradability, renewability, affordable price, and ease of due to alteration, pectin emerging as a prospective participant in a variety of industries, including the textile and food sectors, pharmaceutics, tissue engineering, bioimplants, drug delivery, and cosmetics. Pectin-based movies about food have strong oxygen barrier qualities, as well as solid adhesion and hardness. films made of pectin have benefits, but they also have drawbacks, including rigidity, due to their extreme water sensitivity, they are fragile and have poor water barrier properties **[6]**. Pectin films need to be improved as a result **[7]**.

Pectin is necessary for cross-linking enhance its physical qualities and water resistance because it has a significant hydrophilic feature. Common techniques include chemical and physical cross-linking. A flexible technique, chemical cross-linking creates strong, stable coatings by establishing covalent bonds. The most often utilized cross-linkers include glutaraldehyde, diamines, and divalent ions (Ca2+, Mg2+). Meanwhile, secondary and reversible bonds that form during physical cross-linking make films less stable **[8]**. However, they are preferred when encapsulating dynamic bioactive compounds, inside hydrogels are antimicrobial, antioxidant, anti-browning, scent, and colorants, especially if the creation occurs under benign conditions without the use of organic solvents **[9, 10]**.

Pectin has been suggested to enhance the functionality of Pectin-based goods with uses in the food industry, medication delivery **[7]**. In the presence of transglutaminase, whey protein/pectin films were created that effectively inhibited microbial growth **[11]**.

The solution casting process produced intriguing bio-inspired edible films out of Pectin and poly (ethylene glycol), which are competitive with synthetic packaging materials now on the market **[12]**.

With a linear chain of D-glucopyranose repeating units that are -1-4 connected and several hydroxyl groups, with cellulose being the most prevalent renewable, likewise an ecofriendly polysaccharide. Because it is inexpensive, highly biodegradable, biocompatible, nontoxic, chemically stable, and has exceptional thermal stability, and high mechanical strength, it finds extensive use as packaging material within the paper, pharmaceutical, businesses related to food, cosmetics, and biomedicine **[13]**.

1.1.Pectin Polymer:

Pectin is a complex polysaccharide that mostly consists of esterified D-galacturonic acid residues in and a-(1-4) chain, with the chain-helix shape being broken up by the short Rhamnose inserts. As side chains, it also contains additional neutral sugars. Due to these side chains, the molecule seems to have smooth (side chain-free) and "Hairy" (side chain-containing) areas. Different amounts of methyl esterification occur in the galacturonate residue. According to X-ray and electron microscopic investigations, pectin is crystalline, and citrus pectin has a crystallinity of roughly 6%. Pectin that is produced naturally includes fibrillar crystals.

1.2.Microcrystalline Cellulose:

The refined form of wood pulp is microcrystalline cellulose $(C_6H_1O_5)_n$. The substance is a white, flowing powder. It is a chemically inert material that has no discernible absorption and does not break down when consumed. In excessive doses, it gives food bulk and may have a laxative effect. An excipient that is frequently utilized in the pharmaceutical business is microcrystalline cellulose. It is employed in solid dosage forms, such tablets, and has excellent compressibility characteristics. It is possible to create hard tablets that dissolve quickly. Similar to cellulose, microcrystalline cellulose complies with USP criteria **[14]**.

It can be used as a stabilizer, texture adjuster, anti-caking agent, suspending agent, and other things in many processed food products. When used in reasonable amounts, microcrystalline cellulose is generally recognized as safe, according to the Select Committee on GRAS Substances.

MATERIALS

Pectin is purchased from Hi Media laboratories Pvt. Ltd. Mumbai (INDIA). Micro Crystalline Cellulose is bought via Loba Chem. Pvt. Ltd. in Mumbai (INDIA), Antibacterial Drug (Bamboo Leaf)

METHODS

1.Preparation of PMCC Composite Films

A solution was made by stirring the pectin solution at 60 °C for two hours while microcrystalline cellulose powder is dissolved. Pectin (1-2 g) was added to a combination of 1 ml Glycerin. Then, the microcrystalline cellulose/pectin combination was cast onto 90 mm diameter acrylic plates for 30 minutes at 4 degrees Celsius, then immersed in a solution for 6 hours before being rinsed with deionized water.

