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# CHITOSAN AND PLA COMPOSITE FILMS ENRICHED WITH BUTTERFLY PEA EXTRACT: A MULTIFUNCTIONAL APPROACH TO WOUND CARE, FOOD SAFETY, AND ANTIBACTERIAL DEFENSE

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## ABSTRACT

This work demonstrates the preparation or synthesis of a biodegradable polymeric film of chitosan and Poly lactic acid using the solvent-casting method. Using natural medicine extracts from the Butterfly Pea (Clitoria ternatea) seed in combination with chitosan and polylactic acid (PLA) composite sheets, a new field in materials science and medicinal applications is emerging. First, we synthesize separate polymeric films of chitosan and PLA after that make different ratio films. This study explores the versatile possibilities of these composite films, looking at their use as effective antibacterial agents, novel food packaging materials, and wound dressings. Chitosan and PLA composite films containing Butterfly Pea seed extract were tested for antibacterial activity to inhibit Escherichia coli (*E. coli*) bacteria. A key finding of the study is that composite films are capable of mitigating microbial growth in a variety of settings, thanks to their antibacterial properties. In addition to providing a comprehensive foundation for further research and development, the characterization techniques used shed light on the material's crystal structure, surface morphology, chemical composition, and thermal behavior. The research highlights the composite films' ability to reduce microbial growth in a variety of situations while also demonstrating their antibacterial activity. The material's crystalline structure, surface morphology, chemical composition, and thermal behavior were all clarified by the characterization methods used, creating a solid platform for further study and development.

**Keywords: Chitosan, Poly Lactic acid, Butterfly pea, glycol, Antibacterial activity**

## 1.0 INTRODUCTION:

Researchers from academia and industrialist are actively developing bio composites, which are composite materials that are biodegradable and environmentally benign., in response to environmental concerns and governance legislation **[2]**. Chitosan and Polylactic Acid (PLA), two biodegradable polymers, have drawn a lot of attention because of their distinctive qualities and biocompatibility, making them promising candidates for a variety of applications, such as drug delivery systems, tissue engineering, and sustainable packaging. In recent years, there has been an increasing interest in exploiting the antibacterial capabilities of natural chemicals for different purposes, including wound healing, food preservation, and healthcare. The Butterfly pea (*Clitoria ternatea*), a flowering plant native to Southeast Asia, has attracted substantial interest in this regard because of its high amount of bioactive chemicals with confirmed antibacterial activities. The coupling of butterfly pea extracts with Chitosan biodegradable polymeric films gives an intriguing path for the creation of novel antibacterial materials. By putting butterfly pea extracts into Chitosan films, researchers may construct controlled-release systems that harness the inherent antibacterial capability of butterfly pea while taking use of Chitosan's film-forming and biodegradable qualities. Recent research has studied the composition and characterization of these films, indicating their promise in wound care, food packaging, and medicinal applications **[1]**. In light of its unique applications as a polymer matrix, polylactic acid (PLA) is processed, renewable, biodegradable, and biocompatible **[4, 5]**. Another biodegradable polymer that has received a lot of attention recently is polylactic acid (PLA). It is made from renewable resources like sugarcane or corn starch. PLA possesses traits like strength, stiffness, and transparency that are comparable to those of conventional plastics made from petroleum. It has uses in the biomedical, agricultural, and packaging industries. In more recent research, efforts have been made to enhance the mechanical strength, thermal stability, and biodegradability of PLA by copolymerizing, blending, and adding nanofillers **[6, 7]**. A Chitosan is biodegradable, non-antigenic, non-toxic polymer and Due to chitosan's numerous health benefits, which include strong antioxidant and antibacterial properties, the natural polymer formed from chitin is biocompatible **[8]**. Chitosan is a cationic linear polysaccharide made of N-

