



Green Synthesis of Titanium Dioxide Nanoparticles Using Extraction of *Psidium Guajava* for Smart Packaging Application

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ABSTRACT

This research aims to investigate the application of smart packaging in exhibited antimicrobial activity against bacteria such as *E. coli* and *S. aureus*. An alternative of non-degradable plastic based on the green synthesis of titanium dioxide nanoparticles (TiO₂NPs) and biofilm of Chitosan-TiO₂ NPs has been developed. TiO₂ NPs are known to be effective antimicrobial agents synthesized from *Psidium Guajava* leaves. Chitosan is a natural carbohydrate polymer employed in smart biofilm for packaging for a long time due to its biodegradability, biocompatibility, and low toxicity. Chitosan-Titanium dioxide Biofilm underwent characterization study through XRD, FTIR and FESEM. The analysis has shown the spectra minima at 380 nm from UV- vis analysis represents TiO₂ bands, the small size of titanium dioxide nanoparticle at 5-10 nm obtained from FESEM analysis, the crystallographic nature is "plane of TiO₂ anatase" through XRD analysis, and the main functional group involved are carboxylic group O–H and Ti–O–Ti from FTIR analysis. The titanium dioxide nanoparticle was incorporated into chitosan, and the effectiveness of antimicrobial properties of the incorporated packaging was studied and recorded by observing the inhabitant zone. Consequently, TiO₂-Chitosan efficiently suppresses the growth of bacterial colonies. The study only considers the antimicrobial packaging used, which will be disposed of generally for biodegradable packaging.

Keywords:

Green Synthesis; Titanium Dioxide Nanoparticles; *Psidium Guajava*; Smart Packaging

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1. Introduction

Food packaging is critical in modern commercial trade as it protects food quality and safety, facilitates transportation, allows safe storage, and prevents product damage and spoilage while minimizing economic losses [1]. A critical opportunity is a demand for innovative packaging

materials that are both acceptable and safe. These materials were combined with sensors embedded in smart packaging to control the disposal of active substances. Biodegradable plastic may be a viable option for food packaging to address plastic waste issues because it degrades upon removal. Plastic biodegradation rates are temperature, humidity, and microbe population-dependent [2]. While it is critical to producing biodegradable and antibacterial green-based packaging materials, the trade-off between biodegradability and antimicrobial activity must also be addressed [3].

In recent years, the use of NPs as antibacterial agents has grown in popularity. TiO₂ NPs are desirable antimicrobial agents for food packaging as they improve the physical properties of bioplastic packaging and gain primary scientific interest among all metal oxides due to their favourable properties in photocatalytic, antimicrobial, and antibacterial applications [4]. The FDA has approved TiO₂ for use in medications, cosmetics, and food contact surfaces [5] due to its non-toxicity and antibacterial action against various microorganisms. The use of TiO₂ as a capping agent is to prevent harmful processes and provide a cost-effective choice. As a result, it is possible to create a safe antibacterial biofilm. In general, the polysaccharide-TiO₂ hybrid materials have enhanced physical and chemical properties as a function of the TiO₂ content. The polysaccharide-TiO₂ also showed antimicrobial action against gram-negative and gram-positive bacteria, yeasts, and moulds and increased UV protection [6-8] for food and non-food packaging.

Packaging garbage, particularly non-biodegradable polymers, has become a considerable portion of municipal solid waste, raising environmental issues. Additionally, the discarded packaging material is a visible source of litter, providing a significant waste management concern [9]. It is well established that trash created by smart packaging is primarily unsuitable for recycling, posing a significant problem for businesses. One of the primary problems in the design and production of smart packaging is identifying a suitable material that enables the application of biodegradable sensors and communication features [10]. Additionally, plastic fragments continuously result in micro-and nano-plastics that harm human health.