Characterizations

The physical characteristics of the PMCC the composite membranes were examined using a JSM-7600F scanning electron microscope, 10-15 kV of rising voltage, and under extremely high vacuum, a thin layer of platinum was applied to the samples (20 mA, 100 s) **[1]**.

A Spectrum FTIR spectra were captured using a two-spectrometer utilizing a 4 cm1 resolution and a wave number range of 4000-400 cm^1. A TA Instruments model Q5000 TGA was used to conduct the thermo gravimetric (TG) analysis. The samples were heated from 25 to 600 °C in a nitrogen atmosphere at a rate of 25°C/min. **[1]**.

1. XRD

X-beam diffraction investigation is a material science method for deciding the crystallographic design of a material. This technique includes presenting the example to X-beams and estimating the powers and dispersing of the X-beams as they leave the example. The XRD range of a Pectin/Micro Crystalline Cellulose composite film is protuberance was seen in the range at 25.5 Å, which compares to the shapeless idea of the film, as affirmed by an X-beam diffractometer **[17]**.

2. Expansion Study

Studying the expansion of the wound dressing film on the wound surface involved measuring the change in diameter of a circular film sample in a 10% gelatin solution. In a nutshell, 100 ml of warmed distilled water was used to continuously mix 10 g of gelatin powder into a transparent solution. All ratio films with known diameters were then inserted into the gelatin solution in a petridish, and the sample's diameter was periodically checked until it reached a consistent value. The following expression was used to determine the Expansion ratio (ER)

ER= Dt/D0

Where, Dt= diameter at time and D0 = initial diameter

Table 1: Expansion study of all ratio films

Time	Pectin/MCC (50:50)	Pectin/MCC (60:40)	Pectin/MCC (70:30)
0 min	2	2	2
15 min	2.11	2.14	2.14
30 min	2.2	2.24	2.2
45 min	2.3	2.3	2.26
60 min	2.3	2.3	2.26

