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Value-Added of Sweet Orange Peel Waste into TiO_2 Nanoparticles via Green Synthesis for Antibacterial Applications

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Abstract

This research focuses on the TiO_2 nanoparticles synthesized via a green chemistry approach using extracts from sweet orange (*Citrus sinensis*) peels. The quality of the synthesized titanium dioxide nanoparticles was enhanced through an annealing process at 350°C for 6 hours. It was found that this thermal treatment significantly improved the particle quality. The antibacterial activity of the nanoparticles was investigated at concentrations of 0.1, 0.3, and 0.5 mg/mL in deionized water using the disc diffusion technique against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli)* were used as representative strains for Gram-positive and Gram-negative bacteria, respectively. The results indicated that the TiO_2 at a concentration of 0.5 mg/mL exhibited the most effective antibacterial performance against both types of bacteria.

Keywords: Sweet orange peel, TiO_2 nanoparticles, Green synthesis, Antibacterial

1. Introduction

Environmental issues have become a major global concern, with food waste emerging as one of the most pressing challenges associated with the growing world population. Food waste encompasses materials discarded or lost throughout various stages of the food supply chain, including production, transportation, processing, and consumption. Common disposal methods such as incineration, landfilling, and composting—release significant amounts of CO_2 and methane, two primary greenhouse gases that contribute directly to climate change. Moreover, these practices lead to unnecessary loss of valuable resources such as water, energy, and agricultural land [1]. Food waste can generally be classified into two types: approximately 40% is edible, while 60% is inedible, such as fruit peels. Fruit peels, which are by-products of the fruit processing industry, are often discarded despite being rich in biologically active compounds. These bioactive molecules offer high potential for value-added utilization, particularly in the food, cosmetic, and pharmaceutical industries [2], [3].

In green chemistry and nanotechnology, *Citrus sinensis* peel extract is often used as a natural reducing and stabilizing agent in the biosynthesis of metal oxide nanoparticles (like TiO_2 or ZnO). This is because its phytochemical content (flavonoids, phenolics, ascorbic acid, etc.) can reduce metal ions and stabilize the formed nanoparticles in an eco-friendly and cost-effective manner. The sweet orange is a hybrid species, believed to have originated from a cross between the pomelo (*Citrus maxima*) and the mandarin (*Citrus reticulata*). It produces round, bright orange-colored fruits with a sweet, juicy pulp and a fragrant peel that contains essential oils and bioactive compounds, such as flavonoids, limonene, and vitamin C [4], [5].

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This research focuses on adding value to sweet orange peel by utilizing it as a reducing agent in a green chemical process for the synthesis of TiO_2 nanomaterials with antibacterial properties. Flavonoids were extracted from sweet orange peel using 5 different volume concentrations for 1, 5, 10, 15, and 20 mL. Additionally, we studied the purity of synthesized TiO_2 by comparing the annealed process with FT-IR, XRD, and FE-SEM techniques. Finally, the antibacterial activity of the pure titanium dioxide nanoparticles (TiO_2 NPs) was evaluated using the disc diffusion method to determine the inhibition zone against bacterial growth.

2. Experimental

2.1 Preparation of flavonoid extract

The peels of sweet orange or *Citrus sinensis* were washed three times with tap water to remove dirt and dust. They were 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 peel powder was then mixed with deionized (DI) water as a solvent and shaken at 250 rpm for 3 hours. Subsequently, the mixture was heated in a water bath at 60 °C for 1 hour, and the extract was filtered through Whatman No. 1 filter paper, as shown in Figure 1.