Smart packaging uses innovative materials for surrounding food goods that can overcome plastic's failure to protect light, oxygen, and other gases from entering and degrading consumables, as well as to extend the shelf life of food and preserve human health [11]. Thus, the green synthesis technique for producing nanoparticles provides additional benefits due to its non-pathogenic and cost-effective nature. Herein, TiO₂ NPs-chitosan as an antimicrobial for smart packaging was formulated using aqueous *P. Guajava* leaf extract. The production of TiO₂ NPs uses biological reduction, chitosan, which acts as a capping agent for the produced and exhibits outstanding antibacterial action. This project aimed to formulate TiO₂NPs-chitosan as an antimicrobial for smart packaging produced using aqueous *P. Guajava* leaf extract for smart packaging, including chitosan as a capping agent. This approach may help develop innovative packaging. The production of TiO₂ NP uses biological reduction, which acts as a capping agent for the produced and exhibits outstanding antibacterial action. The method could aid in the development of novel packaging.

2. Methodology

2.1 Materials

Acid acetic (98%), Chitosan powder, Glycerol (98%), Ethanol (96%) and Titanium Tetraisopropoxide (TTIP) were purchased from Sigma-Aldrich and used without prior purification.

2.2 Preparation of aqueous *Psidium Guajava* Extract

Fresh *P. Guajava* leaves were collected from a community farm in Jelatek, Kuala Lumpur. The accumulated *P. Guajava* leaves were double-washed with tap water and distillation water to remove dirt and sand on the surface of the leaves. Then, the leaves were dried for one day at 70°C in EscoIsotherm Forced Convection Laboratory Oven. Then, the dried leaves were crushed into powder using a blender and stored in an airtight container inside a glass vacuum desiccator to ensure no moisture and prevent mould and fungus. Next, 20g of powdered *P. Guajava* were mixed with 100 mL distillate water at 50°C for 1 hour. Then, the solution was filtered using Whatman filter paper to synthesize nanoparticles to produce leaf extract.

2.3 Synthesis of Titanium Dioxide Nanoparticles

20 mL TTIP and 80 mL of Ethanol were stirred continuously to get titanate acid, $\text{TiO}(\text{OH})_2$. Approximately 80 mL $\text{TiO}(\text{OH})_2$ was constantly mixed with 20 mL leaf extract in an Erlenmeyer flask. The solution changed from green to milky tea colour, indicating the formation of TiO_2 NPs. The synthesized NPs were separated by centrifugation of the mixture at 5000 rpm for 15 minutes. The centrifuged particles were washed with Ethanol and again subjected to centrifugation at 5000rpm for 10 minutes to ensure only TiO_2 NP was synthesized. The steps were taken from a previous study by Dias *et al.*, (2019) [12] and modified according to this work. The separated TiO_2 NPs were dry and ground to calcinate at 500°C in a muffle furnace for 3 hours. The dried nanoparticles were characterized using various analytical techniques and subjected to antimicrobial investigation.

2.4 Synthesis Titanium Dioxide Nanoparticle-Chitosan

1g of chitosan powder was dissolved in a mixture of 1M acetic acid aqueous solution, 1M glycerol was stirred constantly with a magnetic stirrer and heated for 20 minutes at 90°C. The dissolved solution was strained through eight layers of cheesecloth to remove undissolved debris and then sonicated for 30 minutes in a bath-type ultrasound sonicator. 15mL of the solution was cast onto a plastic plate with a 90mm diameter. The solution was dried at room temperature for 72 hours. A thin film was stripped out from the petri dish once dried. The solutions were mixed well with continuous stirring at 500rpm for 15 minutes, then sonicated for 10 minutes in a bath-type ultrasound sonicator to obtain an NPs solution [13].

2.5 Characterization study

2.5.1 UV-Vis Spectrophotometer (UV-Vis)

The absorption spectrum of TiO_2 was recorded using UV-Vis Shimadzu UV-2600 to monitor the formation of TiO_2 NPs that has been reduced using *P. Guajava* with a scanning range of 200–800 nm.

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis of green synthesized TiO_2 NPs was performed using a Shimadzu IRTracer-100 analysis. The spectrum will record in the wavenumber range of 400–4000 cm^{-1} . FTIR spectroscopy was carried out to observe the interactions between Chitosan and TiO_2 .

2.5.3 X-ray Diffraction Analysis (XRD)

Crystal structures of TiO₂, Chitosan, and chitosan-TiO₂ nanocomposite films were analyzed using an X-Ray diffractometer in the range of $2\theta = 10^\circ - 80^\circ$ with a Cu K α radiation source at 0.15 nm. The working parameters are voltage of 40 kV, current of 40 mA, and scanning rate of 2 minutes⁻¹. The speed scan used for the study is at 2° per minute.