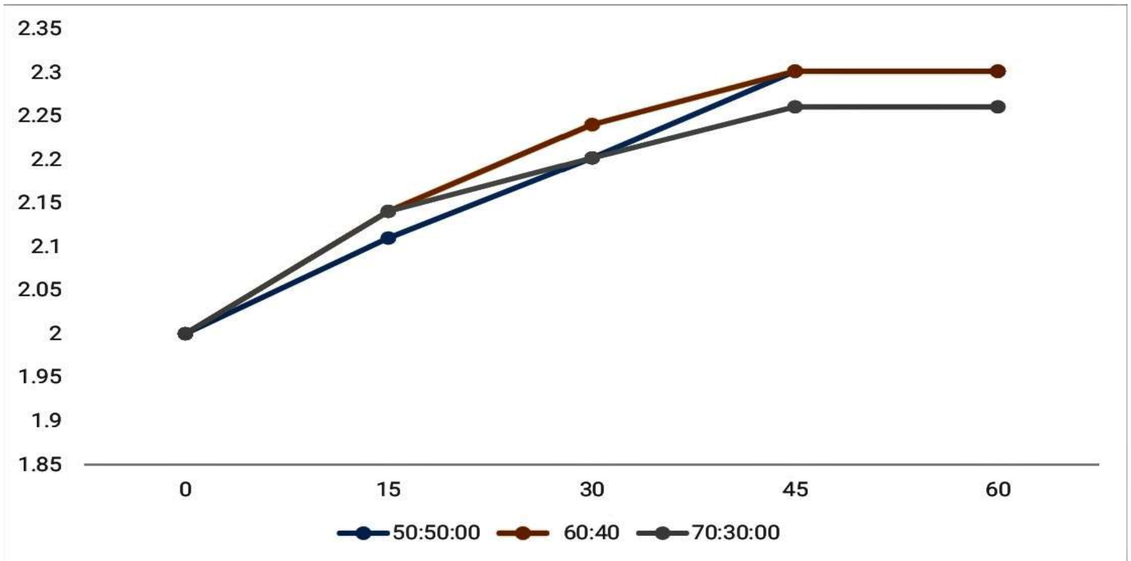


Figure 1: Expansion Ratio of composite PMCC film. (a) PMCC1 (b) PMCC2 (c) PMCC3

3.Antibacterial

By using the disc diffusion method, the antibacterial and antifungal effects of the PMCC composite films were investigated against *E. coli, C. albicans* and *S. aureus*. Using PMCC composite films, which have been sliced into 10 mm-diameter rounds and sanitized for 60 minutes under a UV lamp. On Tryptone Soya Agar plates, test bacterial lawns (approximately 1 105 CFU/plate) were created. After being sanitized, the films were carefully positioned on the grass and kept at °C for 24 hours. Afterward, evaluate how the tested components affected bacterial growth inhibition **[19]**.

RESULTS

1.SEM Morphology

Figure 2 shows SEM pictures of PMCC and PMCC2 films at a 10,000x magnification. PMCC film's surface morphology **(Figure 2A)** shows that it has a uniformly smooth surface devoid of holes. **Figure 2B** depicts the PMCC3 film, which in this instance had a rough surface and some particles present. It is evident that the addition of Glycerin and tea Bamboo leaf to PMCC film had no major impact on the morphology of the dense surface other than to increase roughness. The results of the SEM investigation show that the surfaces of the PMCC and PMCC2 films exhibit thick, poreless surface morphologies.

3. FTIR Characterization

Figure 3A displays the cellulose FTIR spectrum (Curve a). The hydroxyl groups and intramolecular hydrogen bond are represented by a wide peak with a range of 3500 to 3200 cm1. The CH2 groups are responsible for the peak at 2687 cm1 **[14]**. For Curve, displayed common bands at 1600 and 1300 cm1, which are associated with the C=C vibration of the benzene skeleton. C=O stretching is suggested to be responsible for the peak at 1590 cm1 **[15]**. According to previous reports on glycerin **[16]**, the three distinctive bands for glycerin (Curve a) are assigned to the respective aromatic vibrations at 3160, 2137, and 1684 cm1.Two bands, at 1342 and 1396 cm1, are ascribed to the absorptions of pectin's esterified and free carboxyl groups, respectively (Curve a). All the microcrystalline cellulose, pectin, and Glycerin characteristic bands can be seen in the FTIR spectra of PMCC3 for PMCC composite films **(Figure 3A)**. This verified that the PMCC composite films had been prepared successfully.

3. Thermo gravimetric Analysis

According to the method of scheduled heating in the vicinity of 25-600C when surrounded by nitrogen, to investigate how the weight of PMCC composite film changed, thermogravimetry was performed. as temperature increased. Figure respectively, shows the Different Thermal Gravity (DTG) and TG spectra of PMCC films. For PMCC films, there is a slow prior to 100°C, there is weight loss and a rapid one between 250 and 400 °C. The primary reason of physicochemical adsorption and hydrogen bonding between water molecules caused the first weight reduction, and the primary cause of the second weight loss was the temperature decline and disintegration of the combination of cellulose and pectin formation of C, CO. The PMCC film residue is 4.35%. The diagram demonstrates that the PMCC film's maximum breakdown temperature (Tmax) is 370.8 °C **[18]**.

5.530%. The TGA curve of PMCC3 films likewise showed two stages of weight reduction. Due to the presence of glycerin, the residue of the PMCC3 film rose to 8.47%. This finding was further supported by the DTG peak's change from.746.17to 756.17 °C. These findings indicate that following loading with Glycerin, the thermal stability marginally diminishes.

4. XRD

A material science technique for determining the crystallographic structure of a material is X-beam diffraction analysis. This method entails giving the example to the X-beams and calculating their powers and dispersions as they exit the example. **Figure 5** shows the XRD spectrum of a composite film made of microcrystalline cellulose and pectin. An X-beam diffractometer confirmed that a protuberance was visible in the range at 25.5, which compares to the film's shapeless notion **[17]**.

