

Research article

Flavonoid Extraction from Mango Peels for Nanoparticles by Green Synthesis Process

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Abstract

This research study explored the extraction of flavonoids from ripe and raw mango peels using different solvents (deionized water, methyl alcohol, and ethyl alcohol) over varying durations. The extracted compounds were then employed in synthesizing titanium dioxide nanoparticles (TiO₂ NPs) via a green chemical process using titanium isopropoxide. Optimal extraction, yielding the highest flavonoid content, was achieved with deionized water after 3 h for ripe peels and 4 h for raw peels. The titanium dioxide nanoparticles synthesized exhibited an anatase crystal structure, as confirmed by XRD, Raman, and FTIR techniques. SEM images showed that the nanoparticles were evenly distributed and had a smooth surface. The titanium dioxide nanoparticles demonstrated stronger antibacterial activity against *Escherichia coli* than *Staphylococcus aureus*. Furthermore, raw mangoes were found to be more effective in inhibiting bacterial growth than ripe mangoes.

Keywords: green synthesis; flavonoids extraction; mango peel extraction; titanium dioxide nanoparticles

1. Introduction

Green synthesis refers to the development of chemical processes or methodologies that minimize the use of hazardous substances and maximize efficiency and sustainability. It focuses on reducing the environmental impact of chemical reactions, typically by using environmentally friendly solvents, reducing waste generation, and utilizing renewable resources whenever possible (Singh et al., 2018; Huston et al., 2021; Álvarez-Chimal et al., 2024).

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Mango peel extraction involves isolating beneficial bioactive compounds such as polyphenols, carotenoids, vitamins (like A and C), and dietary fibers from the outer skin of mango fruits. These compounds offer numerous health benefits, including antioxidant properties that help neutralize free radicals and reduce oxidative stress, potentially lowering the risk of chronic diseases. Additionally, mango peel extraction contributes to sustainability by utilizing a part of the fruit that is typically discarded, thereby reducing food waste. Extracts from mango peel have applications as food additives, functional foods, cosmetics, and pharmaceuticals, highlighting their value in promoting health and minimizing environmental impact through waste reduction (Yang & Li, 2013; Garcia-Mendoza, 2015).

Flavonoids are a diverse group of phytonutrients (plant chemicals) that belong to the larger class of polyphenolic compounds. They are found naturally in a wide variety of fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Flavonoids are characterized by their antioxidant properties, which help protect cells from damage caused by free radicals.

Flavonoids are extracted from various natural sources, including mango peel, due to their significant health benefits and therapeutic properties. These compounds are a subclass of polyphenolic compounds widely distributed in plants, and are known for their antioxidant, anti-inflammatory, and anti-cancer properties. Specifically, flavonoids extracted from mango peel have been found to exhibit strong antioxidant activity, helping to protect cells from damage caused by free radicals and oxidative stress. This antioxidant capability is crucial in reducing the risk of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders. Moreover, flavonoids contribute to overall health by potentially improving vascular function, reducing inflammation, and supporting immune function. Their extraction and utilization underscore their importance in both preventive and therapeutic aspects of health care, making them valuable compounds in nutrition, pharmaceuticals, and functional foods (Duthie & Crozier, 2000; Pietta, 2000).

This research study holds significant importance as it was focused on the study of the extraction of flavonoids from mango peels using various solvents, including deionized water, methyl alcohol, and ethyl alcohol. Extracting flavonoids from mango peels provided valuable insights into the obtained extracts, which were subsequently utilized in synthesizing titanium dioxide nanoparticles through a green chemical approach. The choice of employing a green chemical method for nanoparticle synthesis is essential as it aligns with the principles of sustainability and may have positive environmental implications. Characterization of the TiO₂ NPs was conducted using X-ray diffractometry (XRD), Fourier transform-infrared spectrometry (FT-IR), and Raman spectrometry, revealing a consistent anatase crystal structure across all samples with no impurities detected.

2. Materials and Methods

2.1 Extraction of flavonoid compounds from mango peels

To extract flavonoid compounds from the peels of two types of mangoes (raw or green mangoes and ripe or yellow mangoes), the mangoes were rinsed with deionized water and peeled. Then, the peels were cut into thin, small pieces for easier drying. The peels were dried at 60°C overnight until they became crisp, then ground using a mortar or a blender. A 0.5 g sample of the ground mango peels was placed into an Erlenmeyer flask and added with 25 mL of solvent. The solvent was deionized water (DI water), methanol (MeOH) or ethanol (EtOH). Each flask was shaken in a shaker for 1, 2, 3, 4, or 5 h. After each extraction hour, the flasks were heated at 60°C for 1 h in a water bath. Finally, each extract was filtered to remove unwanted residues, as shown in Figure 1.