acetyl D-glucosamine and covalently linked D-glucosamine (deacetylated unit). Since they appear to degrade as new tissues are formed, scaffold composites made with chitosan, an antimicrobial component, are appealing materials. Chitosan has been widely utilized in bone tissue engineering because it encourages osteoblast growth and mineral rich matrix deposition in nature **[3]**. Chitosan has uses in food packaging, biologic medication delivery systems, and wound treatment. To improve its functionality and broaden its possible uses, recent research has concentrated on investigating the mechanical, thermal, and barrier properties of chitosan as well as its modification through the mixing and integration of nanoparticles **[9, 10]**. Biofilm, a natural food packaging material made of chitosan, can increase the quality and shelf life of melons. It can also stop the development of bacteria, fungi, and other microorganisms. Chitosan is a polysaccharide and amino group-containing antimicrobial agent. To enhance the antibacterial action of chitosan, a number of other compounds, including a biopolymer, polysaccharides, lipids, or a mixture of these, as well as fatty acids and essential oils, were added. Additionally, chitosan exhibits exceptional film-forming capacity, significant antibacterial action, selective gas permeability ($CO_2$ and $O_2$), and compatibility with various compounds, including vitamins and minerals. Additionally, recent research has focused on the application of antimicrobial compounds in biodegradable food packaging systems to stop microbial development on food surfaces. Chitosan has been studied previously by many researchers who have looked into its use as extra material in package production. The notion of biodegradable food packaging is being revived with the usage of chitosan in food packaging, according to which the distribution of packaging can lessen the number of microorganisms in foods and stop their growth. Biofilm, a natural food packaging material made of chitosan, can increase the quality and shelf life of melons. Inhibiting the growth of microorganisms is another benefit **[11]**.

## 2.0 MATERIALS AND METHODS:

### 2.1 Materials

Suvidhinath Laboratories in Vadodara procured the chitosan, and according to the supplier's specifications, it was deacetylated to a degree of 80–85%. Banka Bio Limited (IND) offered poly (lactic acid) in pellet form (PLA: 92% L-lactic and 8% meso-lactic). Intrinsic viscosity measurements in chloroform at 25 C were used to get the average molecular weight of 49,000. BRM Chemicals' Polyethylene Glycol (PEG 400).

We bought acetic acid and chloroform from SRL Pvt. Ltd. in India.

**2.2 Methods**

**2.2.1 Preparation of Chitosan Film**

Chitosan was dissolved in a 1% (v/v) aqueous acid solution to create the film-forming solution, which included 1% (w/v) chitosan. Chitosan was dissolved in 1% acetic acid for 30 minutes while being stirred magnetically. Next, a homogeneous coating of the film-forming solution measuring 1 mm thick was applied to a petri dish. For three days, the casting took place at 25 °C or room temperature.

**2.2.2 Preparation of PLA films**

Polylactic acid 1% weight/volume was dissolved in chloroform for 30 minutes while being heated and magnetically stirred. Layers of glass Petri dishes, each 1 mm thick and uniform, were cast to create the films. Film was cast at a room temperature environment.

**2.2.3 Preparation of Chitosan-PLA Blends based films**

Before being combined, Chitosan was dissolved in 1% acetic acid, while PLA (1% by weight) was dissolved in chloroform. Then both solutions were mixed under magnetic stirring to create a homogeneous solution. The blended films were made by pouring on an acrylic petri dish and letting it evaporate for three days at room temperature. Then make different ratio films of chitosan and PLA (50/50,40/60,30/70).

**2.2.4 Preparation of extracts of Clitoria ternatea seeds**.

Clitoria ternatea seed extraction was obtained by drying powder of seeds 3 grams in 30 ml methanol solvent for 3 days at room temperature. The mixture was then centrifuged for 15 minutes at 3000 rpm on a magnetic stirrer or until the mixture became homogeneous. After being macerated, the extracts were filtered using No. 1 Whatman filter paper. Filtrate was used as extract and for further use stored at 4 °C.

**2.2.5 Preparation of Antibacterial drug loaded Chitosan-PLA composite film**

In chloroform, poly lactic acid (PLA) was dissolved in acetic acid solution at 1% w/v under magnetic stirring for fifteen minutes while chitosan was dissolved in 1% w/v acetic acid solution. Both solutions dissolved separately after that add the 3 ml of extraction. Then mix both solutions under magnetic stirring until the mixture becomes homogeneous. This solution was then poured onto a Petri dish, dried overnight and the resulting film was peeled off to obtain the required loaded polymeric composite

**3.0 Chemical Analysis**

**3.1 Swelling Study**

To study the swelling of the composite films, physiological fluid was employed (pf). This fluid was made by dissolving 8.307 g of sodium chloride (NaCl) in which 0.367 gram of CaCl2 in 1 liter of purified water. The pf solution was created by combining both of these. Now each ratio film was divided into separately weighted tiny parts. After being dipped into the PF solution, the films were taken out at various intervals to be dried on filter paper and weighed.