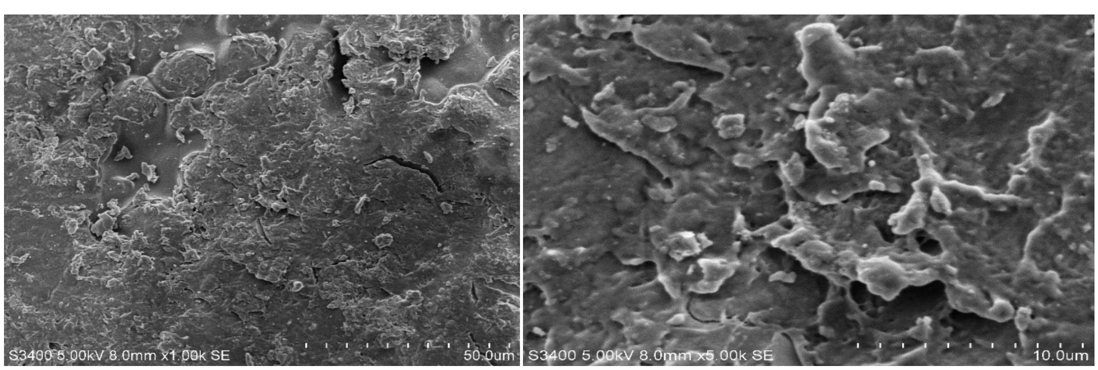
5. Bacterial Inhibition Zones

The ability of PMCC composite films to prevent bacterial growth was assessed using the disc diffusion method. The sizes of the inhibition zones surrounding the samples after 24 hours were evaluated in order to determine the effectiveness of bacterial inhibition.

images of the inhibitory zone are shown in **Figure 6**. The absence of any inhibiting regions against the following pathogens: S. aureus on PMCC films (a) suggests that PMCC films have no capacity to suppress bacterial or fungal growth. However, after being loaded into PMCC films with glycerin and bamboo leaf, they show distinct inhibitory zones surrounding *Staph aureus, C. albicans* and *E. aureus*. In the meantime, the inhibitory capacity also grows as loadings rise. The dimensions of PMCC composite films' typical inhibitory zones are shown in Figure 6. The inhibitory zones on PMCC1 films had the smallest widths, Compared to *E. coli, C. albicans* and *S. aureus*, they were smaller, measuring 24, 14, and 22 mm. Against *E. coli, C. albicans* and *S. aureus*, respectively, the highest bacterial and fungal content was found in PMCC2 films. inhibitory capabilities. It is obvious that as The PMCC composite films' loads include glycerin and bamboo leaves. an inhibitory zone widens. The current work amply demonstrates the outstanding bacterial and fungal inhibitory properties of PMCC composite films **[14]**.

6. Activity of the antibacterial

The antibacterial efficacy of a film containing bamboo leaf extract was tested on *E. coli*. A petri dish containing an *E. coli* culture was placed with one piece of the Pectin/MCC film (labelled as (A) in the image) and the bamboo leaf extract loaded Pectin/MCC film (labelled as (B) in the **Figure 7**). The process for making *E. coli* cultures is covered in the study's Characterization section. In contrast to the Pectin/MCC film, the zone of inhibition in the composite film incorporating bamboo leaf extract was larger, measuring 4 cm in diameter. This suggests that there is a bioactive ingredient present **[19]**.



