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# The Influence of Glycerol on Preparing Tapioca/$TiO_2$ Bionanocomposite Films

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## Abstract

This study focuses on the preparation of bionanocomposite films incorporating $TiO_2$ nanoparticles synthesized through a green chemistry approach using extracts from sweet orange (Citrus sinensis) peels. Tapioca starch was used as the biopolymer matrix, while $TiO_2$ nanoparticles served as the dispersed phase. The films were fabricated with varying glycerol-to-starch ratios (25%, 50%, 75%, and 100%) to determine the optimal formulation. Among these, the film containing 75% glycerol exhibited the most desirable mechanical and physical properties. The obtained films were subsequently characterized for their physical properties, water wettability via contact angle measurements, antibacterial activity using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*, and biodegradability under sunlight exposure.

**Keywords:** Bionanocomposite films, $TiO_2$ nanoparticles, Green synthesis, Antibacterial, Degradable films

## 1. Introduction

Nowadays, plastic waste has emerged as one of the most critical global environmental issues. Consequently, the search for alternative materials that are biodegradable and environmentally friendly has gained significant worldwide attention. Conventional or commodity plastics are widely used in various applications, such as water bottles, food packaging, food wraps, seedling bags, and garbage bags. However, these plastics are non-degradable and cannot be decomposed naturally, leading to the continuous accumulation of plastic waste and severe environmental impacts. Thailand is among the countries that heavily rely on commodity plastics and is currently facing similar environmental challenges. According to the Pollution Control Department (2020), Thailand consumes approximately 2.331 million tons of plastic annually for packaging and single-use products, equivalent to an average of 7,000 tons per day, accounting for 41.4% of total plastic usage in the country [1]-[3].

There are various methods for disposing of these plastics, such as landfilling (Landfill), which takes up space and takes hundreds of years to decompose completely. Recycling (Recycle) or reuse (Reuse) is the most environmentally friendly method; however, it is a complex process and can be expensive. Burning, this method will result in toxic substances accumulating in the atmosphere, etc. Considering the current campaign to protect the environment and the problems mentioned above, biodegradable plastics have garnered considerable attention as a potential replacement for plastics derived from petroleum-based chemicals. They are environmentally friendly and help reduce the amount of waste generated. Additionally, biodegradable plastics are produced from natural raw materials, including cassava, corn, sweet potatoes, and wheat. These biodegradable plastics help reduce environmental pollution and conserve various resources. However, biodegradable plastics still have

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limitations in their use, especially in consumer applications, where they should not only exhibit good biodegradability but also possess effective antibacterial properties. Biocomposite films have been developed to possess desirable properties for use in various fields, particularly antibacterial properties. This can be achieved by incorporating other materials with excellent antibacterial properties. It is popular to add nano-substances with antibacterial properties, such as zinc oxide (ZnO), titanium dioxide ($TiO_2$), silver oxide ($Ag_2O$), etc. [4]-[6]

Titanium dioxide ($TiO_2$) is a material with three crystal structures: anatase, rutile, and brookite. Due to the variety of crystal structures, titanium dioxide has outstanding properties in terms of electrical, chemical, thermal, and optical properties. In addition, titanium dioxide is a crucial catalyst for the decomposition of organic substances by light (Photocatalysts) and is also environmentally friendly and safe. Therefore, titanium dioxide is widely used in many fields, such as in the coating industry, paint production, ink, textiles, cosmetics, water treatment, antibacterial agents, etc. In particular, $TiO_2$ plays a crucial role in enhancing the photodegradation efficiency of biocomposite films through its photocatalytic properties. When exposed to UV light from sunlight, $TiO_2$ (especially in the anatase phase) acts as a photocatalyst by absorbing photons with energies equal to or greater than its band gap (~3.2 eV). This absorption generates electron-hole pairs ($e^-/h^+$), which subsequently react with water and oxygen molecules to produce highly reactive oxygen species (ROS), including hydroxyl radicals (•OH) and superoxide radicals ($•O_2^-$). These ROS can oxidize and break down organic polymer chains in the starch matrix, disrupting the film structure and accelerating its biodegradation. This photocatalytic degradation mechanism is highly beneficial for the development of environmentally friendly packaging materials that can decompose more rapidly after disposal, thus reducing the accumulation of plastic waste. The integration of $TiO_2$ nanoparticles into biocomposite films therefore serves two purposes: enhancing the antibacterial properties for food packaging and improving the film's ability to fully degrade under natural sunlight [7]-[11].