2.5.4 Antimicrobial Activity

The bacterial culture samples were lyophilized and suspended in nutrient broth containing 0.5% sodium chloride at 37°C for 24 hours to create a viable culture source [14]. Each of these strains was cultivated in tryptic soy broth. Unless otherwise mentioned, the themes are grown aerobically at 37° with 10 mL of media in 18-150 mm borosilicate glass culture tubes while shaking at 200 rpm under standard laboratory lighting conditions. An overnight culture of a single colony in nutrient broth and turbidity adjustment to 0.5 McFarland standards will produce bacterial inoculums. This bacterial suspension containing produced TiO₂ nanoparticles (20g/mL) was plated on Mueller-Hinton agar plates with a diameter of 8 mm. These plates were incubated at 4 degrees Celsius for 15 minutes to allow diffusion [15] and then at 37 °C for 24 hours to cultivate bacteria.

2.5.5 Field Emission Scanning Electron Microscopy (FESEM)

FESEM was used to determine plant-synthesized nanoparticles' morphology and size. 1nm resolution at 15kV brand FE1(USA) model NOVA NANOSEM 23 was used for the FESEM images of cross-sectional and surface TiO₂ NPs and Chitosan- TiO₂ nanocomposite films.

3. Results and Discussion

TiO₂ NPs formation can be noticed based on the colour change from green to milky tea (Figure 1), indicating that the *P. Guajava* plant contains an antibacterial component that can be used to control foodborne infections and spoiling organisms [16]. Figure 1 shows the TiO₂ colour transformation for leaves extracted with TTIP solution. The green synthesis of TiO₂ NPs using *P. Guajava* extract was effectively achieved.

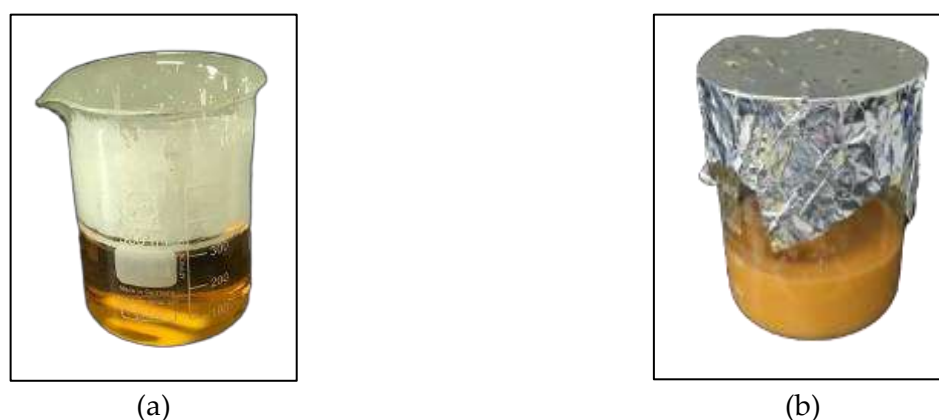


Fig. 1. TiO₂ Color Transformation a) Leaves extract mixed with TTIP solution b) Leaves extract mixed with TTIP solution after 8 hours stirred

UV-vis absorbance spectroscopy determines the molecular size and band gap of nanoparticles containing TiO₂. The synthesis of TiO₂ NPs shows a strong absorption band at 391 nm, indicating that *P. Guajava* represents a TiO₂ band between 380 to 400 nm, which is similar to results by previous work by Rekha *et al.*, (2019) [17], as shown in Figure 2.

The peaks and functional groups responsible for synthesizing chitosan film and chitosan-TiO₂ nanocomposite film were determined using Fourier Transform Infrared Spectroscopy (FTIR) (Figure 3). The OH and NH stretching bands were identified at 3363 cm⁻¹ and 3135 cm⁻¹, respectively. The peak of C-N at 1569 cm⁻¹ and C-H at 1422 cm⁻¹ of chitosan reduced when TiO₂ was added, most likely because the functional groups of chitosan form hydrogen bonds with titanium. A similar observation was reported by Thakur *et al.*, (2019) [18]. As the concentration of TiO₂ in the chitosan film increased, the peaks between 530 and 720 cm⁻¹ indicated Ti-O-Ti bending vibrations. The bands at 1015 cm⁻¹ and 1186 cm⁻¹ correspond to C-O-C stretching oscillations and Ti-O-C bending vibrations, respectively.