**3.2 Gelatin Expansion Study:**

we simulated the expansion of the wound dressing film by measuring the alteration in the circumference of a circular film sample immersed in a 10% gelatin solution. We utilized 2 grams of gelatin powder dissolved in 100 mL of purified water. The gelatin was completely dissolved by heating the solution. Subsequently, 20 mL of the gelatin solution was placed in a Petri plate, and the film was submerged in the solution. On the gelatin surface, a sheet of sample with a specified diameter was placed, and its circumference was measured. The sample's diameter was regularly modified until it reached a consistent size or different time interval. Next, the calculation for the Expansion Ratio (ER) was determined using the given equation:

$$\mathbf{ER} = \frac{\boldsymbol{diametere\ at\ time\ (DT)}}{\boldsymbol{Initial\ Diameter}}$$

where, Dt = t time diameter and Do = Diameter at initial time.

**4.0 Characterization:**

**4.1 UV-Visible of Clitoria ternatea seed extract**

The light-absorbing properties of the developed plant seed extract of *Clitoria ternatea* were analyzed using a UV-1900 series Instrument provided by Shimadzu at Parul Institute of Applied Science, Parul University, Vadodara. The wavelength was set between 200-800 nm.

**4.2 Fourier-Transform Infrared Spectrophotometry (FTIR)**

FTIR spectroscopy, also known as Fourier transform in was used to confirm the existence of functional groups in a Chitosan-PLA mixed film. FTIR analysis was employed to assess the polymeric blend of Chitosan-Poly (lactic acid) at Parul Institute of Pharmacy and Research, Parul University, Vadodara. The FTIR spectrum utilized for this assessment has an aperture of 4 $cm^{-1}$ and a range of 3500 to 1000 $cm^{-1}$.

**4.3 Scanning Electron Microscope (SEM) Analysis**

In order to make images of the material, an electron magnifying lens known as a filtering electron magnifying instrument checks the surface of an example with activated light emission. The interaction between the

electrons and the sample's atoms results in different signals that show the sample's surface topography and chemical composition. An SEM analysis was performed to evaluate the surface morphology and determine how the Chitosan and PLA composite films were disseminated. PNP Analytical Solutions in Vadodara performed the SEM study, taking all required safety procedures **[12]**.

**4.4 Thermogravimetric Analysis (TGA)**

TGA is a method of material analysis that assesses the mass of an experiment as a function of time or temperature when controlled heating is present. Temperature and weight loss are noted as the material's constituent parts gradually volatilize over time. Because TGA testing can measure weight loss at very high temperatures, it is useful for evaluating polymers. In a nitrogen environment with a flow rate of 10 °C/min, samples were studied at temperatures ranging from ambient to 800 °C at 25 °C/min **[12]**. Taking all required precautions, the TGA analysis was carried out at PNP Analytical Solution in Vadodara.

**4.5 Crystalline Material Characterization**

The basic characterization of material properties such as crystal structure, crystallite size, and strain is done by X-ray diffraction (XRD). The Composite film and natural resources X-ray diffraction patterns were obtained using a Phillips X-Pert diffractometer. The equipment was run at 40 kV and 40 mA, and the nickel-filtered Cu X-rays had a wavelength of 0.1542 nm. This technique involves exposing the sample to X-rays while measuring the sample's X-ray intensity and scattering **[13]**.

**5.0 RESULT AND DISCUSSION:**

**5.1 Swelling Study:**

The swelling test establishes the maximum time that composite film may be kept wet. Swelling is described as an increase in a gel or solid's volume brought on by the absorption of a liquid or gas. The first stage is swelling in the interaction of molecules in liquid and polymer's polymeric network disintegration, which typically occurs after chain solvation in polymers. The PLA/chitosan film was submerged in the PF solution for 30 minutes, removed, and then dried. after which it was measured for weight. then repeat the procedure 3 or 4 times.