The most widely known nanomaterial synthesis processes are physical and chemical. These two methods mainly use chemicals that are harmful to the environment. However, the world is currently focusing on reducing the use of chemicals and promoting environmental friendliness. As a result, biological methods are increasingly used in nanomaterial synthesis, which is known as green synthesis. This process has the advantage of not using chemical reducing agents but rather using reducing agents derived from natural extracts, thereby ensuring the stability of the synthesized substances. Additionally, the green process is environmentally friendly, making it particularly suitable for medical applications. Sweet orange peel (Citrus sinensis) was selected as a bio-reducing agent for environmentally friendly $TiO_2$ nanoparticles synthesis due to its high content of natural phytochemicals, particularly flavonoids, phenolic compounds, and organic acids. These bioactive compounds can simultaneously act as reducing agents, capping agents, and stabilizers during nanoparticle formation, reducing the need for hazardous chemicals. Furthermore, utilizing sweet orange peel waste adds value to an agricultural byproduct, supporting waste reduction and the circular economy concept. Therefore, the use of sweet orange peel not only promotes environmentally friendly synthesis methods but also enhances the sustainability of $TiO_2$ nanoparticle production [12]-[15].

This research focuses on the use of $TiO_2$ nanoparticles synthesized via green chemistry exhibiting self-degradation behavior, particularly light absorption. Photocatalytic reaction testing was conducted over various time periods to determine the optimal light exposure time for efficient degradation of organic compounds in the extract. These nanoparticles were then used as enhanced materials in tapioca starch-based bionanocomposite films. The study specifically investigated the influence of glycerol concentration on the physical properties, surface wettability, degradation, and antibacterial activity of the resulting bionanocomposite films. The $TiO_2$ sample exhibiting the strongest antibacterial performance was selected for incorporation with tapioca starch to develop agricultural bioplastics. Therefore, this research aims to explore the potential application of environmentally friendly synthesized $TiO_2$ nanoparticles with both photocatalytic and antibacterial properties in the production of agricultural biofilms. The ultimate goal is to create environmentally friendly, cost-effective, and practical materials suitable for sustainable agricultural applications.

## 2. Experimental

### 2.1 Preparation of flavonoid extract and synthesis of $TiO_2$ nanoparticles

The peels of sweet orange (*Citrus sinensis*) were washed three times with tap water to remove dirt and impurities, then cut into small pieces and dried at 70 °C for 24 hours or until completely dry. The dried peels were ground into a fine powder using a blender. The resulting peel powder was mixed with deionized (DI) water as a solvent and shaken at 250 rpm for 3 hours. The mixture was subsequently heated in a water bath at 60 °C for 1 hour and filtered through Whatman No. 1 filter paper to obtain the extract. A 10 mL portion of the sweet orange peel extract was then mixed with 2 g of titanium (IV) isopropoxide precursor and stirred for 1 hour, followed by heating in a water bath for an additional hour. The obtained sample was dried and annealed at 350 °C for 6 hours to yield $TiO_2$ nanoparticles.