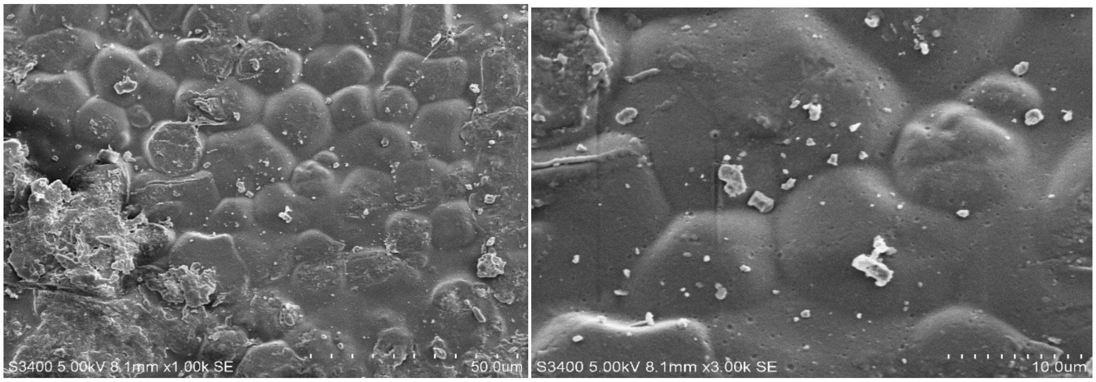


Figure 2: SEM images of PMCC films

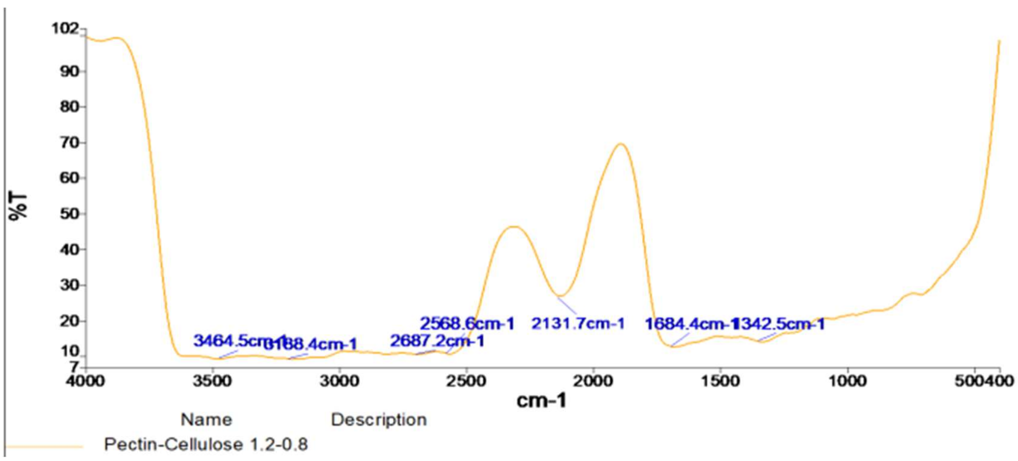


Figure 3 (a): FTIR spectrum data for PMCC1 composite films: (a) Pectin (b) Glycerin and (c) Microcrystalline cellulose

Sample Details 1

Analyst	X-Axis Units	X-Axis start value	X-Axis end value	Number of points	Y-Axis Units
Analyst	cm-1	4000	400	3601	%T

Single Peak Table 1

Peak Number	1	2	3	4	5	6
X (cm-1)	3464.45	3188.41	2687.17	2568.60	2131.66	1684.38
Y (%T)	9.25	9.14	10.46	10.58	26.90	12.55

Single Peak Table 1

7
1342.48
14.05

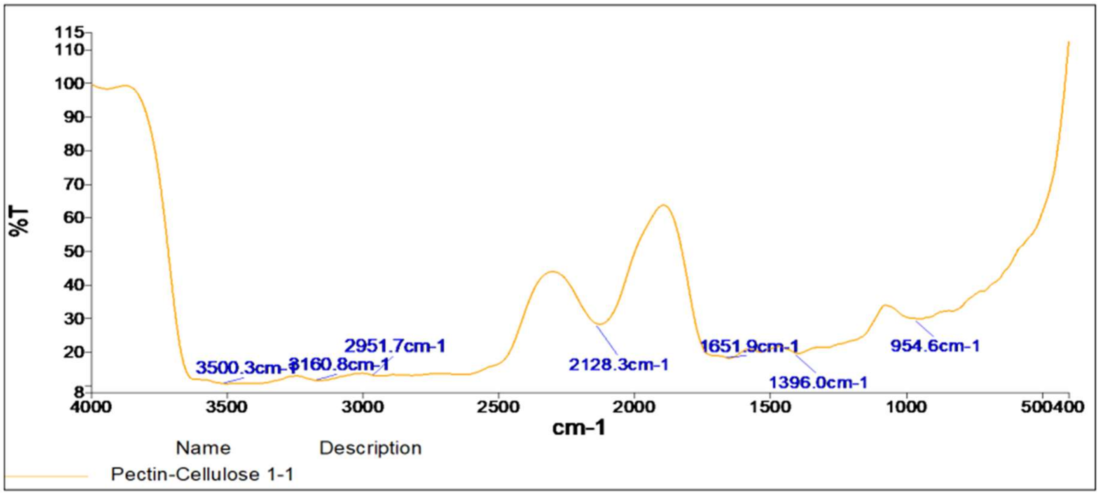


Figure 3 (b): FTIR spectrum data for PMCC2 composite films: (a) Pectin (b) Glycerin (c) and Micro crystalline cellulose