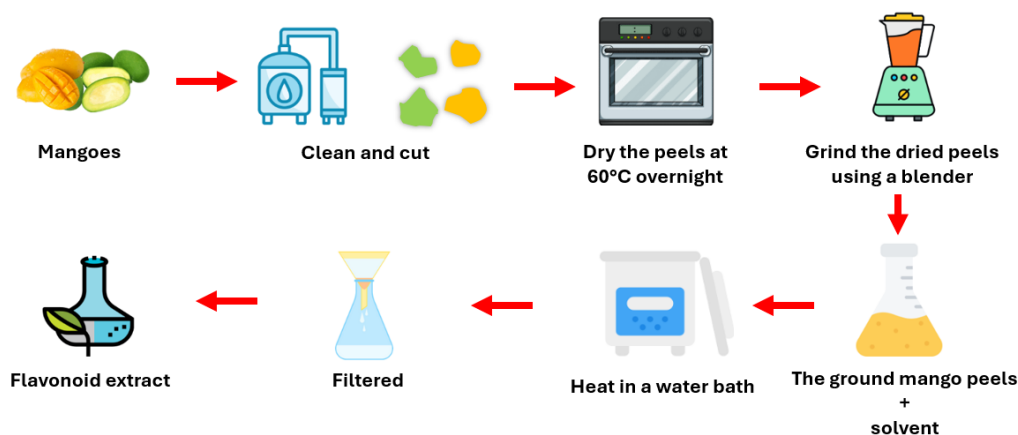


Figure 1. Diagram of extraction of compounds from mango peels

2.2 Method to determine total flavonoid content

The total flavonoid content was characterized by UV-Vis measurement and expressed in terms of mg quercetin/100 g, as outlined by Lees & Francis (1971). The total flavonoid content was measured at 374 nm using distilled water as a blank and then calculated as follows (Rigby & Dana, 1972).

$$\text{Total flavonoid content (mg quercetin/100g)} = \frac{\text{Absorbance at 374 nm} \times \text{dilution factor}}{76.5}$$

2.3 Synthesis of TiO₂ nanoparticles using green chemical process

To synthesize titanium dioxide nanoparticles through a green process, titanium isopropoxide was mixed with flavonoid extract by weighing 2 g of titanium isopropoxide into a 100-mL beaker and adding the flavonoid extract in varying amounts (5, 10, 15, 20, 25, and 30 mL). Each mixture was stirred for 1 h using a magnetic stirrer, then heated in a water bath at 60°C for 1 h. The mixture was then dried at 150°C overnight and finally calcined at 500°C for 2 h, as illustrated in Figure 2.

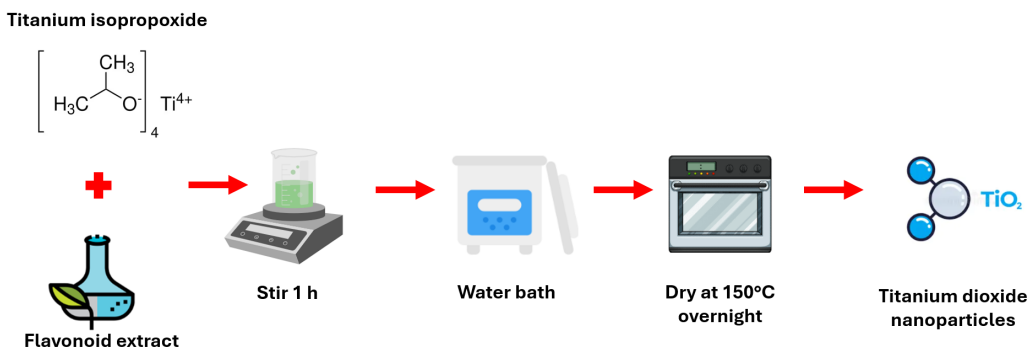


Figure 2. The synthesis of TiO₂ nanoparticles by green chemical process

The reduction of flavonoids with titanium isopropoxide ($\text{Ti}(\text{OiPr})_4$) is a process that typically involves the hydrolysis and condensation of titanium isopropoxide to form titanium dioxide (TiO_2), where flavonoids may act as stabilizers, reducing agents, or surface modifiers. The mechanism can be broken down into key stages (Nasrollahzadeh & Sajadi, 2015; Rodríguez-Jiménez et al., 2021), as follows:

1) Hydrolysis of titanium isopropoxide

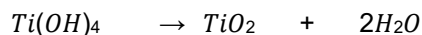
Titanium isopropoxide reacts with water (hydrolysis) to replace the isopropoxide groups ($-\text{OiPr}$) with hydroxyl groups ($-\text{OH}$):



In this step, the flavonoids may play a role in influencing the extent of hydrolysis. They contain hydroxyl groups that could coordinate with titanium and limit uncontrolled hydrolysis, forming a more controlled reaction environment.

2) Condensation to form TiO_2

The hydroxylated titanium species undergoes condensation, where the hydroxyl groups ($-\text{OH}$) are eliminated as water, resulting in the formation of Ti-O-Ti bonds, which eventually yield TiO_2 nanoparticles:



This process generally occurs at elevated temperatures to facilitate the crystallization of the TiO_2 from amorphous forms.

2.4 Characterization and measurements

The flavonoid extracts from the samples were measured by the absorbance from a UV-VIS spectrophotometer PG Instrument with model T92+ Spectrophotometer at the wavelength of 374 nm. The flavonoid contents were determined by the phase, composition, and structural characteristics of titanium dioxide nanoparticles analyzed from X-ray diffraction (XRD) patterns obtained from scanning -2θ from $80-10^\circ$ at a fixed incident angle of 0.4° using X-ray diffractometer (Rigaku Smartlab) with $\text{CuK}\alpha$ radiation ($\lambda = 0.15406\text{nm}$). Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of synthesized titanium dioxide (TiO_2). The FT-IR spectra of the films were measured from the wave number ranging from $500-4,000\text{ cm}^{-1}$ by Perkin Elmer UATR 2 spectrophotometer. Raman Spectroscopy was the technique used for the identification of the extracted flavonoid and possible interactions between their materials. The technique can provide valuable information of the synthesized TiO_2 nanoparticle purity and molecular structure. The morphology of the synthesized TiO_2 was studied by field emission scanning electron microscopy (FE-SEM) (Apreo S-Thermo Fisher Scientific).

2.5 Bacterial inhibition assay

To prepare the culture medium, 5.4 g of nutrient broth (Criterion, USA) was mixed with 600 mL of water and the pH was adjusted to approximately 6.8-7 using sodium hydroxide or

hydrochloric acid solutions. Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* cultures (obtained from Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang) were prepared by inoculating a loopful of bacteria into 5 mL of sterilized liquid nutrient broth and incubating overnight in a shaker using a speed of 250 rpm and temperature of 27°C. The bacterial cultures were then spread evenly onto prepared agar plates and allowed to sit for 3-5 min. Sterilized filter paper discs (7 mm diameter) were placed on the agar, and the synthesized titanium dioxide nanoparticles dispersed in deionized water at concentrations of 0.3, 0.5, and 0.7 mg/mL were placed onto the discs, with 30 µL per spot. Deionized water was used as a control. The plates were then incubated at 37°C for 18-24 h. After the end of incubation period, the diameter of the inhibition zones (mm) was measured from one edge to the opposite edge.

This process allowed for the assessment of bacterial inhibition using the synthesized titanium dioxide nanoparticles (Santhoshkumar et al., 2014). The presence and effectiveness of the nanoparticles in inhibiting bacterial growth was indicated by the size of the inhibition zone around the nanoparticle-loaded filter paper disc. Mean (\bar{X}) and standard deviation values obtained from the three iterations were statistically analyzed.