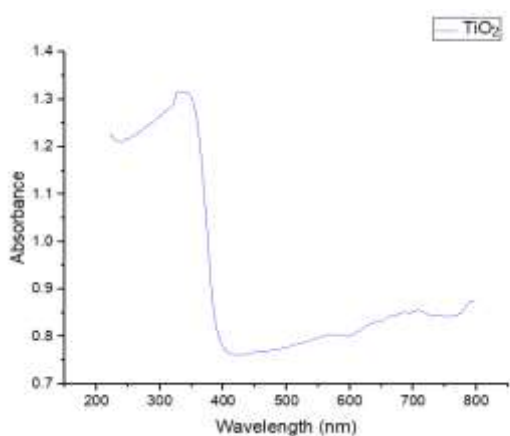


Fig. 2. UV-vis Analysis of TiO₂NPs

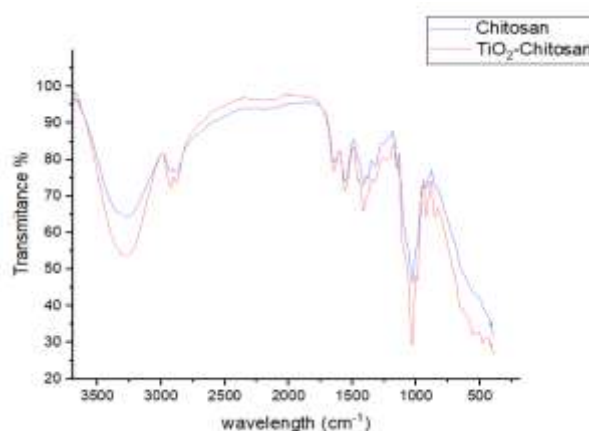


Fig. 3. FTIR result of Chitosan and TiO₂-Chitosan biofilm

The Chitosan diffractogram displays a strong peak at 25.70°, indicating a high degree of crystallinity (Figure 4). Crystalline TiO₂ may be the outcome of the crystalline structure of chitosan reduced with TiO₂ NP synthesis using *P. Guajava* [19] gives four peak values at 2θ, which are associated with 23.36°, 37.85°, 48.04° and 54.12°. The result is consistent with Kumar *et al.*, (2020). Meanwhile, peak values of TiO₂-Chitosan were 25.70°, 37.66°, 48.06° and 54.09°. The TiO₂ XRD pattern corresponds to the (250), (105), (111), and (104) planes. These planes correspond to the anatase phase in the crystalline form, as discussed by Xing *et al.*, (2020), [20] and Samy *et al.*, (2020) [21]. The 2θ peak at 25.42 and 49.11 confirm the TiO₂ anatase structure.

Figure 5 depicts the uniform distribution and smooth surface of the TiO₂-chitosan film shown by FESEM analysis. In the case of the TiO₂-chitosan composite film case, the composite particles are highly distributed and agglomerated, indicating that granular particles are interlocked [22]. The results demonstrate that the particle size distribution of the NPs generated at the lowest TiO₂ concentration has a more extensive size range, with a mean particle size of 5.88nm.

