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Preparation of New Biofilms Suitable for Manufacturing Processes for A sustainable Environment

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Abstract: Biodegradable biofilms are sustainable environmental solution and are increasingly important with the increasing awareness of consumers about the environment. This study aimed to prepare and characterize biodegradable poly-3-hydroxybutyrate (P3HB) films with improved physical and chemical properties after incorporation with plant extracts. These biofilms are antibacterial for use in food packaging and medical supplies. The new (P3HB) bioplastic films were prepared using casting technique. The prepared biofilms were the first P3HB/G (resulting from P3HB and ginger extract) and the second P3HB/C (resulting from P3HB and clove extract). All the mentioned biofilms were compared to the P3HB/M which was prepared from P3HB and chloroform only. The thickness, moisture, dissolvability and swelling of the new resulted biofilms were measured. Their chemical and morphological properties were studied using SEM and FTIR. Their ability to inhibit *Staphylococcus aureus* and *Escherichia coli* were evaluated, as well as their efficiency in food packaging and preservation. The obtained results demonstrated that the P3HB/C and P3HB/G films exhibit good chemical, physical and antibacterial properties due to the phytochemicals incorporated into the polyester polymer or loaded into their matrix. Thickness, moisture and swelling for P3HB/C and P3HB/G were significantly different; compared to P3HB/M. Yet, when comparing P3HB/C and P3HB/G, the moisture was the only markedly various. The prepared biodegradable packaging materials are great alternatives to conventional plastics since it could be friendly ones to environment.

Keywords: Biodegradable polyester, Bioplastic films, Plant extracts, Poly-3-hydroxybutyrate.

Introduction

A Bio-based plastic are the focus of recent research to reduce pollution from petrochemical plastics. Nowadays, consumers are need for foods produced using the least rate of unnaturally made preservatives, fit for longer consumption (Abrha *et al*., 2022; Al-Hilphy *et al*., 2024). Astimulation occurs in the migration of antimicrobial compounds

from prepackaged packaging materials which could dominate the microorganisms in food. As a result, a considerable endeavor has been made to develop biopolymer packaging systems as carriers for plant-derived antimicrobials (Oda *et al*., 2022).The presence of new food packaging materials inhibits or inactivates microorganisms, reducing the use of preservatives (Zanetti *et al*., 2018, Deshmukh& Gaikwad, 2024, Srisa *et al*., 2022). Medical supplies made from biodegradable polymers represent a major leap forward in protecting the environment from plastic and thermal pollution (Yang, 2022, Oda *et al*., 2022). They can be disposed of after being sterilized by environmentally friendly methods (such as radiation) and then, analyzed by microorganisms (Hiep *et al*., 2023). Recently, the possibility of using bioplastic polymers as drug carriers inside the body of living organisms. (Satchanska *et al*., 2024; Ahmad *et al*., 2024). Biopolymers are non-toxic and do not stimulate the immune system.

Medicinal herbal extracts were known as antiseptics and remedies for various diseases in ancient times (Saleh *et al*., 2023). Nowadays, many researches handle the use of these herbals in, vanishing the damages of skin, antimicrobial and antioxidant agents (Azeem *et al*., 2024)

Ginger and cloves are local spices that help keep foods from spoiling, which indicates their high biological effectiveness (Yousif & Hassan, 2023). However, undesirable parameters exist, such as: their rapid release in biological systems, and their volatility and agglomeration when pure. Besides, they lose their antibacterial effectiveness when being exposed to environmental influences (Premjit *et al*., 2022). Bioactive chemical compounds present in medicinal plants have been encapsulated in typical polymeric matrices from depleted resources (Saifullah *et al*., 2019). Polycaprolactone (PCL), polyglycolide (PGA), and polybutylene succinate (PBS) have been commonly used as drug carriers in the medical field (Romero-Castelán *et al*., 2023). These biopolymers preferred in such industries because of :

- Having nonpoisonous effect when packaging ,
- Recording no negative biological harm with plant extracts, and
- Being able to be biodegraded in a short span of time (Dutta & Sit, 2023).

The bioplastic polymers, such as P3HB, are able to keep plant extracts away from undesired interactions (Acharjee *et al*., 2024). P3HB are large bio-polyester molecules produced within bacteria and archaea in the form of granules. They can serve as energy reservoirs in time of starvation (Sharma *et al*., 2022). In 2022, Luciano reported that the use of P3HB as a carrier for bioactive plant compounds. P3HB carrier provided positive support for bioavailability and half-life that is sensitive to external conditions without causing cytotoxicity.

These properties enhance the use of P3HB as an auspicious material for encapsulating natural compounds to protect their activities. The encapsulation preserves the effectiveness of antimicrobial compounds, prevents them from interacting with food and releases them at specific times. (Ladhari *et al*., 2023). P3HB has suitable mechanical properties and is resistance to water vapor permeability. It is a suitable substitution to polyethylene of hydrocarbon origin used currently in the process of packaging (Sharma *et al*., 2022). It seems that the use of P3HB polymer could be a solution to the problem of environmental pollution by plastic. In addition, the goal is to

obtain a "green" material with entirely renewable resources. These materials carry antibacterial phytochemicals interwoven with the biopolymer matrix. This study aims to prepare and synthesize a novel biofilm with good manufacturing properties as a promising alternative to hydrocarbon plastics.