Table 2: TGA

Temperature	24.97	94.97	154.97	204.97	314.97	424.97	554.97	654.97	784.97
Weight	99.973	96.347	90.800	82.674	56.994	46.038	41.215	39.207	27.699

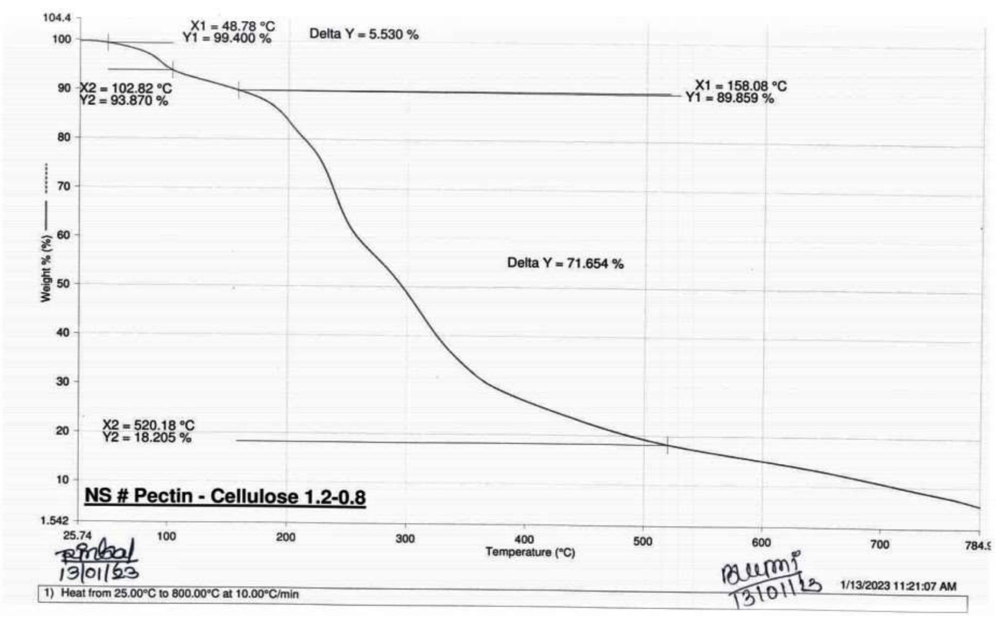


Figure 4: TGA analysis of PMCC films

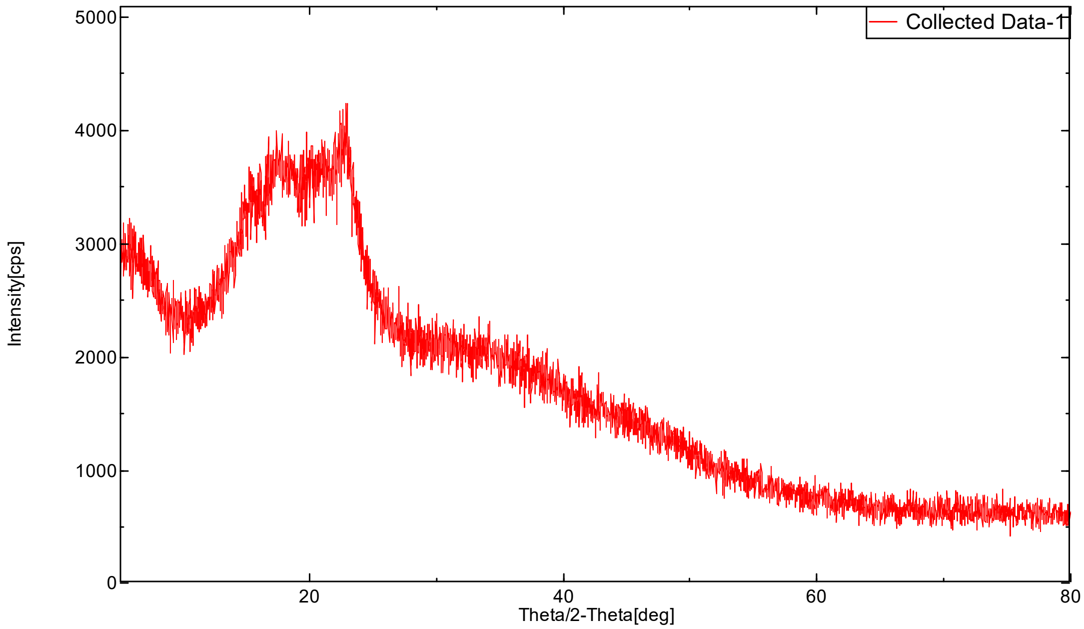


Figure 5: XRD analysis of PMCC films

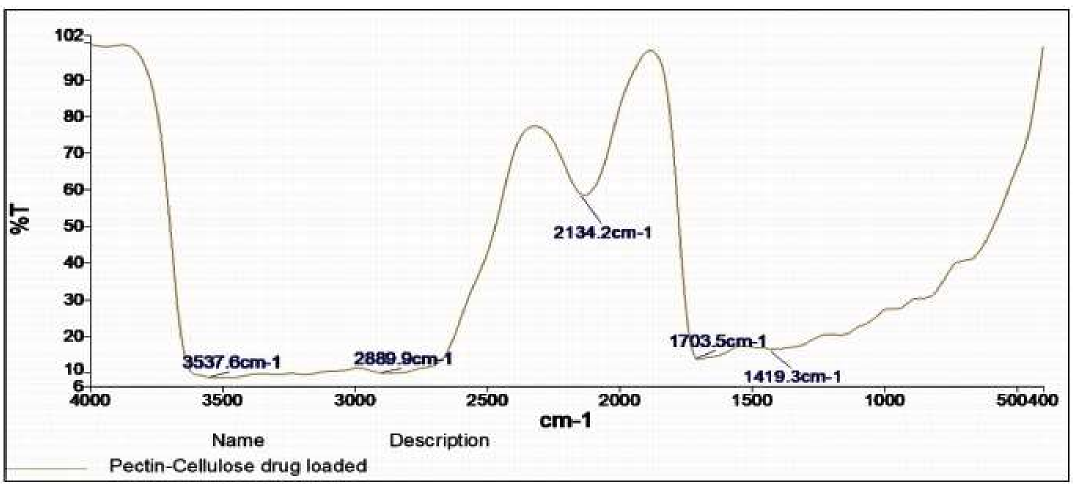


Figure 6: FTIR spectra of PMCC Antibacterial composite films. (a) Pectin (b) Glycerin (c) Bamboo leaf and (d) Micro crystalline cellulose

Sample Details 1

Analyst	X-Axis Units	X-Axis start value	X-Axis end value	Number of points	Y-Axis Units
Analyst	cm-1	4000	400	3601	%T

Single Peak Table 1

Peak Number	1	2	3	4	5
X (cm-1)	3537.59	2889.88	2134.18	1703.47	1419.30
Y (%T)	8.51	9.73	58.58	13.53	16.35

Figure 7(A): Pectin/MCC Composite film

Figure 7(B): Extract loaded Pectin/MCC film

CONCLUSIONS

The study looked at how the antibacterial qualities of biodegradable PMCC composite films were affected by the addition of bamboo leaf and glycerin. TG curves demonstrated that the loading of bamboo leaf and glycerin marginally reduced the overall thermal stability of the PMCC composite film.

E. coli, Candida albicans and *Staphylococcus aureus* all showed a notable inhibitory impact when exposed to the generated PMCC composite films. They also demonstrated effective scavenging actions against Free radicals of hydroxyl and DPPH. The developed PMCC Composite films may be regarded in the field as a promising candidate of Based on food preservation and packaging on the results.

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