2.6 Antibacterial assay for commercial and synthesized TiO₂

This setup allowed the evaluation of the antibacterial properties of both commercial and synthesized TiO₂ compared to the negative control. To assess bacterial resistance, experiments were conducted on petri dishes to limit environmental variables. Positive and negative controls were established for these tests. In this study, commercial TiO₂ and synthesized TiO₂ were used as positive controls, as they are known to have antibacterial properties. The negative control was deionized water, which does not exhibit antibacterial effects. Commercial TiO₂ was prepared at a concentration of 0.3 mg/mL, while synthesized TiO₂ was prepared at varying concentrations (0.3, 0.5, and 0.7 mg/mL), and all solutions were sterilized using an autoclave. These solutions were applied to filter paper discs placed on bacteria-inoculated agar plates, with 30 µL of each solution carefully dispensed. The plates were incubated for 18-24 h, after which the antibacterial effects of both commercial and synthesized TiO₂ were compared to the negative control.

3. Results and Discussion

3.1 Effect of flavonoid extraction volume with various solvents

Figure 3 illustrates the total flavonoid content (as calculated by Lees & Francis, 1971 and Rigby & Dana, 1972) obtained from the extraction of ripe and raw mango peels using various solvents, each with different extraction times. The results show that the deionized water solvent extracted the highest amount of flavonoids because it is a polar solvent that effectively extracts polar compounds like flavonoids from plant materials. Flavonoids typically have hydroxyl groups and other polar functional groups that interact well with water molecules, facilitating their extraction. In contrast, methyl alcohol (methanol) and ethyl alcohol (ethanol) are polar solvents but may not be as selective or efficient in extracting flavonoids from mango peels due to differences in polarity and solvent-solute interactions. Additionally, DI water is non-toxic and less likely to cause degradation or modification of flavonoid compounds during extraction, preserving their integrity and bioactivity. In contrast, alcohol solvents can sometimes denature or alter flavonoid molecules, potentially reducing the total amount extracted. Interestingly, ripe mangoes

(DIY) required 3 h (Figure 3a) for extracting the highest amount of flavonoids, whereas raw mangoes (DIG) required 4 h (Figure 3b) because ripe mango peels were softer and contained higher moisture levels compared to raw mango peels, which likely facilitated easier and quicker extraction of flavonoids. The softer texture and higher moisture content in ripe mango peels allowed solvents to penetrate and extract flavonoids more efficiently.

Figure 3c shows that the highest percentage of titanium dioxide yield was achieved using different volume (5, 15, 15, 20, 25, 30 mL) of the extract as a reducing agent in the synthesis of titanium dioxide. It was found that ripe mango peels produced the highest yield with a 20 mL volume of flavonoid extract, while raw mango peels achieved the highest yield with a 25 mL volume. Ripe mangoes typically have higher water content, which can affect the solubility of flavonoids and make them more readily available for extraction in smaller volumes of solvent compared to raw mango peels, which are drier and may require more solvent to achieve higher yields. For raw mangoes, a 20 mL volume of extract exhibited the dominant lowest %yield because raw mango extract contains not only reducing agents but also compounds that may act as capping agents (substances that stabilize nanoparticles). At 20% volume, there might be an imbalance where the capping agents inhibited the growth of TiO_2 particles, resulting in fewer particles forming, and hence a lower yield.

3.2 The characteristics of synthesized titanium dioxide (TiO_2)

The extracted compounds were then employed in synthesizing titanium dioxide nanoparticles (TiO_2 NPs) via a green chemical process using titanium isopropoxide. Characterization of the TiO_2 NPs was conducted using X-ray diffractometry (XRD), Raman spectrometry and Fourier transform-infrared spectrometry (FT-IR).

3.2.1 X-ray diffraction (XRD)

To verify the uniqueness of titanium dioxide, the X-ray diffraction technique was used and the results are shown in Figure 4, where the peak corresponding to the anatase phase (JCPDF file No. 00-021-1272) can be observed. The diffraction peaks were observed at an angle of 2θ , corresponding to the planes (101), (103), (004), (112), (200), (105), (211), (213), (204), (116), (220), (107), (215), and (301). It can be seen that the titanium dioxide synthesized using ripe mango peel extract differed from that synthesized using raw mango peel extract. The titanium dioxide obtained from ripe mango peel extract displayed in a combination of the (105) and (211) planes, which are characteristic of the anatase phase of TiO_2 , which is known for its photocatalytic properties. The presence of these planes suggests that the TiO_2 synthesized from ripe mango peels was of the desired anatase phase due to its superior activity compared to other TiO_2 phases (such as rutile or brookite).