Materials & Methods

Chemicals /Biological Materials and Instruments

Poly (3hydroxybotyrate) (CAS No.: 29435-48-1) was purchased from Sigma-Aldrich, USA. The chloroform was brought from Daejung chemical & metal Co.Ltd (Siheung, Korea). Additionally, Mueller Hinton Agar (MHA), MacConkey agar, Mannitol Salt agar, Nutrient Broth and McFarland standards solution 0.5, were acquired from Oxford (Hampshire, UK). Fourier-transform infrared spectroscopy (FTIR) spectral data were recorded on a Shimadzu and using a KBr disc. The physical description was implemented using a Scanning Electron Microscopy analysis (SEM) (Czech Republic).

Source of Bacterial Isolates

The means of gathering samples from garden soil was a sterilized tube which was sent to the laboratory. The serial dilutions of the collected samples were made, with 0.1 ml of each dilution being spread on culture agar. MacConkey medium and mannitol salt agar were used to isolate *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*). The purified colonies were transferred to a specialized medical laboratory accredited by the Ministry of Health in Karbala, Iraq. The diagnosis was achieved by using the VITEK system following (AlKhafaji *et al*., 2024 & Oudah *et al*., 2024).

Preparation of Clove and Ginger Extracts

Ten grams of both ginger and clove were used in the experiment. After drying properly, they were cut into small pieces and ground to powder in an electric grinder. Three grams of both ginger and cloves powders were weighed and placed in separate flasks. Then, 100 ml of chloroform were added on each flask. They were placed on a magnetic stirrer hot plate at 70°C for 10 minutes. Later on, the mixture was left for two hours with continuous stirring using a magnetic stirrer. The mixtures were centrifuged at 3,000 rpm for 6 minutes. The supernatants were passed through a 0.45 mm whatman filter paper. After that, the extracts were used to prepare P3HP films (as in Abbas & Al-Subaihawi (2022).

P3HB/M, P3HB/G and P3HB/C Films Preparation

Three grams of P3HB powder was dissolved in 60 ml of pure chloroform to prepare P3HB membrane (P3HB/M) (control membrane). To prepare ginger membrane (P3HB/G), 3 grams of P3HB was dissolved in 60 ml of ginger chloroform extract. To prepare clove membrane (P3HB/C), three grams of P3HB powder was dissolved in 60 ml of clove extract. The samples were exposed to a temperature of 70 ± 5 °C for 2 h. The mixture was stirred using magnetic solvents to ensure that the polymer did not decompose during the polymerization process. The resulted mixtures were cooled to a room temperature after the end of the polymerization period. After that, glass substrates (20cm×20cm) lined with non-stick wax paper were used in order to mold the solution over them. The casting molds were placed in a closed hood containing cotton pieces saturated to allow polymerization to occur properly. This could produce consistent, uniform films. The chloroform evaporation was for 30 min at

room temperature (as explained in Oda *et al*., 2022).

The Thickness of Bioplastic Films

The thickness of the films was measured by means of a micrometer screw. The measurements of center and the four corners of films were obtained. Consequently, the average of previously mentioned measurements was stated in the experiments which were implemented repeatedly four times for each film (following Kanmani & Rhim, 2014).

The Moisture Content of Bioplastic Films

The moisture content of bioplastic films was measured by using the thermogravimetric analysis (as followed by Pech-Cohuo *et al.,* 2022). The very basics of this method was dependent on the evaporation of water which exists in the films. This evaporation was done by exposing them to a heat and then, weighing them constantly. The weight of the pieces of the film of (3 cm × 3 cm) were (W1). After that and to gain a constant weigh of (W2), they were dried in an oven at 105 ˚C. for four times for each film. The moisture content in the experiments was obtained by applying the below equation:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1} \times 100 \quad \text{..... Equation (1)}$$

Water Solubility of Bioplastic Films

The determination of the water solubility related to the biofilms was done by the use of film pieces of (2 cm × 2 cm). They were dried at 105 ◦C. The films weighted (F_{W1}). The researcher submerged the pieces of the biofilms in beakers of 100 ml with 30 ml of distilled water. They were sealed and stored in a room temperature for 20 hrs. with a constant agitation up to 150 rpm. The films were dried out with filter papers (weight before) in an oven at 105 ˚C for 20 h so as to gain the final films weight (F_{W2}) (as it was clarified in Arifin *et al*., 2022). The experiments were fulfilled in four repetitions for each film. Then, the water solubility of the films was analyzed by applying the below equation (Jawad *et al.,* 2025).

$$\text{Water Solubility (\%)} = \frac{F_{W1} - F_{W2}}{F_{W1}} \times 100$$

Bioplastic Films Swelling Degree

For the determination of bioplastic films swelling degree, pieces of (4 cm × 4 cm) were dried in an oven at 105 ˚C and films weighted (S_{W1}). Then, they were soaked in 100 mL beakers with 60 mL of distilled water at 28 ˚C for 2 hrs. The extra water of highly swollen samples was removed using filter paper and the samples were weighed (S_{W2}) (as explained in Rui *et al*., 2022 & Alali *et al*., 2024). Experiments were carried out in four repetitions of each films. The average value was measured as well as the swelling ratio. The degree of bioplastic films swelling was calculated using the below equation:

$$\text{Swelling degree (\%)} = \frac{SW2 - SW1}{SW2} \times 100$$

Characterization of P3HB/M, P3HB/G and P3HB/C Films

The study used FTIR spectroscopy to determine the functional groups present in the P3HB/M, P3HB/G and P3HB/C films. Moreover, the FTIR spectra were calculated. Their range was from 4000 to 600 'cm−1' (similar to Khaleel *et al*., 2024). The physical properties of the surface of the prepared films were studied by using SEM device in the SEM Center unit in the College of Veterinary Medicine- University of Karbala. The specifications were: quantity: 450, cutting

capacity: 2KA, main voltage: 230 VAC, and main frequency: 50/60 Hz. The acceleration voltage of this room was 20-30 kV (similar to what was mentioned in Sun *et al*., 2022).