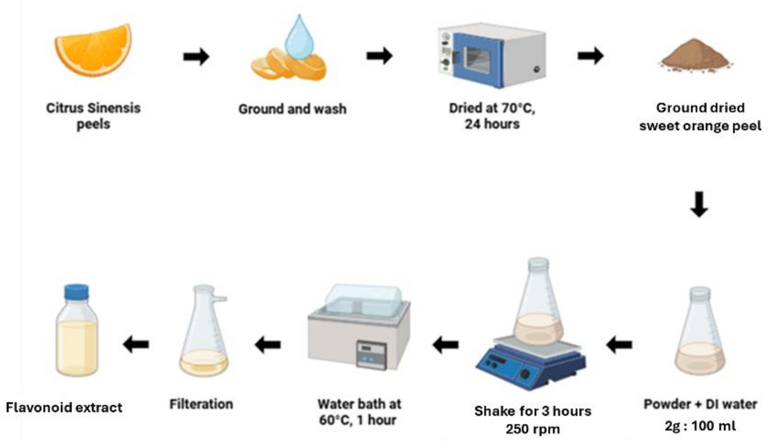

Fig. 1. Preparation of flavonoid extract from sweet orange peel

2.2 Synthesis of titanium dioxide nanoparticles

A 10 mL sweet orange peel extract was prepared and mixed with 2 g of titanium isopropoxide precursor. The mixture was stirred for 1 hour and then placed in a water bath for an additional 1 hour. The sample was then dried and annealed at 350 °C for 6 hours, as shown in Figure 2.

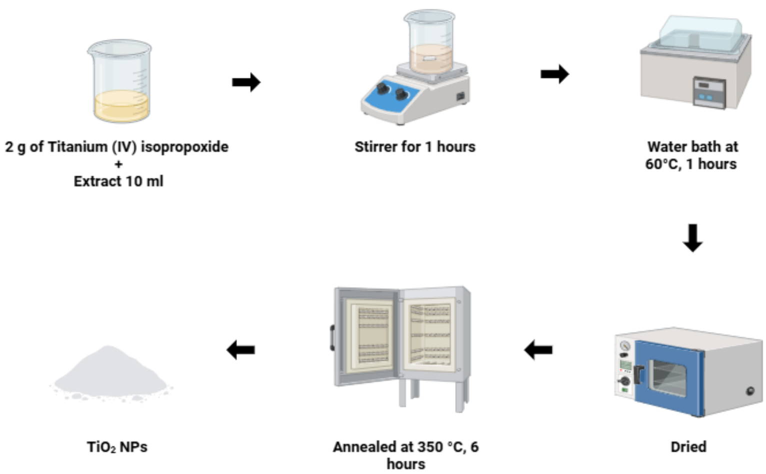

Fig. 2. Synthesis process of titanium dioxide nanoparticles

2.3 Characterization of titanium dioxide nanoparticles

The crystal structure characteristics of the particles were examined by an X-ray diffractometer at a wavelength of 1.54 Angstrom with a measurement rate of 5 degrees per minute from a 2θ angle of 10 degrees to a 2θ angle of 80 degrees. When the X-ray diffraction pattern of the synthesized powder particles was obtained, the X-ray diffraction pattern was examined for the type and characteristics of the resulting phases compared to the X-ray diffraction patterns of various substances in the standard database (JCPDS files). After that, the data obtained from the X-ray diffraction pattern was used to calculate the crystallite size using the Scherrer equation. The functional groups were studied using a Fourier transform infrared spectrometer (FT-IR), starting with measurements at wave numbers ranging from 4000 to 400 cm^{-1}, and repeating the measurements eight times. The morphology of the synthesized powder particles was analyzed using a field emission scanning electron microscope (FE-SEM) to determine their shape and size.

2.4 Antibacterial activity

The antibacterial activity of TiO_2 NPs was tested via disc diffusion techniques. The inhibition zone of bacteria was tested using *Staphylococcus aureus (S. aureus)*, a gram-positive bacterium, and *Escherichia coli (E. coli)*, a gram-negative bacterium. The prepared bacteria were incubated at room temperature in a shaker with shaking at 250 rpm for 24 hours. For the antibacterial activity test, the bacteria were swabbed across the entire surface of the prepared solid media in a culture dish. Leave it for 3-5 minutes and then place a sterile filter paper disc with a diameter of 5 mm on the culture dish. Then, the synthesized TiO_2 NPs were dispersed in deionized water at concentrations of 0.1, 0.3, and 0.5 mg/mL and dropped onto sterile filter paper at specified positions, 30 µL each, including 30 µL of deionized water as a reference point. The culture plates were then incubated at 37 °C for 18-24 hours. After the time was up, the diameter of the clear zone (Inhibition zone) was measured.