The swelling ratio (SR) has been calculated with the help of the following equation;

$$\mathbf{SR = (Mt - Mo) / Mo\ g/g}$$

Where Mo = initial mass and Mt = mass at different time intervals.

**5.2 Gelatin Expansion Study:**

An expansion study is carried out to estimate the expanding diameter of the polymeric

composite **(Table 2)**. Below formula for ER; ER= Dt/Do

Where Dt = Diameter measured at time t,

Do =diameter at initial,

**5.3 Spectrum of UV-Visible Absorbance**

This information most likely refers to light absorption at various wavelengths, which is often employed in UV-Visible spectroscopy to ascertain the amount of or the characteristics of compounds in a sample. This UV spectra which is shown in **Figure (3)**.

**5.4 FTIR Analysis:**

Fourier transform infrared spectroscopy (FTIR) is a very flexible materials examination tool that may help find organic and inorganic pollutants that might contaminate or damage products. As a consequence, FTIR is widely used to identify the earliest stage of any material. The analysis was performed within the range of 400 to 4000 $cm^{-1}$, utilizing scans with a resolution of 4 $cm^{-1}$. The FTIR spectra were recorded in transmittance mode. We prepared a film, dried and subsequently crushed it, followed by preparing a powder for FTIR analysis. The FTIR spectrum of the chitosan-PLA composite film exhibits absorption peaks corresponding to chitosan and PLA. The results are shown below; We can see chitosan's infrared spectrum in **Figure (4).** A strong band in the region 3000-3500 cm−1 at 3291 and 3368.51 relates to N-H and O-H stretching, as well as the intramolecular hydrogen bonds. Stretching of the C-H atoms in symmetric and asymmetric ways are responsible for the absorption bands at around 2921 and 2871 $cm^{-1}$, respectively. These bands may be seen in various polysaccharide spectra and have polysaccharide-specific properties. The absorbance at around 1647 cm-1 refers to the C=O functional group. A peak shown nearly at 1559 $cm^{-1}$ corresponds to the N-H bending of the primary amine. The peaks between 1460 and 1375 cm-1, respectively, verified the CH2 bending and CH3 symmetrical deformations. The peak at 1066 cm−1 corresponds to C-O stretching. It is possible to explain the absorption band around at 1250 cm−1 by the asymmetric stretching of the C-O-C bridge. Now we focus on **Figure (5)**, In this the carbonyl (C=O) stretching vibration in PLA, which has ester bonds, which is a sharp peak at 1749.85 nm. C-H bending sharp peak at wavelength 1453.91 nm. In this spectrum, we can observe the stretching vibration of the C-C-C backbone at 1183.51 nm which is sharp peak. A chemical change in the film was examined using FTIR spectroscopy. The composite film's FT-IR spectrum is shown in **Figure (6)**. In IR spectra of a composite film of PLA and Chitosan the broad peak around at 3000 to

3500 $cm^{-1}$ shows the presence of O-H stretching band. The absorption at around 2919 $cm^{-1}$ sharp peak it indicates the presence of a C-H stretching band. The sharp peak at 1748 $cm^{-1}$ which refers to the presence of the C=O functional Group. The possibility of a C-O (Ester) stretching vibration of the carbon-oxygen bond is suggested by the existence of a prominent peak in the spectrum at 1078 $cm^{-1}$.

**5.5 Morphological characterization:**

The link between the surface morphology and the film's composition was examined using SEM. SEM provides information on the nanoparticle dispersion within the continuous matrix, the composite's homogeneity, the presence of voids, the amount of aggregate, and the possible nanoparticle orientation**.** In **Figure (7-a)** shows the surface morphology image of Chitosan/PLA. The abbreviation EHT stands for "Electron High Tension" or "Accelerating Voltage." It shows that the microscopy's electron beam was accelerated to a voltage of 10,000 volts. In electron microscopy, higher accelerating voltages may provide improved penetration and resolution. The abbreviation "In Lens Mag" stands for "In-Lens Magnification." It says that 25,000 times (25.00 K X) of magnification were used to get the picture. There was a 4.1 millimeter working distance which is shown in **Figure (7-b)**. Controlling the focus and depth of field in electron microscopy is a crucial parameter.