Antibacterial Activity

The P3HB/M, P3HB/G and P3HB/C films were tested against *S.aureus* and *E.coli* which were isolated from the previously mentioned soil. Both isolates were activated by being cultured in nutrient broth medium for 24 hrs. The bacterial suspension at a concentration of (about 10^7CFU/ml) was grown on the surface of three MHA plates by swab. On their surface, one of the types of pre-prepared films was placed. One type of bacteria was on one half of the plate and the second type of bacteria was on the other half. The plate which was on the surface of P3HB/M was used as a control. The cultured plates were incubated for 24 hrs. under a temperature of 37°C.

Bioplastic Bags Manufacturing Method and Application in Food Packaging

Bioplastic bags of P3HB/M, P3HB/G and P3HB/C films were manufactured by folding a piece of film (measured:8cm long and 4cm wide). The edges were glued with a hot knife to produce a bag with dimensions of (4×4) cm. The efficiency of the bags produced in preserving food was tested by placing a previously soaked and boiled bean. The purpose behind it was to represent the food item which would be preserved when using the manufactured bags. The beans were stored in three bags made of P3HB/M, P3HB/G and P3HB/C films. One bean was left unpreserved by placing it on filter paper with three replicates. After that, all samples were stored under the same conditions and at room temperature. The samples were being humidified every two hours using sterile water spray for all samples at the same time. After 72 hrs., the results were recorded.

In Vivo Wound-healing Study and Ethics Statement

The ability of the prepared membranes to contribute to wound healing were tested using live animals in the laboratory. The instructions and notices stated by the Institutes of Health literally were followed as stated in Couto and Cates, 2019, regarding the care and the handling of animals in the laboratory. The experiment was applied on the animal house at the College of Pharmacy, University of Karbala. The test animals were twenty male rats; weighing (250 g) and aged between 5 and 7 weeks. These rats were grouped into four divisions having five rats in each group arbitrarily. And that depended on the materials used to bandage the skin wounds. The rat's skin was cut about 1.5 cm (wound area) from the dorsal side after removing hair from the area. The wounds of three groups were covered with an equal size of the prepared membrane in three groups. The wounds of the second group were covered with P3HB/M, the third group with P3HB/G, while the fourth group with P3HB/C. The cotton gauze was the only dress of the rats with bare wounds in the first group and the control group. Under the room temperature, the rats were placed individually in cages after applying the dressing materials. After each three days, the membrane dressings under study were changed. The external appearance of the wound was photographed in (0, 4, and 12) days to measure the rate of wound healing.

Measurements of Wound Healing

Wound healing percentage (WHP) was calculated by noticing the difference in wound area (WA). Wound areas were

measured using a ruler from the first day until the end of the experimental days, according to the following equation stated in Salim *et al*., (2022):

$$\mathbf{WHP} = \frac{WA\ at\ day\ 0 - WA\ at\ day\ 10}{WA\ at\ day\ 0} \times 100$$

….. Equation (4)

Statistical Analysis

Statistical differences were obtained by SPSS version 22- IBM / USA software using independent-sample t-tests. All results were shown as mean ± standard error (SE). $P \leq 0.05$ was considered statistically significant (Hoehndorf *et al*., 2015). The significance level between the groups was specified as (*).

Results & Discussion

Preparing New Bioplastic Films

P3HB/M, P3HB/G and P3HB/C films were prepared by casting technique. The P3HB/M appeared transparent, whitish in color, with a very soft and somewhat thin texture. P3HB/C appeared in a transparent, yellowish-white color with a very slightly rough texture. It was more durable and flexible than P3HB/M. P3HB/G appeared in a transparent, white-brown color, with a very slightly rough texture. It was more durable and flexible than P3HB/M, and somewhat similar in durability and flexibility to P3HB/C (as shown in the Fig. (1)). The addition of ginger and clove extracts led to a change in the nature of the bioplastic films in terms of texture and color with a noticeable difference in the thickness and strength of the membranes. Thus, the strength of the clove extract was higher. Plant extracts are characterized by the presence of compounds such as flavonoids, sterols, alkaloids, tannins, and glycosides. Their type and proportions differ according to the type of plant. Therefore, the difference found in the physical characteristics of the membranes is attributed to the addition of clove or ginger extract. These results are consistent with (Tan *et al.,*2021), as the addition of clove oil to PVA/chitosan film led to a change in the thickness, and transparency of the film.

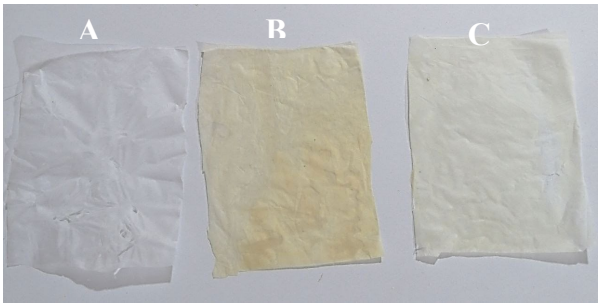

Fig. (1): The External Appearance of the Prepared bioplastic Films, A= P3HB/M, B= P3HB/G, C= P3HB/C

Physicochemical Properties of Bioplastic Films

The thickness, moisture, swelling and solubility results of P3HB/M, P3HB/G and P3HB/C films are mentioned in Table (1). Thickness, moisture and swelling for P3HB/C and P3HB/G were significantly different; compared to P3HB/M. Yet, when comparing P3HB/C and P3HB/G, the moisture was the only markedly various. The easiness of preparation of the bioplastic films was the reason behind their thickness. As the thickness of the bioplastic films was increasing, the forming of the films would be more rigid and difficult. However, a better mechanical protection for the packaging and doing other applications for the food could be available. Generally, the thickness of the P3HB/G and P3HB/C films were significantly higher than that of the P3HB/M. This was possible because the phytochemicals loaded or combined with them could maximize the viscosity of the film solution.When being dried, the resulted film became thicker. In addition, ginger and clove-plasticized films were not significantly different in isolation.