3. Result and discussion

This results and discussion part consists of three main analytical sections. The first concerns the purity of titanium dioxide nanoparticles, which is further studied in terms of the crystalline structure and functional groups. The second focuses on the effect of annealing times on the synthesis of titanium dioxide nanoparticles (TiO_2 NPs) by FT-IR, XRD, and FE-SEM techniques. The last studies the antibacterial activity.

3.1 The purity of synthesized TiO_2 NPs

The purity of TiO_2 nanoparticles was characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) techniques. The utilized titanium (IV) isopropoxide as the precursor and a sweet orange peel extract as the reducing agent shows as equation 1.

$$Ti_4(OCH_3)_{16} + Extraction \rightarrow 4TiO_2 + Organic\ by\ product \quad (1)$$

Based on Equation 1, the required reaction molar ratio between the precursor and the extract was 1:1. Consequently, the experiment was designed by varying the volume of the sweet orange peel extract (SE) (5, 10, 15, and 20 mL) to determine the corresponding volume equivalent to 1 mole. The synthesized powder samples from all conditions were subsequently characterized by their characteristic crystalline structure using the X-ray Diffraction (XRD) technique, as presented in Figure 3.

Figure 3 illustrates the X-ray diffraction (XRD) patterns of titanium dioxide nanoparticles synthesized via a green chemistry approach using varying volumes of sweet orange peel extract. It was observed that the SE volume of 10 mL, the XRD pattern begins to match the standard diffraction pattern of titanium dioxide (JCPDS file No. 01-071-1166), which corresponds to the anatase phase with a tetragonal crystal structure. The characteristic diffraction peaks appear at 2θ values of 25.08°, 37.89°, 47.71°, 54.85°, 62.90°, 69.52°, and 74.79°, corresponding to the (101), (004), (200), (105), (204), (116), and (215) crystal planes, respectively. This result suggests that the 10 mL volume of sweet orange peel extract may be the optimal amount for facilitating the formation of titanium dioxide compounds.