**5.6 Thermal Properties:**

Thermogravimetry was used to examine how the weight of the Chitosan and PLA composite film altered using the technique of planned heating in the range of 25°C-800°C. While in the presence of nitrogen. When the temperature increased. Here TGA analysis was conducted by heating the sample from 25-800°C at a rate of 10°C per minute. In this analysis at the temperature point X1=89.75°C and X2=154.12°C where weight loss occurred and matching weight percentages Y1=99.053% and Y2=95.313% as well as deltas showing how much the weight changed which is shown in below **Figure (8)**. Particularly, a substantial weight loss of 94.580% was seen at about 246.39°C, and an additional change with a weight loss of 2.653% was seen at about 355.83°C. The above data provides indications of the stability and behavior of the sample's thermal destabilization.

**5.7 Crystallographic Characterization Using X-ray Diffraction:**

A flexible and effective analytical method utilized in many scientific and commercial domains is X-ray Diffraction (XRD) analysis. By observing how X-rays interact with the sample, its main goal is to examine the atomic

and molecular structure of crystalline materials. A graph with intensity (commonly shown on the y-axis) and the 2θ angle (often represented on the x-axis) is generally produced by the XRD analysis. The crystalline phases included in the composite film would be determined by the locations and intensities of these peaks. The graph below displays peaks for a composite made of PLA and chitosan that match to their respective crystal structures. Here 2θ degree value on the X-axis at 17.596 shows a sharp peak which corresponds to higher Crystallinity and at the 2θ value 20.086 is less crystalline. After this point no sharp peak which shows the amorphous region. Amorphous areas in XRD patterns may be recognized by their lack of identifiable peaks. Instead, they show up in the pattern as wide "humps" or diffuse dispersion. This XRD graph is shown in **Figure (9)**.

**5.8 Activity of the Antibacterial**

The antibacterial activity of Chitosan and PLA film which contains a natural drug Butterfly pea seed extract which tested against the *Escherichia coli (E. coli)* bacteria. Log phase culture of *Escherichia coli* in nutrient broth used for antimicrobial activity. A standard concentration was then achieved by diluting the bacterial culture. Now we sampling our solution via well diffusion method in well. The zones of inhibition were assessed during an overnight incubation which was observed measuring around **3.2 to 3.6 cm** which is shown in **Figure (10)**.

**Table 1: Swelling Study of Separate film and Composite film**

| Time | Chitosan | PLA | Ch/PLA (50:50) |
|---|---|---|---|
| 0 min | 0.5 | 0.6 | 0.8 |
| 30 min | 1.3 | 1 | 1.5 |
| 60 min | 1.5 | 1.5 | 1.8 |
| 90 min | 1.8 | 1.55 | 2 |
| 120 min | 1.9 | 1.6 | 2.1 |
| 150 min | 1.9 | 1.6 | 2.1 |

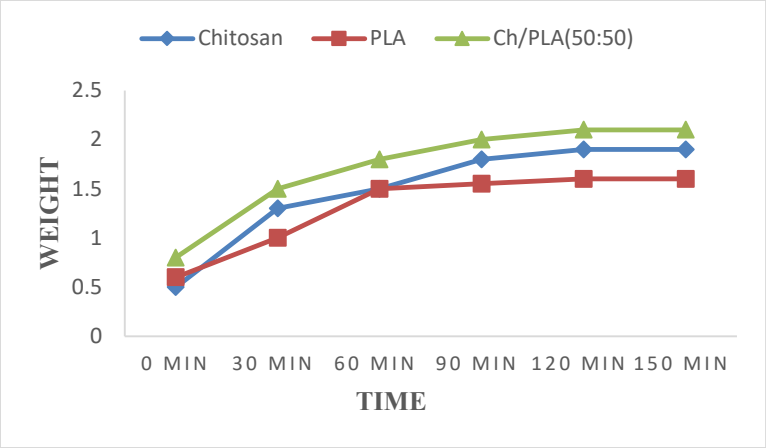


**Figure 1: Graph of Swelling Study**

**Table 2: Gelatin Expansion Study**

| Time | Chitosan | PLA | CS/PLA (50:50) | CS/PLA (30:70) |
|---|---|---|---|---|
| 0 min | 1.5 | 1.6 | 1.5 | 1.5 |
| 20 min | 2.3 | 1.9 | 1.6 | 1.61 |
| 40 min | 2.5 | 1.91 | 1.75 | 1.69 |
| 60 min | 3.7 | 1.95 | 1.85 | 1.7 |
| 80 min | 4.3 | 2.01 | 2.3 | 1.8 |