Actually, they were so before adding plant extracts. The results of water solubility, moisture and swelling ratio showed significant variation among the manufactured films, which supports the occurrence of an interaction between the compounds present in the plant extracts with the bioplastic film matrix. The relationship between P3HB/G and P3HB/C films was direct as the proportion of plant extracts were being increased. This happened because of plant extracts which had hydrophilic properties. Accordingly, an interaction with water would be available by the aid of establishing and creating H-bond. The more different the types of hydrophilic compounds are and the higher their concentration in the composition of the biofilms were, the greater was the swelling and the moisture of those membranes (Jing *et al*., 2023).

Table (1): Physical Properties: Thickness, Moisture, Water-solubility, Swelling of P3HB/M, P3HB/G and P3HB/C Films

Characters	P3HB/M	P3HB/C	P3HB/G	SD1	SD2	SD3
Thickness (mm)	0.002 ± 0.021	0.005± 0.013	0.006± 0.04	*	*	NS
Moisture (%)	1 ± 0.03	11± 0.05	7± 0.31	*	*	*
Solubility (%)	0.1± 0.001	0.5± 0.06	0.5± 0.02	NS	NS	NS
Swelling (%)	1 ± 0.07	4± 0.08	5± 0.12	*	*	NS

Data are means ± standard error of the mean. P3HB/M =P3HB membrane, P3HB/G= ginger membrane, P3HB/C= clove membrane, SD= indicate Significant Difference ($p \leq 0.05$), SD1= between P3HB/M and P3HB/C, SD2= between P3HB/M and P3HB/G. SD3= between P3HB/C and P3HB/G, NS= Not statistical significance.

This is consistent with the results of Wiset *et al.,* (2014), who compared the addition of glycerol in the preparation of edible films. It was found that films with added glycerol had a higher dissolution rate than those without glycerol. Furthermore, Warkoyo *et al*. (Warkoyo *et al*., 2023; Warkoyo et al., 2024) mentioned that the difference in the type of edible film making materials may be the reason for the increased thickness. Accordingly, the total amount of dissolved solids in the film could be increased. This causes a difference in the viscosity of the film solution causing the thickness to vary. Cano *et al*. (2020) found that the addition of tannins to caseinate or gelatin films resulted in differences in the microstructural, physical and chemical properties of the resulting films. The film thickness and wettability after addition varied without significant differences in water solubility or increase in swelling of the tannin-caseinate/gelatin films. This was attributed to the establishment of hydrophobic interactions and hydrogen bonding in the prepared biofilms. This happened especially when adding tannin because of the greatest phenolic content.

External Morphology of Bioplastic Films by SEM

The scanning electron microscopy results showed different morphologies and structures of the prepared films surfaces. The surface of P3HB/M shown in Figure (2.A) is characterized as being less rough compared to the other two types, in addition to containing clear cracks in the membrane tissue structure. Its general characteristic is that the molecules composing the membrane surface are not tightly packed. The surface of the P3HB/C membrane shown in Figure (2.B) is characterized by being compact and does not contain any cracks. The outer surface consists of polycrystalline pentagonal shapes of a large size compared to the surface of other two membranes. This means that its surface is

rougher, compared to the surface of the P3HB/M membrane. The surface of the P3HB/G membrane shown in Figure (2.C) is characterized by being rough, free of cracks, and having particles arranged in a star-shaped pattern and having a wavy nature. The addition of plant extracts of ginger and cloves in the manufacturing of the prepared P3HB/G and P3HB/C membranes led to a change in the chemical composition of the membrane tissue, which led to a significant change in the surface properties of the membrane, such as surface roughness and external crystalline shape.

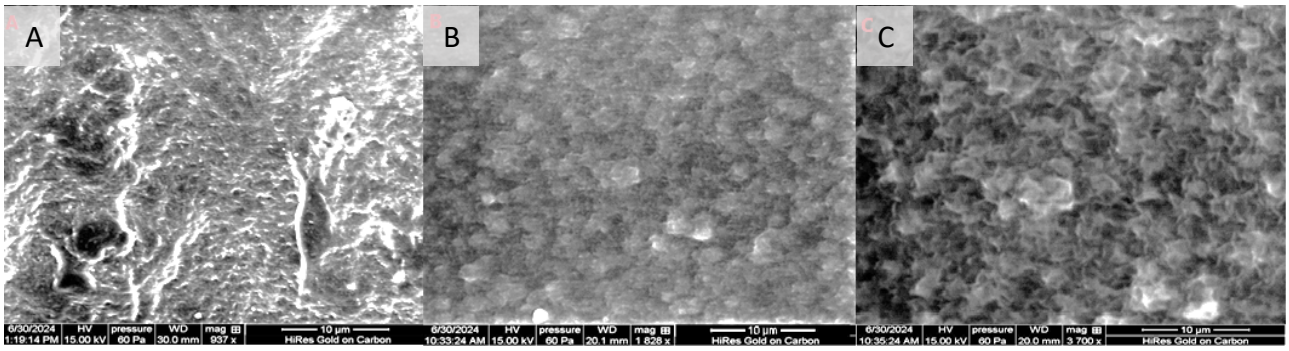

Fig. (2): Structure of the External Morphology by Scanning Electron Microscope Images: A: P3HB/M, B: P3HB/G, and C: P3HB/C