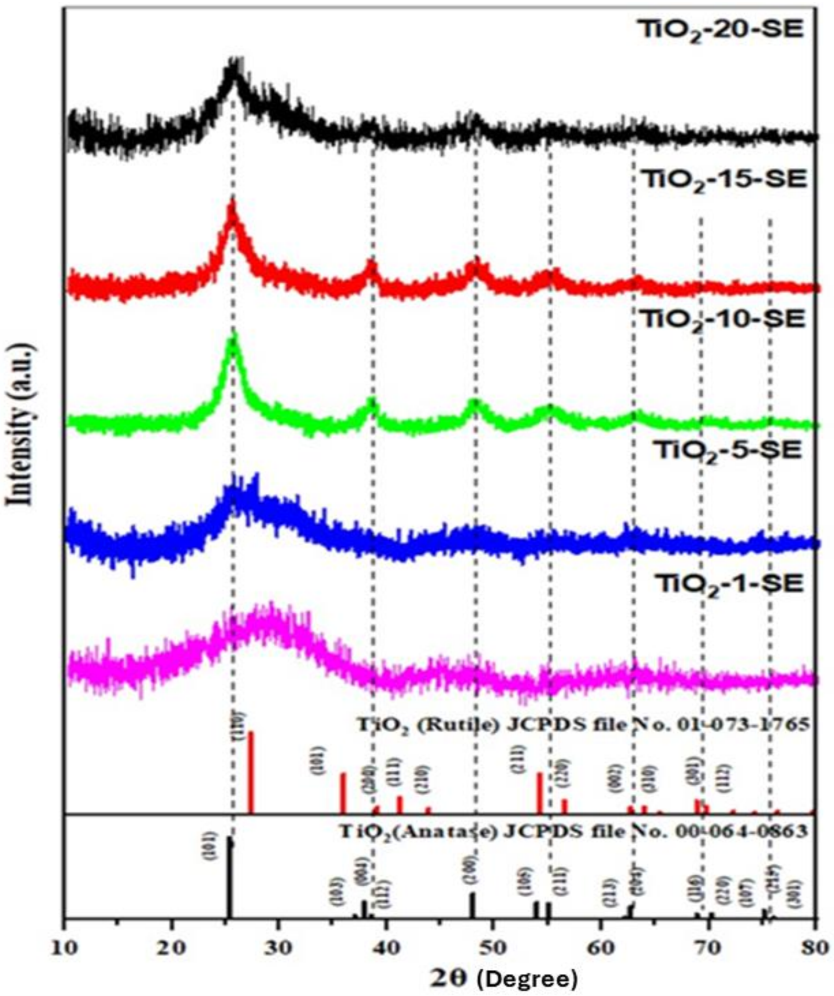

Fig. 3. X-ray diffraction patterns of titanium dioxide nanoparticles under different volume concentrations of sweet orange

However, it is important to note that XRD analysis only provides information regarding the crystallinity of the material. If secondary products, particularly organic compounds in an amorphous state, are present, they may not be detectable by XRD due to the inherent limitations of this technique. Therefore, it is essential to characterize the synthesized titanium dioxide nanoparticles using Fourier transform infrared spectroscopy (FT-IR) to identify possible functional groups and confirm the presence of organic constituents.

The morphology of the synthesized powder particles was analyzed using Field Emission Scanning Electron Microscopy (FE-SEM) to determine their shape and size.

Figure 4 illustrates the relationship between the transmittance percentage (%T) of titanium dioxide nano powder and wavenumber as measured by Fourier Transform Infrared Spectroscopy (FTIR) before the annealing process. A notable decrease in transmittance is observed in the wavenumber range of 3100–3400 cm^{-1}, corresponding to the O–H stretching vibrations of hydroxyl groups [6]. Additionally, a reduction in transmittance at approximately 1630 cm^{-1} is associated with O–H bending vibrations [7]. Bending vibrations of hydrocarbon groups (CH, CH_2, CH_3) are detected in the wavenumber range of 1300–1400 cm^{-1}. The C–O stretching vibrations appear in the range of 1030–1100 cm^{-1} [8]. Furthermore, O–Ti–O stretching vibrations, indicative of the anatase crystalline phase of titanium dioxide, are observed in the range of 400–800 cm^{-1} [9]. When the annealing time was increased to 6 hours, these residual O-H impurity peaks vanished. Only the pure phase characteristic of the O-Ti-O stretching vibration of the TiO_2 anatase crystal lattice was observed in the 400-800 cm^{-1} region. The same pure phase result was also found for annealing durations of 12 and 24 hours.

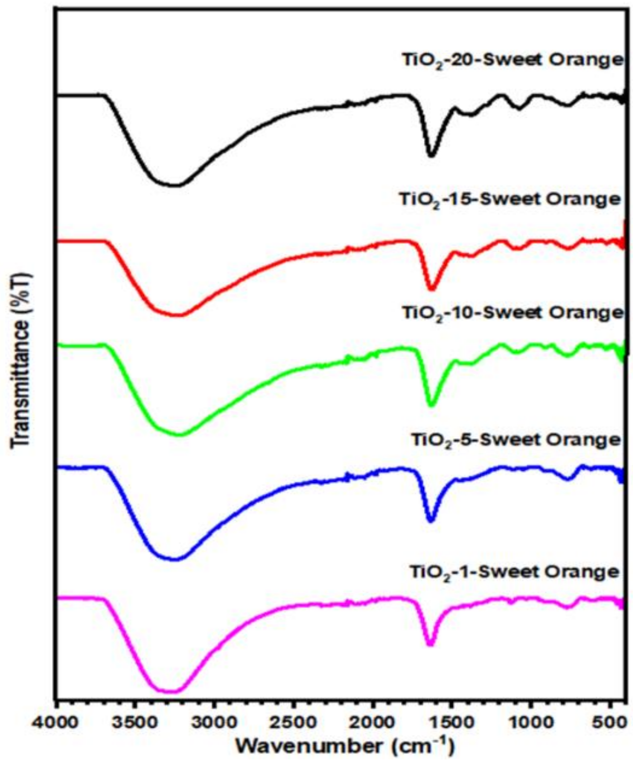

Fig. 4. The effect of annealing times on synthesized TiO_2 nanoparticles

3.2 The effect of annealing times on synthesized TiO_2 nanoparticles

The effect of annealing times on TiO_2 nanoparticles with sweet orange extracted studied by 3 techniques: FT-IR, XRD, and FE-SEM, respectively.