### 2.2 Preparation and physical characterization of bionanocomposite films

Tapioca starch (5% w/v) was dissolved in deionized water, and the synthesized $TiO_2$ nanoparticles were added at a concentration of 0.5 mg/mL. Glycerol was incorporated at various ratios to the starch solution 25%, 50%, 75%, and 100% (%w/w relative to the starch content), and the mixture was heated at 95 °C with continuous magnetic stirring for 30 min until homogeneous. The solution was further homogenized for 1 min, poured into 2 × 2 cm silicone molds, and dried at 60 °C for 18 hours after standing at room temperature for 1 hour. The resulting films were characterized for physical properties using an optical microscope (OM) and for surface wettability using water contact angle (WCA) analysis.

### 2.3 Degradability, Solubility, and Swelling Ratio Tests of Bionanocomposite films

#### 2.3.1 Degradability:

The degradability of the synthesized bionanocomposite films composed of $TiO_2$ nanoparticles ($TiO_2$ NPs) and tapioca starch was evaluated based on their physical characteristics, film solubility, and water swelling ratio. The films were exposed to sunlight for 1, 10, 20, and 30 days, respectively, to investigate their degradability behavior. For all tests, the film samples were standardized to a size of 2 × 2 cm.

#### 2.3.2 Film Solubility Test:

After determining the appropriate ratio of glycerol to starch during film preparation, the solubility of the nanocomposite films was analyzed. Each 2 × 2 cm dry film sample was weighed ($W_o$) and immersed in 50 mL of distilled water with continuous stirring for 24 hours. The resulting film residues were filtered through pre-baked filter paper (baked for 2 hours), and the combined filter paper and residues were dried at 80 °C for 24 hours. After cooling to room temperature, the dry films were weighed again ($W_d$). The percentage of film solubility was calculated using equation 1:

$$Film\ Solubility\ (\%) = \frac{W_0 - W_d}{W_d} \times 100 \quad (1)$$

Where $W_0$ is the initial dry film weight (grams), and $W_d$ is the weight of dry film after soaking in distilled water (grams).

#### 2.3.3 Swelling Ratio Test:

The water swelling behavior of the $TiO_2$–tapioca starch bionanocomposite films was evaluated using 2 × 2 cm film samples. Each sample was initially weighed dry ($W_o$), placed in a desiccator for 48 hours, and then immersed in 50 mL of distilled water for 24 hours. After soaking, the films were removed with forceps, and excess surface water was gently blotted using paper towels to prevent evaporation. The wet films were then weighed ($W_e$), and the swelling ratio was determined using equation 2:

$$Swelling\ Ratio\ (g/g\ dry\ film) = \frac{W_e - W_0}{W_e} \quad (2)$$

Where $W_0$ is the initial dry film weight (grams), and $W_e$ is the weight of dry film after soaking in distilled water (grams).

### 2.4 Antibacterial activity

The antibacterial activity of $TiO_2$ nanoparticles was evaluated using the disc diffusion method against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Bacterial cultures were incubated at 250 rpm for 24 hours and spread on agar plates. Sterile paper discs (5 mm) were placed on the plates, and 30 µL of $TiO_2$ nanoparticle suspensions were added to each disc, with deionized water as a control. The plates were incubated at 37 °C for 18–24 hours, and the inhibition zones were measured.

## 3. Result and discussion

### 3.1 Physical properties of bionanocomposite films

The bionanocomposite films were prepared using titanium dioxide nanoparticles synthesized via a green synthesis, utilizing 10 mL of sweet orange peel extract. The synthesized nanoparticles were subsequently annealed at 350°C for 6 hours to enhance their quality before being incorporated into the tapioca starch matrix. As shown in Table 1, at 0% glycerol concentration, the resulting films were rigid, brittle, and prone to breakage. Increasing the glycerol concentration to 25% led to improved film flexibility and reduced hardness; however, the films remained brittle and fragile. Further increases in glycerol content to 50%, 75%, and 100% progressively improved the softness and flexibility of the films while reducing their brittleness and fragility. At 100% glycerol concentration, the films exhibited high softness and elasticity, making them difficult to remove from the molds. Even after successful development, the films tended to stick together, making their separation complicated.