The addition of plant extracts in the manufacturing of P3HB/C and P3HB/G membranes led to a change in the membrane texture. This led to a significant change in the surface properties of the membrane, such as surface roughness and external crystalline shape. The difference in the external crystalline shapes of the P3HB/C and P3HB/G membrane surfaces indicates that the physical and chemical properties of the prepared membranes differ according to the type of extract used, which may be due to the difference in the compounds present in each of them. These results are consistent with Tan *et al.,* (2021) who demonstrated that adding clove oil and ginger oil extracts to the PVA/chitosan film made it mostly homogeneous and compact texture. This is consistent with what Pech-Cohuo *et al*. (2022) found when examining using an electron microscope. He stated that adding corn starch and ramen starch to the chitosan membrane led to a change in the texture. The membranes became rougher and had different surface shapes depending on the type and concentration of the added substance.

Characterization of Biofilms Using FTIR

The FTIR analysis results for the P3HB/M film elucidated a distinct and clear peak at wavelength (1730-1715) resulted from the rigid property of carbonyl vibrations (C = O) which characterizes the ester groups, as well as the stretching frequency of the (O–C) bond with two or more peaks in a region between (1300-1000 cm) (Escobar *et al*., 2024). An absorption peak also appears at wavelength (1454.38) which represented the absorption peak of the C = C bond. The appearance of absorption band at (3430, 2935.76, 1454.38, 1381.08) cm−1 confirms the presence of OH, CH, CH2 and CH3 groups, respectively. In addition, the peaks in the region from 956.72 to 810.13 cm belong to the bending and stretching of the (C–H) bond (as shown in Figure (3)). The results of FTIR analysis for the prepared membranes manifested a clear

difference in the type of the present peaks, as peaks appeared at wavelengths which did not appear in the pure membrane with no plant extract. Also, a difference appeared in the strength of the bond, as some peaks which increased in length or width were noted. That was an indication of the formation of bonds in additional numbers, as shown in Figures (4 and 5). The change in the vibration absorption peaks proves the presence of new chemical bonds responsible for new chemical and biological properties (Al-Murshedi *et al*., 2022; Alkhafaji *et al*., 2022). It was resulted from the union of the components of the plant extract with the molecules of the polyhydroxylbutyrate compound. Its parts were composed of a mixture of the compounds presented in the extract, also from the retention of some others within the membrane tissue to be released at a later time. It should be highlighted for its use as a drug carrier and to preserve the chemical properties of the compounds. This is a consistent with what Arifin *et al*., (2022) explained that the appearance of a new absorption band and a change in the absorption band, In addition, the difference in absorption intensity means a new interaction has occurred between the original polymers and the additives. And that affected the properties of the bio-nanocomposite film. Similar result was found in other studies including Tamimi *et al*., (2021).

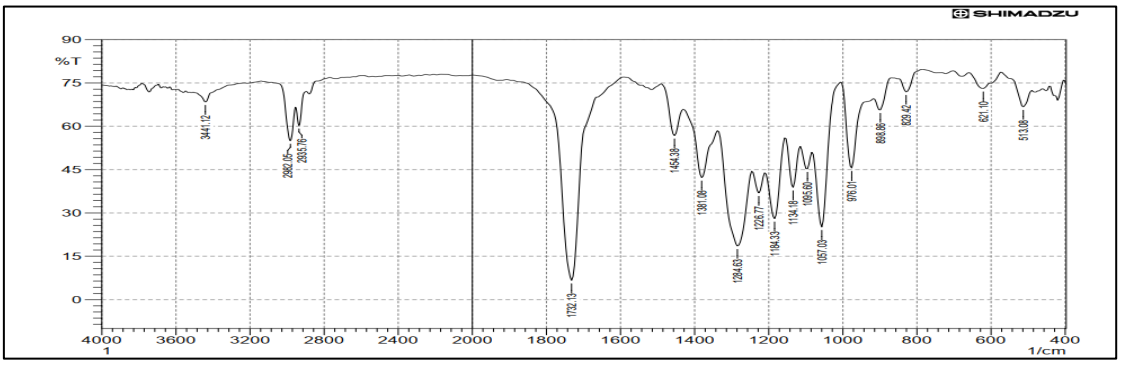

Fig. (3): FTIR Spectra of Bioplastic P3HB/M.

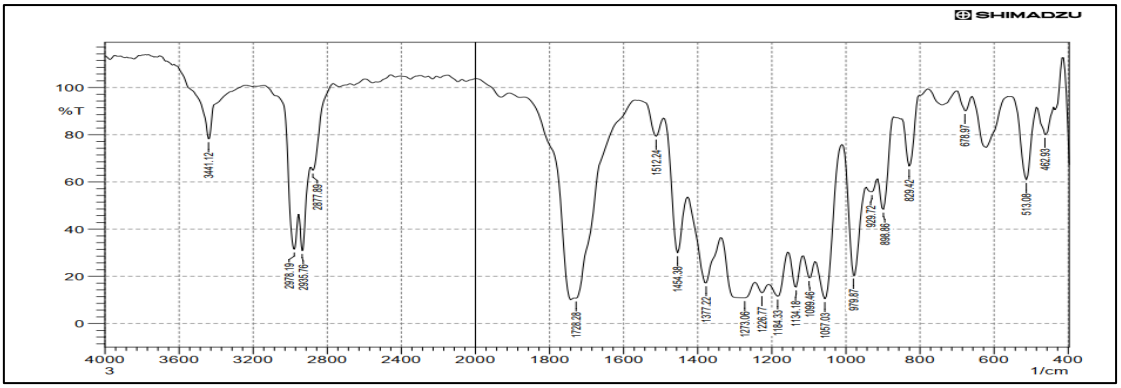

Fig. (4) : FTIR Spectra of Bioplastic P3HB/G.