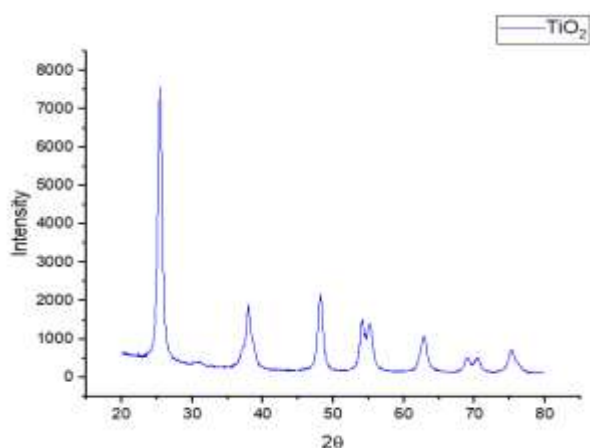


Fig. 4. X-RD pattern of green synthesis TiO₂ NP

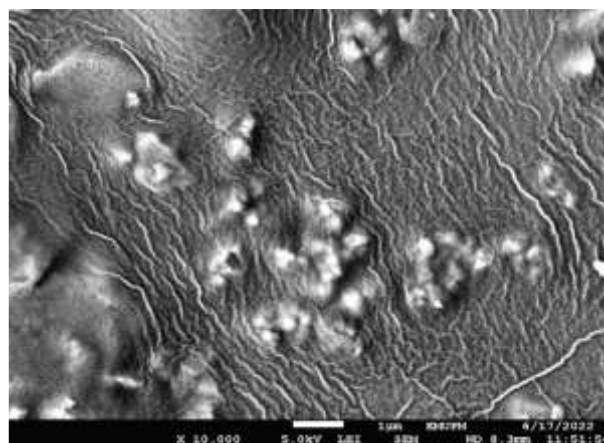


Fig. 5. FESEM images of TiO₂-Chitosan film

The control solution containing no solvated nanometals displayed no such effect, and the zone of inhibition zone for the antimicrobial of TiO₂-chitosan biofilm (Figure 6). Several investigations have revealed that chitosan's rather potent antibacterial activity of TiO₂-chitosan films should be attributable to the killing action of TiO₂ NPs against *S. aureus* [23], and the TiO₂-chitosan film demonstrated a more potent antibacterial activity [24] than TiO₂. Various processes can explain the bactericidal effect of TiO₂ particles. When exposed to sunshine or UV radiation, TiO₂ exhibits antibacterial action due to its significant oxidizing ability [25].

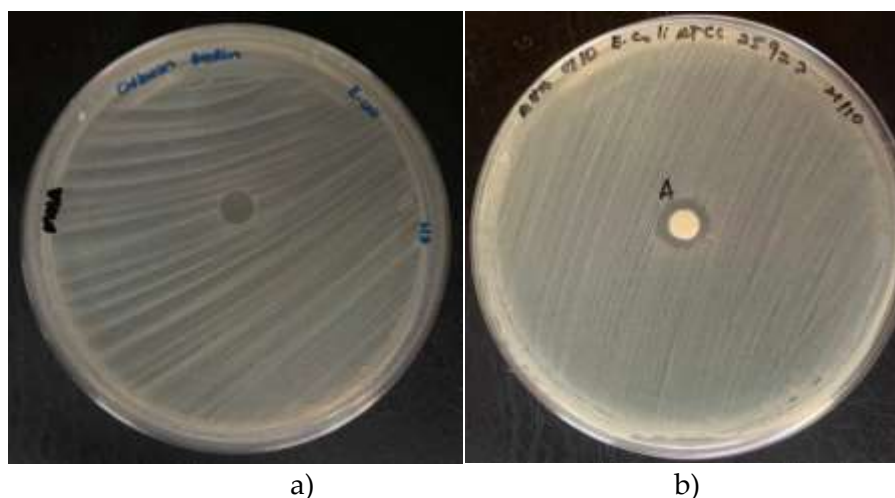


Fig. 6. Photograph of antimicrobial test results against *Escherichia coli*
a) Chitosan b) TiO₂-chitosan biofilm

4. Conclusions

The green synthesis of TiO₂ NP was conducted using extraction of *P. Guajava* and proved their antimicrobial by using chitosan as the biopolymer. A biofilm of Chitosan-TiO₂ was successfully synthesized and characterized using XRD, FTIR, FESEM and antimicrobial activity. The results demonstrate that the particle size distribution of the NPs generated at the lowest TiO₂ concentration has a more extensive size range, with a mean particle size of 5.88nm. The antimicrobial analysis demonstrated that TiO₂ NP-chitosan biofilm shows an inhibition zone against *S. aureus* gram-negative. The objective of using TiO₂ as a capping agent to prevent harmful processes while providing a cost-

effective option has been achieved. The approach could aid in the development of innovative packaging.

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