**Table 1.** Images of the bionanocomposite films using a mobile phone camera and optical microscope (OM) at 40× magnification.

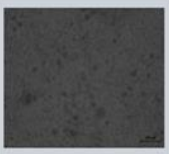
| Equipment | Concentration of Glycerol (%v/v) | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 |
| Mobile camera |  |  |  |  |  |
| Optical microscope (OM) |  |  |  |  |  |

**Table 2.** Images of the bionanocomposite films before and after folding.

| Condition | Concentration of Glycerol (%v/v) | |
|---|---|---|
| | 50 | 75 |
| Before folding |  |  |
| After folding |  |  |

According to Table 1, OM images (bottom row) of the bionanocomposite film samples at 40× magnification revealed that increasing the glycerol concentration led to a reduction in film porosity, along with improved surface smoothness and uniformity. Notably, only films containing 50% and 75% glycerol could be fully detached from the mold. These two samples were subsequently subjected to a folding test. Therefore, the folding test helps determine how well the film can withstand mechanical deformation without losing its integrity. This property is especially important for films intended for packaging, coating, or biomedical applications, where flexibility and resistance to damage during handling are required. From this experiment, the film with 50% glycerol cracked upon folding, whereas the film with 75% glycerol remained intact, as presented in Table 2. Therefore, the optimal formulation was determined to be the bionanocomposite films prepared with 75% glycerol. This formulation was selected for further analysis.

### 3.2 Wetting properties by water contact angle (WCA)

When the bionanocomposite film sample with a glycerol concentration of 75% was subjected to water contact angle measurement, the average contact angle was found to be 88.32°, as shown in Figure 1. This indicates that the film exhibits mildly hydrophilic characteristics, as the contact angle is close to but still less than 90°.

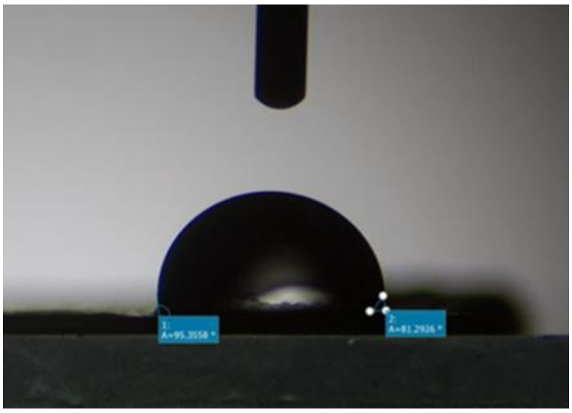


**Fig. 1.** The contact angle measurement of the film surface.

### 3.3 Photodegradability of bionanocomposite films under sunlight exposure

To investigate the biodegradability of the bionanocomposite film sample with a glycerol concentration of 75%, the films were exposed to sunlight and their weights were measured at intervals of 1, 10, 20, and 30 days. The results indicated a progressive decrease in the film weight with increasing exposure time, as illustrated in Figure 2.

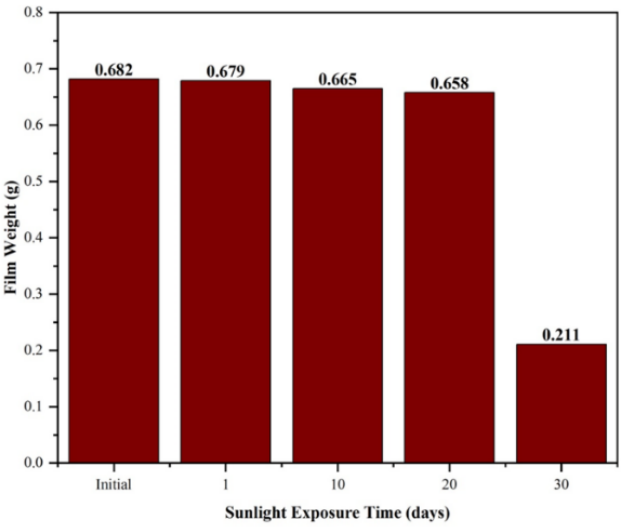
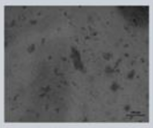
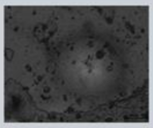
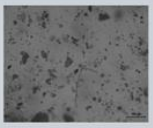
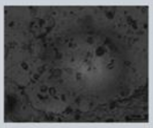