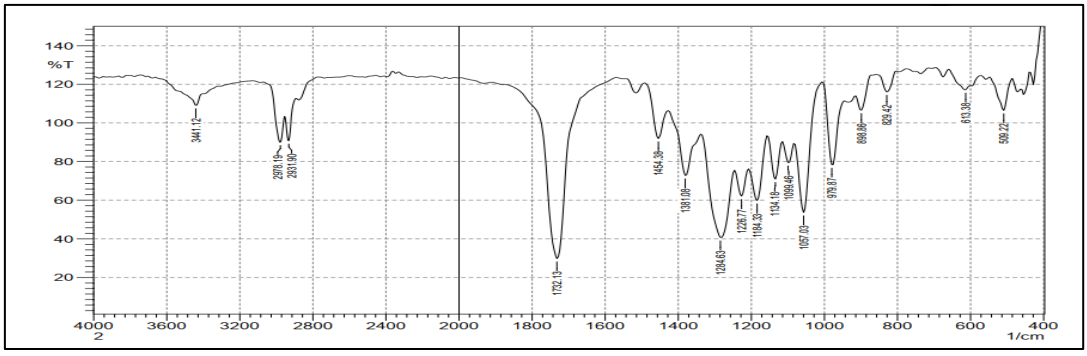

Fig. (5): FTIR Spectra of Bioplastic P3HB/C.

Antibacterial activity of P3HB/M, P3HB/G and P3HB/C films

Figure (6) manifests the results obtained in testing the efficiency of the membranes while possessing the property of resistance to antibacterial. It was found (after the end of the incubation period) that bacterial growth appeared in the control dish, i.e., in the dish which was placed on the surface of the P3HB/M membrane. No colony growth in the dishes which were coated with the P3HB/C membrane. It contained clove bud extract compounds. The result also appeared negative for the dish which was on the surface of the P3HB/G membrane. It contained ginger extract compounds. This test proved the ability of the prepared biofilms to inhibit bacteria due to the antibacterial compounds they contained in their composition, found in plant extracts. They were combined with the membrane compound to form prepared membranes with antibacterial specifications. These criteria must be taken into consideration when manufacturing the : antibacterial plastic bags, medical devices, antibacterial fabrics and fabrics which contain safe and environmentally friendly plant compounds in their composition. Biofilms have previously been used as carriers for antibacterial compounds in several studies, including that on mentioned in Sun *et al.* (2022) where nano-silver was loaded inside a cellulose membrane matrix. On the other hand, (Tan *et al.,*2021) loaded turmeric, clove, and cinnamon inside a PVA/chitosan film, proving the efficiency of the resulting films in inhibiting *S. aureus* bacteria. As for Andrade *et al.,* (2018), the efficiency of the protein film in inhibiting *Staphylococcus aureus*, *Listeria monocytogenes* and *Clostridium perfringens* was proven after loading rosemary extract film texture.

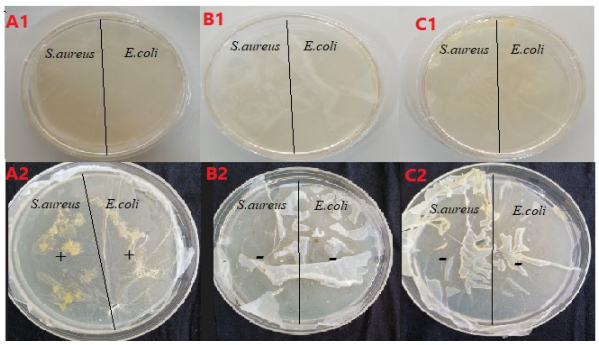

Fig. (6): Testing The ability of the Prepared Membranes to Inhibit Bacteria Isolated from the Soil. A= P3HB/M, B= P3HB/G, C= P3HB/C. The pictures in group A1, B1, C1 Show The plates Before Incubation but the Pictures in Group A2, B2, C2 Appear After Incubation.

Application of Biofilms in Food Packaging

Figure (7) shows the shape of plastic bags after manufacturing. They were manufactured in a simple, very flexible and tightly cohesive way for use in food packaging applications.

The results of the food packaging bag efficiency test presented the efficiency of the P3HB/C and P3HB/G in preserving food, compared to using P3HB/M and beans in Figure (8) which were stored without using any membrane.

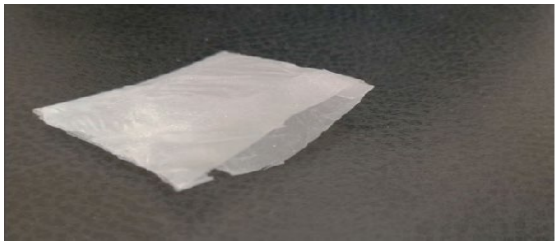

Fig. (7): The Shape of Plastic Bags After Manufacturing Them by Hot-gluing Their Edges and Preparing Them for Food Preservation Testing.

Fig. (8): The Effect of Preserving Beans by Using Prepared Bags with Biofilms: (1) P3HB/M,)2) P3HB/G, (3)P3HB/C, and (4) not Using them as A control Element After 72 hrs.