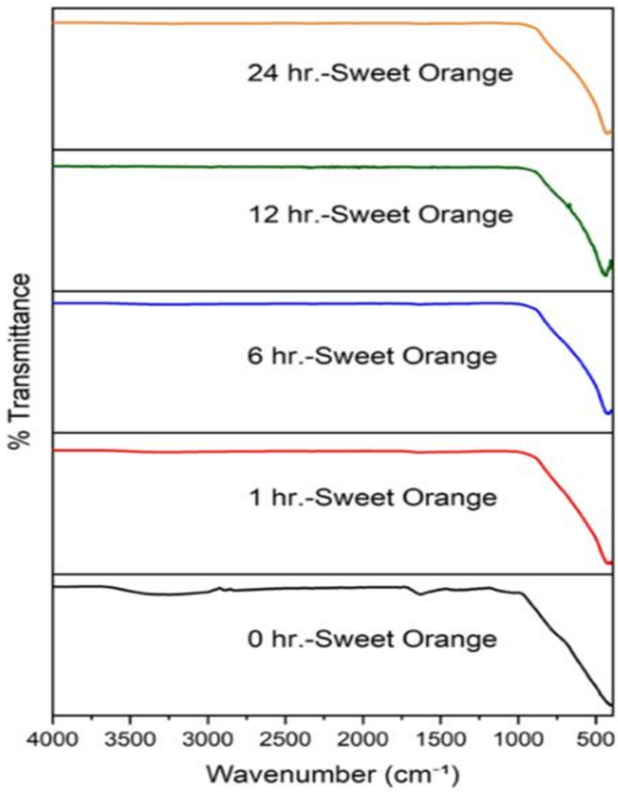

Fig. 5. FT-IR spectra of annealing-treated TiO_2 nanoparticles at different times

As shown in Figure 5, the quality of titanium dioxide nanoparticles synthesized using 10 mL of sweet orange peel extract was improved through an annealing process at 350 °C for 6 hours. After annealing, the characteristic peaks associated with hydroxyl (-OH) groups, observed at wavenumbers 3100–3400 cm^{-1} and approximately 1630 cm^{-1}, disappeared. The pure phase, corresponding to the stretching vibrations of the O–Ti–O lattice network, was observed in the wavenumber range of 400–800 cm^{-1} [9].