**Fig. 2.** The weight of the sample bionanocomposite films after exposure to sunlight.

Furthermore, when the film samples were observed under an optical microscope at 40× magnification, it was found that the surface uniformity of the films decreased and the porosity increased with longer sun-drying durations because the evaporation rate of the solvent becomes faster and less controlled. This rapid and uneven evaporation can cause the polymer chains or particles in the film to rearrange irregularly, leading to the formation of microvoids and cracks on the surface. As a result, the surface uniformity decreases and porosity increases. In addition, exposure to sunlight for a long time may also cause thermal stress or photo-degradation of some film components (especially biopolymers or proteins like zein), further contributing to the roughness and irregular pore structure, as shown in Table 3.

**Table 3.** The weight and images of the bionanocomposite film samples after exposure to sunlight for different times using an optical microscope at 40x magnification.

| Equipment | Sunlight Exposure time (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 10 | 20 | 30 |
| Film Weight (g) | 0.682 | 0.679 | 0.665 | 0.658 | 0.211 |
| Optical microscope (OM) |  |  |  |  |  |

Subsequently, the bionanocomposite films with a glycerol concentration of 75% were exposed to sunlight for 1, 10, 20, and 30 days to investigate their wettability. It was found that the contact angle decreased progressively with longer exposure durations, from 88.32° on the first day (before sun exposure) to 37.64° after 30 days of sun exposure. This indicates that the films became increasingly hydrophilic over time. As shown in Figure 3, the decrease in contact angle corresponds with the surface morphology observed under an optical microscope, where increased sun exposure resulted in a more porous film surface. This enhanced porosity contributes to the observed increase in hydrophilicity of the films, which initially exhibited only slight hydrophilicity before sun exposure, and became markedly more hydrophilic after 30 days of sunlight exposure. The increased porosity caused by prolonged sun exposure enlarges the effective surface area and allows more water molecules to penetrate the pores. This enhances the film's ability to interact with water, thereby increasing its hydrophilicity [16]-[17].