Adding ginger and clove extract had a significant impact on increasing the efficiency of food preservation. These plants contain certain com-pounds which prevent the growth of bacteria and fungi which usually cause food spoilage. Therefore, it is possible to use these types of bags and reduce or eliminate any preservatives for canned foods. Some of the plant compounds were carried in the extract and others were incorporated into a polyhydroxybutyrate matrix. Thus, they acted as a carrier for the antimicrobial compounds released during food preservation and the elimination of bacteria (Oda *et al.,* 2022). That was consistent with Tan *et al.,* (2021); Andrade *et al.,* (2018) ; Pech-Cohuo *et al.,* (2022) *regarding* the use of biofilms as carriers for natural compounds in preserving foods for a longer time with antibacterial properties.

In Vivo Wound Healing Study

The results of the membranes' efficiency test in wound healing are elucidated in Figure (9). The P3HB/G and P3HB/C membranes proved to be efficient in healing rats' wounds completely, in comparison to rats' wounds which were covered with P3HB/M membrane and gauze only. As a result of the P3HB/G and P3HB/C membranes containing chemical compounds found in ginger and clove extracts, an increase in the wound healing rate was recorded. It was estimated at 100% after 12 days. The healing rate reached up to 65% and 72% for the first and second groups, respectively (as shown in Figure (10)). The obtained results indicate that the plant extracts carried in the membranes contributed in increasing the rate of wound healing, compared to the group with P3HB/M membrane and the group with gauze only. This may be due to the fact that P3HB/G and P3HB/C membranes contain phenolic compounds within their plant extract composition (Mathkoor *et al*., 2023). The phenolic carried within the prepared membranes helped in increasing the permeability of the capillaries .Through that, the process of infiltration of anti-inflammatory immune cells in the wound area took place, in addition to the antibacterial property which they possess (Obeed *et al*., 2022). And that was a reason behind not having wound inflammation and accelerating

its healing (Hasan & Kadhim, 2018 ; Al-Madany & Oudah, 2024)

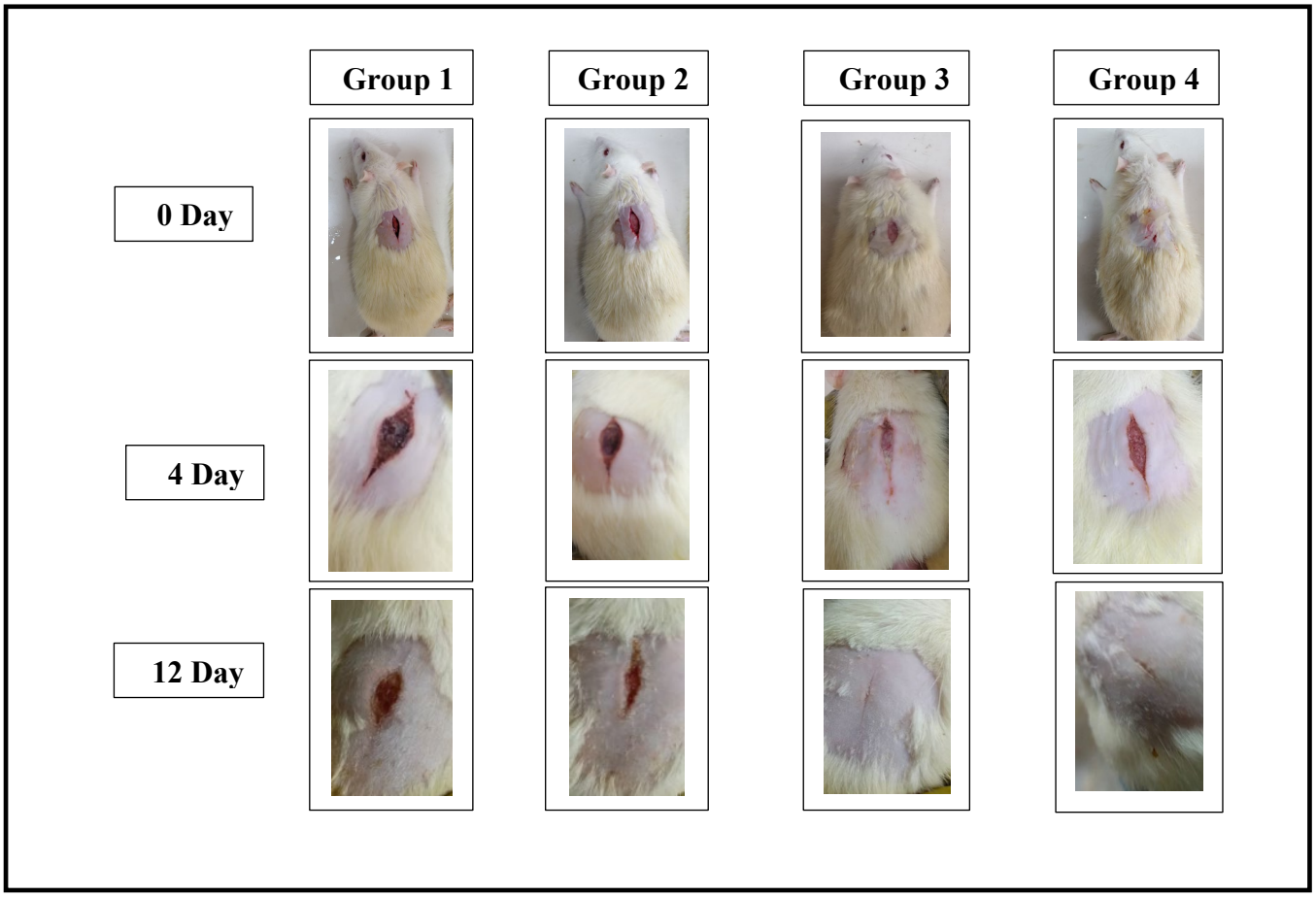

Fig. (9): The External Appearance of Rat Wounds Shows The stages of Wound Healing (1) a control group (2) Using P3HB/M, 3)) P3HB/G, (4) P3HB/C during 12 days.