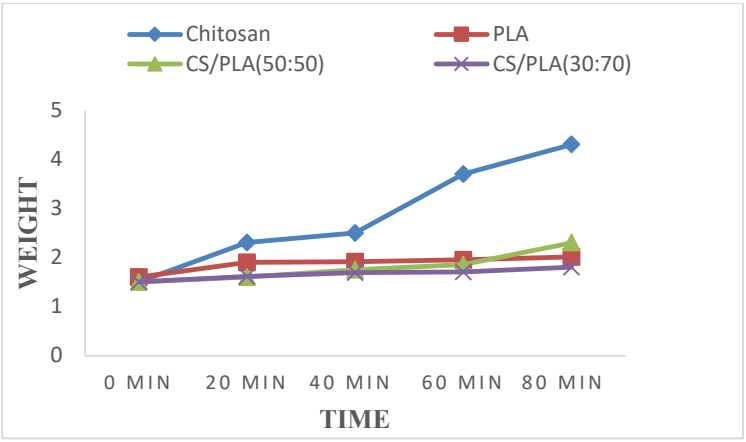


**Figure 2: Gelatin Expansion Graph**

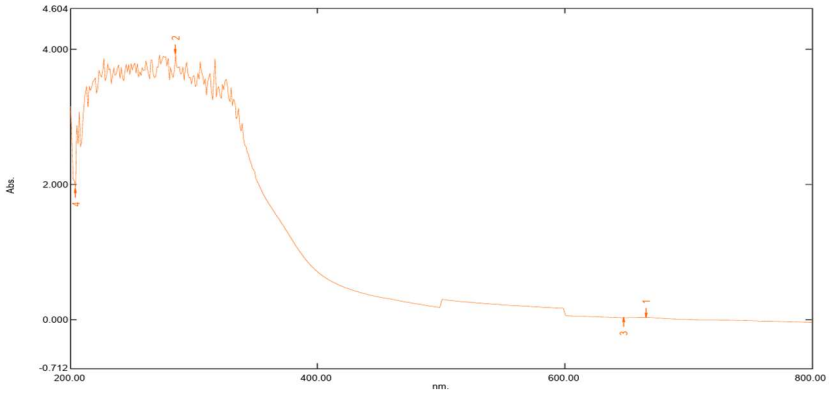


**Figure 3: UV-Vis Spectra**

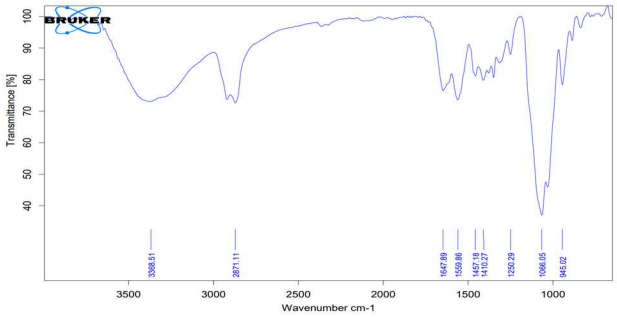


**Figure 4: FTIR Spectra of Chitosan Film**

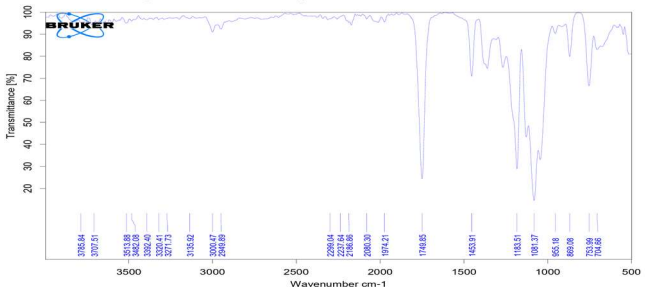


**Figure 5: PLA FTIR Spectra**

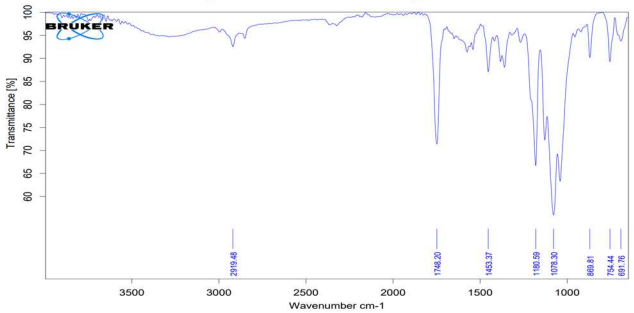


**Figure 6: FTIR Spectra of Chitosan and PLA**

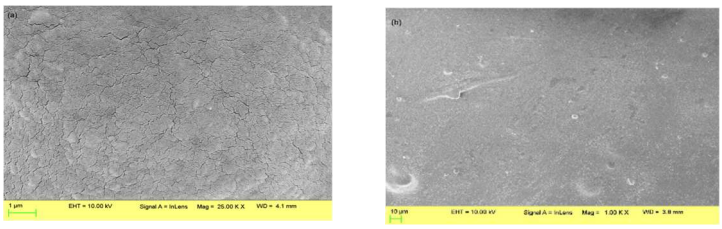


**Figure 7: SEM Analysis of Composite Film**

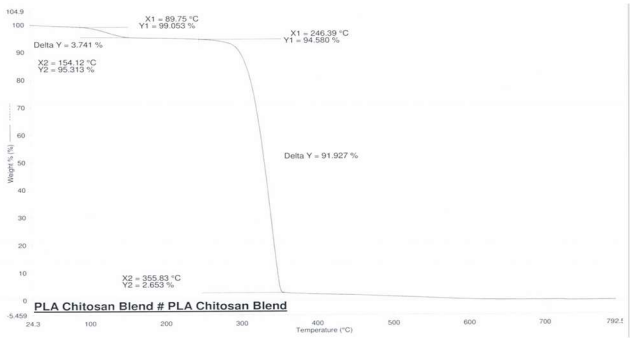


**Figure 8: TGA Analysis of Composite Film**

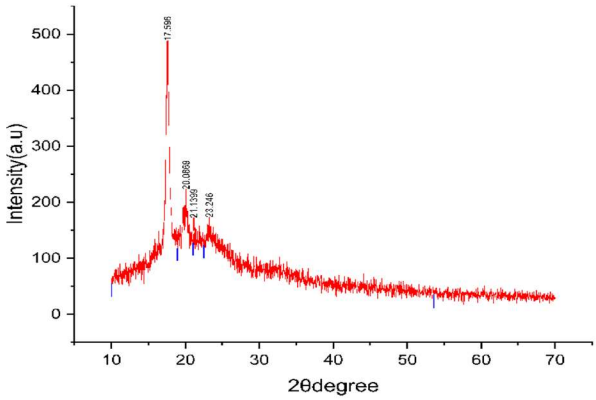


**Figure 9: X-ray Diffraction of Composite Film**

**Figure 10: Antibacterial Activity of composite film against *E. coli***

## 6.0 CONCLUSION:

In this research work**,** the utilization of chitosan and poly lactic acid (PLA) composite films, as well as natural drug Butterfly pea loaded films, for wound treatment, food packaging, and antibacterial activity, has been shown. Their antibacterial action is increased by the use of the natural medicine butterfly pea, making them more efficient in preventing infections and hastening the healing of wounds. The films' ability to withstand oxygen and water makes them excellent candidates for use in food packaging. The crystalline structure and orientation of the composite films have been revealed by characterization techniques such as XRD, and the surface appearance and microstructure of the composite films have been examined using SEM. The films' functional groups have been identified by FTIR analysis, and their thermal stability has been shown by TGA.

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**Conflict of Interest**

No conflicts of interest exist, according to the authors, with the publishing of this work.

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