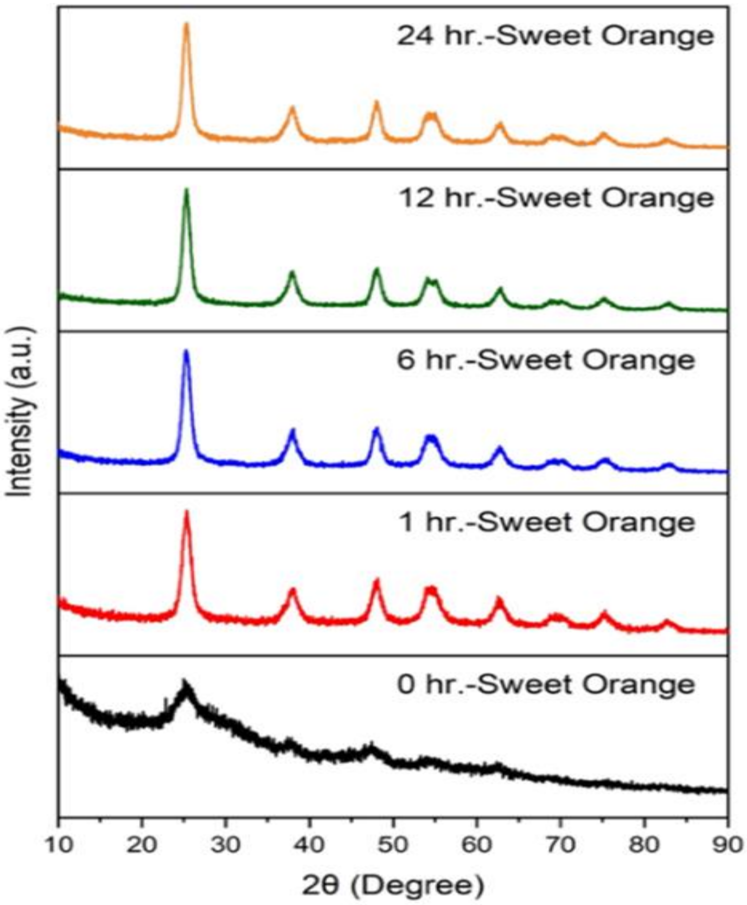

Fig. 6. X-ray diffraction (XRD) patterns of annealing-treated TiO_2 nanoparticles under different times

When the titanium dioxide (TiO_2) nanoparticles were subjected to annealing at 350 °C for 6 hours, as shown in Figure 6, it was observed that the crystallinity of the TiO_2 nanoparticles increased with longer annealing duration because heating can drive it toward a more thermodynamically stable crystalline phase. This phase change is accompanied by a more regular atomic arrangement, hence higher crystallinity. The TiO_2 nanoparticles retained their anatase phase with a tetragonal crystal structure, displaying the primary diffraction planes at (101), (200), and (004), corresponding to 2θ values of 25.08°, 47.71°, and 37.89°, respectively.

Figure 7 shows that the crystallite size of the purified titanium dioxide nanoparticles increased with prolonged annealing time at 350 °C. Specifically, as the annealing duration increased from 1, 6, 12, and 24 hours, the crystallite size grew from 5.617 to 5.683, 5.978, and 5.984 nm, respectively, calculated using the Scherrer equation as shown in equation 2:

$$D = \frac{k\lambda}{\beta cos\theta} \tag{2}$$

where D is the crystallite size, k is the Scherrer constant (k = 0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle corresponding to the peak position [10].

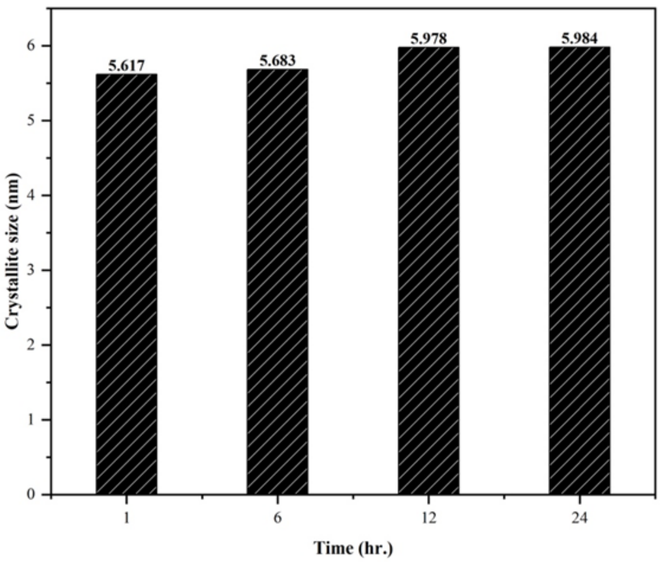

Fig. 7. The crystallite size of annealing-treated TiO_2 nanoparticles under different times

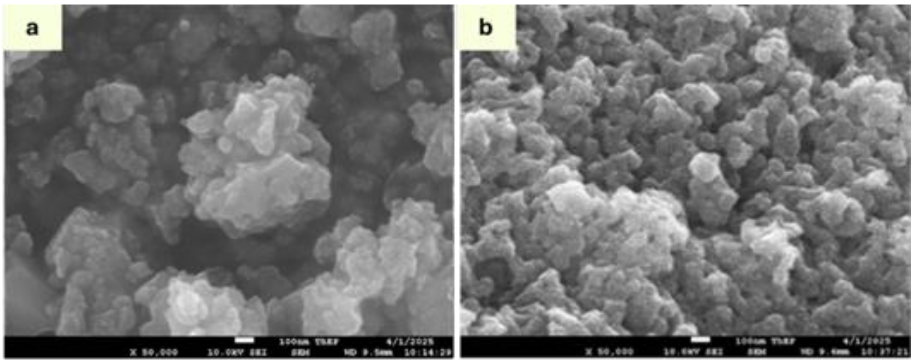

Fig. 8. FE-SEM images of titanium dioxide nanoparticles (a) before annealing and (b) after annealing