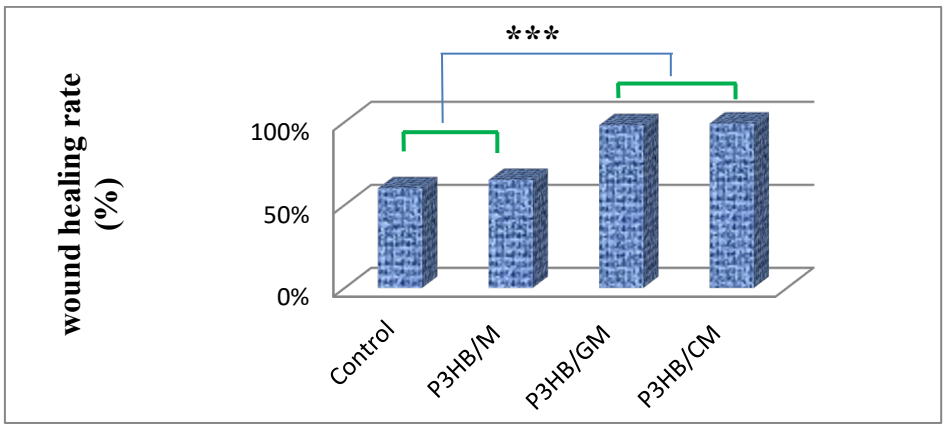

Fig. (10): The Rate of Wound Healing Rate Using and Without Prepared Membranes and the effect of Using Plant Extracts in The Manufactured Membranes

Conclusion

This research has elucidated that P3HB/C and P3HB/G films amalgamated with natural bioactive compounds, such as clove and ginger, could have a vivid prominence. It could be considered as a vital food packages and biomedical applications. The process of integrating plant extracts with the biofilm matrix leads to the production of new compounds with improved specifications; such as increased density and moisture content and increased water absorption capacity. It might be suitable for manufacturing processes. Further studies are recommended to cover the point of improving the chemical and physical properties of P3HB. It can be used as a bioplastic carrier film in order to obtain high quality products suitable for medical and environmental applications.

Study Limitations

The study was conducted during the first six months of 2024 in the laboratories of the College of Applied Medical Sciences and the College of Pharmacy at the University of Karbala.

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R.A.A.: Validation, writing-review, editing and methodology.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

The instructions that must be followed in the care and welfare of animals were adhered to during the tests conducted on them in this research in the scientific laboratories of the College of Pharmacy / University of Karbala.

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تحضير أغشية حيوية جديدة مناسبة لعمليات التصنيع من أجل بيئة مستدامة

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المستخلص: تعد الأغشية الحيوية القابلة للتحلل الحيوي حل بيئي مستدام وتزداد أهميته مع زيادة وعي المستهلكين بالبيئة. تهدف هذه الدراسة إلى تحضير وتوصف أغشية متعدد 3 هيدروكسي بيوتيرات (P3HB) القابلة للتحلل الحيوي ذات الخصائص الفيزيائية والكيميائية المحسنة بعد دمجها مع المستخلصات النباتية. وتكون هذه الأغشية الحيوية مضادة للبكتيريا للاستخدام في تغليف المواد الغذائية والمستلزمات الطبية. تم تحضير أغشية البلاستيك الحيوي الجديدة (P3HB) باستخدام تقنية الصب. تمثل الأغشية الحيوية الجديدة التي تم تحضيرها: أولاً P3HB/G ناتجة عن P3HB ومستخلص الزنجبيل والثانياً P3HB/C ناتجة عن P3HB ومستخلص القرنفل. تمت مقارنة جميع الأغشية الحيوية المذكورة مع P3HB / M التي تم تحضيرها من P3HB والكلوروفورم فقط . تم قياس سمك ورطوبة وقابلية الذوبان وانتفاخ الأغشية الحيوية الناتجة الجديدة. تمت دراسة خصائصها الكيميائية والشكلية باستخدام المجهر الإلكتروني الماسح و .FTIR تم تقييم قدرتها على تثبيط المكورات العنقودية الذهبية والإشريكية القولونية، وكذلك كفاءتها في تغليف الطعام وحفظه. أظهرت النتائج التي تم الحصول عليها أن أغشية P3HB/C و P3HB/G تظهر خصائص كيميائية وفيزيائية ومضادة للبكتيريا جيدة بسبب المواد الكيميائية النباتية المدمجة في المتعدد الأستري أو المحملة في مصفوفتها. كانت السُمك والرطوبة وأنتفاخ لأغشية P3HB/C و P3HB/G مختلفة بشكل كبير؛ مقارنةً بأغشية .P3HB/M ومع ذلك، عند مقارنة أغشية P3HB/C وP3HB/G، كانت الرطوبة هي الاختلاف الوحيد الملحوظ. تعد مواد التغليف القابلة للتحلل الحيوي المُحضرة بدائل رائعة للبلاستيك التقليدي لأنها قد تكون صديقة للبيئة.

الكلمات المفتاحية: متعدد الاستر القابل للتحلل الحيوي، متعدد 3-هيدروكسي بيوتريت، أغشية البلاستيك الحيوي، المستخلصات النباتية.