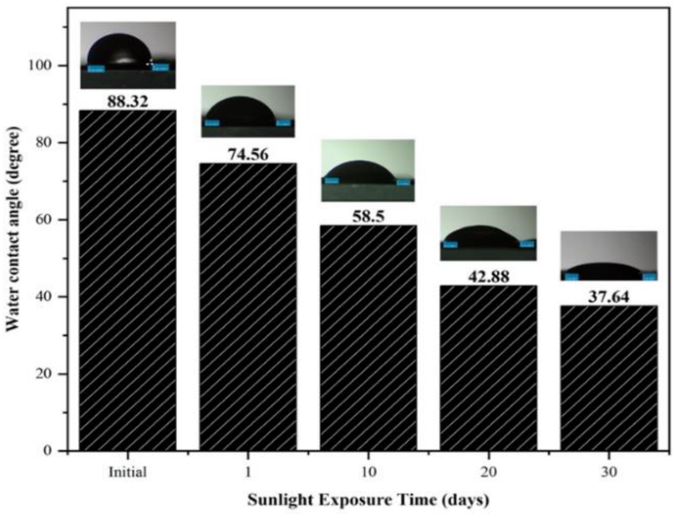



**Fig. 3.** The contact angle of the bionanocomposite films was exposed to different sunlight exposure times.

### 3.4 Solubility of the bionanocomposite films

The solubility of the bionanocomposite films containing 75% glycerol is illustrated in Figure 4, which compares the results before and after sun exposure at 1, 10, 20, and 30 days. The calculated solubility by using equation 2. The initial film exhibited a solubility of 65.20%, which progressively increased with the duration of sun exposure, reaching a maximum of 96.87% after 30 days. This indicates an enhanced biodegradability of the film correlated with prolonged sunlight exposure. Prolonged exposure to sunlight leads to structural and chemical degradation of the polymer matrix. The UV radiation and heat from sunlight can break down polymer chains and weaken the intermolecular interactions (such as hydrogen bonds) between polymer molecules and plasticizers like glycerol. This degradation reduces the film's structural integrity, making it easier for water molecules to penetrate and dissolve the material, resulting in higher solubility [18]-[19].

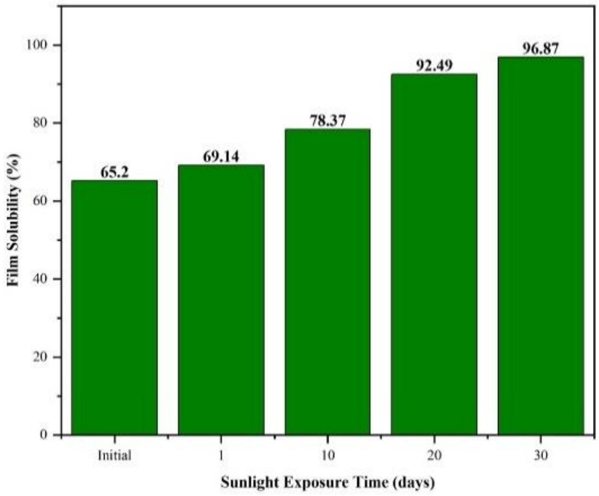



**Fig. 4.** The solubility of the bionanocomposite films wth different sunlight exposure times.

### 3.5 Swelling test

The swelling degree was then calculated using equation 2. The results revealed that before sunlight exposure, the film exhibited a swelling ratio of 0.73 g/g of dry film. However, after 30 days of sunlight exposure, this value decreased to 0.12 g/g of dry film, as shown in Figure 5. This indicates a reduced water retention ability of the films, which correlates with the observed changes in water contact angle, suggesting a decrease in the film's hydrophilicity with prolonged sunlight exposure time. As a result, the film becomes denser and less capable of swelling, leading to a lower swelling ratio.

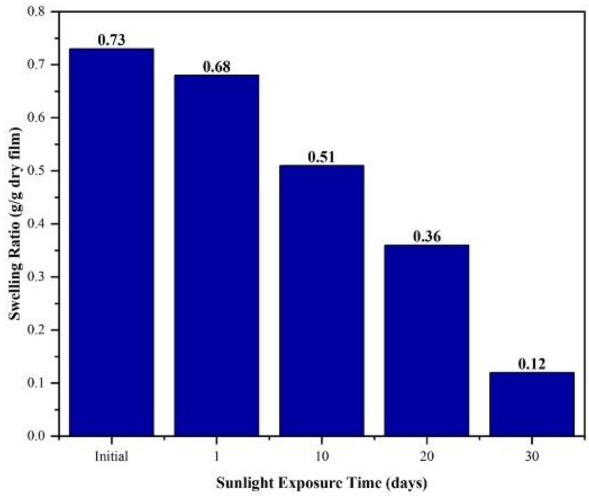



**Fig. 5.** The swelling test of the bionanocomposite films with different sunlight exposure times.

### 3.6 Antibacterial activity of bionanocomposite films

The bionanocomposite films with a glycerol concentration of 75% were tested for their ability to inhibit both gram-positive and gram-negative bacteria using disc diffusion techniques. *S. aureus* was used as a representative gram-positive bacterial and *E. coli* as a representative gram-negative bacterium. The inhibition zone of the bacteria was calculated compared to deionized water as a reference, as shown in Figures 6 (a) and (b). It was found that this bionanocomposite film sample could inhibit both gram-positive and gram-negative bacteria. It was able to inhibit *E. coli*, the gram-negative bacteria, better than *S. aureus*, the gram-positive bacteria, as shown in Figure 6 (c). The bionanocomposite films inhibited *E. coli* more effectively than *S. aureus* because the thinner cell wall, negatively charged surface, and higher reactive oxygen species (ROS) sensitivity of *E. coli* made it more vulnerable to the antibacterial mechanisms of the film [20]-[21].