Figure 8a, before annealing, exhibits a coating of a molten compound on the titanium dioxide nanoparticles. This observation is consistent with the results obtained from Fourier Transform Infrared Spectroscopy (FTIR) in Figure 5, which detected the presence of residual amorphous organic compounds as secondary phases. After annealing, the quality improvement of the titanium dioxide nanoparticles at 350 °C for 6 hours in Figure 8b, the image shows that the nanoparticles became relatively uniform and homogeneous. The average particle size was calculated to be around 195.9 nm.

3.3 Antibacterial activity of TiO_2 nanoparticles

From Figure 9, titanium dioxide (TiO_2) nanoparticles at a concentration of 0.5 mg/mL in deionized water exhibited the highest inhibitory effect on the growth of both Gram-positive and Gram-negative bacteria. Notably, the inhibition of Gram-negative bacteria (*E. coli*) was more effective compared to that of commercially available titanium dioxide nanoparticles. The mechanism that causes cell death occurs when TiO_2 nanoparticles are ionized and diffuse into cells, where they react with bacterial cell walls. The antibacterial mechanism of TiO_2 NPs involves the generation of reactive oxygen species (ROS) when activated by light. These ROS include superoxide radicals (O_2^{-}•) and hydroxyl radicals (•OH). Upon entering bacterial cells, these radicals induce phospholipid peroxidation, disrupting the outer membrane. This damage ultimately destroys internal cellular components and causes cell death [11], [12].

Therefore, this research selected titanium dioxide nanoparticles synthesized via a green chemical method using 10 mL of sweet orange peel extract and 2 g of titanium isopropoxide, followed by annealing at 350°C for 6 hours. The TiO_2 NPs were applied at a concentration of 0.5 mg/mL in deionized water for the preparation of bionanocomposite films in the next research.

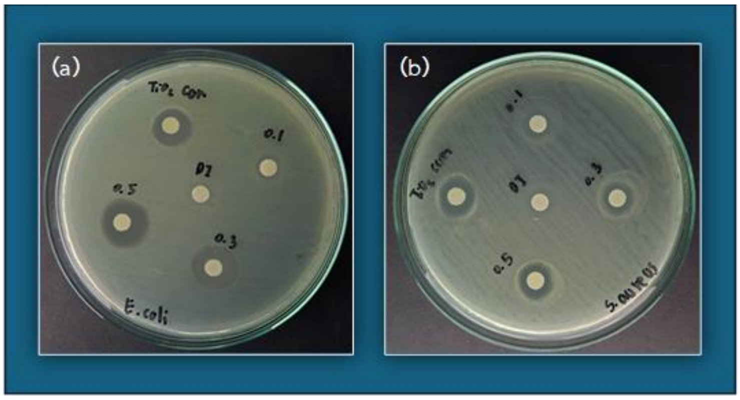

Fig. 9. The inhibition zones of bacterial growth (a) *E. coli* and (b) *S. aureus*

4. Conclusion

This study investigates the synthesis of titanium dioxide (TiO_2) nanoparticles through a green chemistry route utilizing extracts derived from sweet orange (*Citrus sinensis*) peels. The structural quality of the obtained TiO_2 nanoparticles was further improved by annealing at 350 °C for 6 hours, a process that notably enhanced their physical properties. The antibacterial potential of the nanoparticles was evaluated at concentrations of 0.1, 0.3, and 0.5 mg/mL in deionized water using the disc diffusion method against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli)* served as representative bacterial strains for each group. The findings revealed that TiO_2 nanoparticles at a concentration of 0.5 mg/mL demonstrated the highest antibacterial efficacy against both bacterial types.

Acknowledgments

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