Compared to previous research on starch-based bionanocomposite films reinforced with other nanoparticles such as ZnO or Ag, the bionanocomposite films developed in this research exhibit comparable levels of antibacterial activity. Furthermore, the films show improved degradation resistance under sun exposure, which can be explained by the photodegradation properties of the $TiO_2$ nanoparticles. Unlike conventional nano additives, $TiO_2$ can form reactive compounds under light exposure, thus promoting the degradation of the polymer matrix. Moreover, the use of environmentally friendly synthesized $TiO_2$ nanoparticles reduces reliance on hazardous chemicals and aligns with the principles of sustainable and environmentally friendly material development [22].

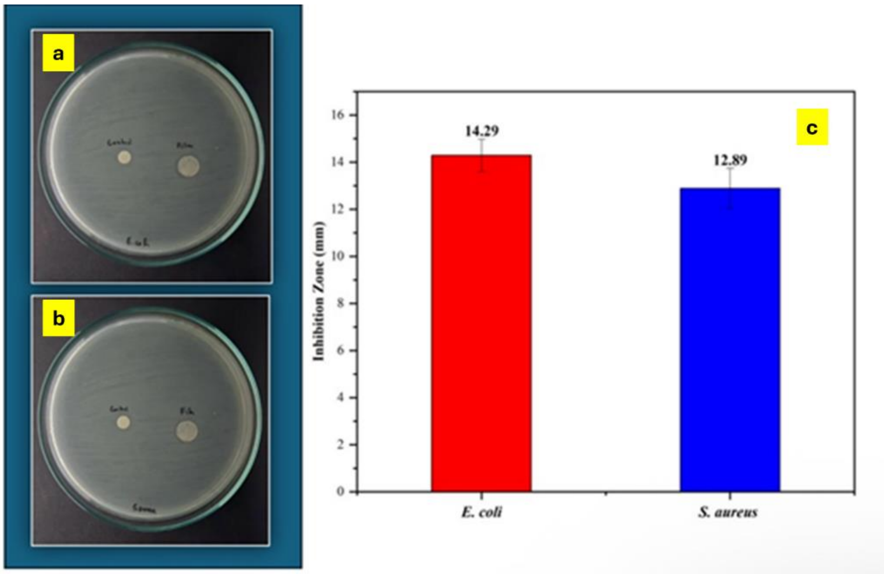



**Fig. 6.** The ability to inhibit bacteria (a) *E. coli*, (b) *S. aureus*, and (c) the size of the clear zone of the bionanocomposite films.

## 4. Conclusion

This research focuses on the preparation of bionanocomposite films composed of titanium dioxide nanoparticles synthesized via a green synthesis method using extract from sweet orange peel and tapioca starch. The study further investigated the optimal glycerol content for film formation, finding that a glycerol concentration of 75% (w/w relative to starch) yielded the most suitable film. The resulting film exhibited a contact angle of 88.32°, indicating mildly hydrophilic properties. The films also showed antibacterial activity against both Gram-positive and Gram-negative bacteria. Biodegradability of the bionanocomposite films was assessed through physical appearance, water wettability, solubility, and swelling behavior. The results indicated that the films were photodegradable under sunlight exposure, with the degradation rate increasing proportionally with exposure time. Future research will focus on optimizing the blending of titanium dioxide nanoparticles, studying long-term degradation behavior under natural outdoor conditions, and evaluating the mechanical performance of the films for practical agricultural applications such as mulch or biodegradable packaging materials.

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