3.2.2 Raman spectroscopy

Figure 5 shows the anatase phase of the synthesized titanium dioxide (TiO_2) from mango peels. The Raman spectra reveal the distinct peaks that correspond to key vibrational modes within its crystal lattice. The E_g mode, observed prominently at around 144, 199, 640 cm^{-1} , signifies the symmetric stretching of oxygen atoms within the TiO_6 octahedral units. Another significant peak, around 399 cm^{-1} , represents the B_{1g} mode, involving

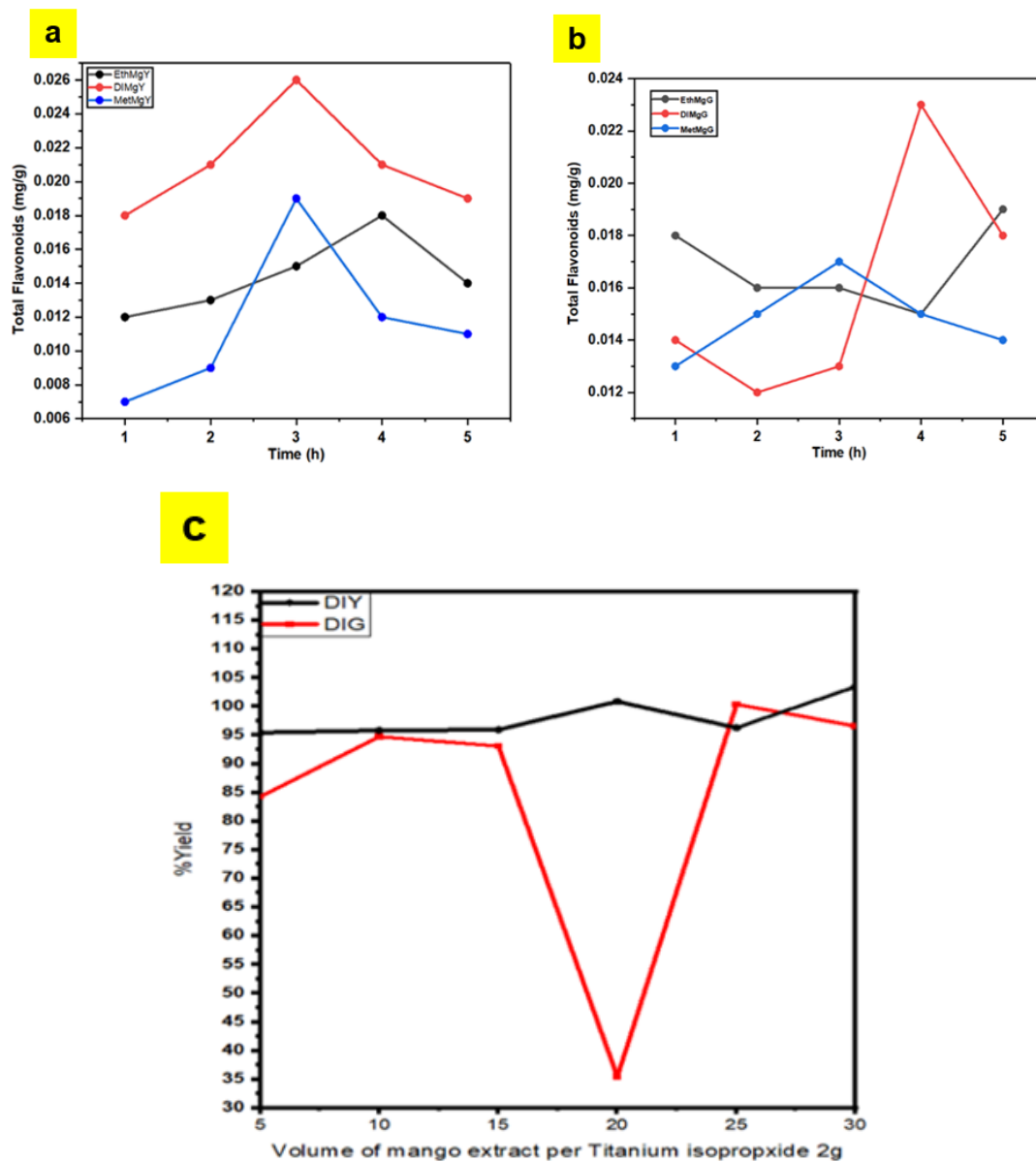


Figure 3. The flavonoid extraction graphs obtained from different solvents:
a) ripe mangoes (DIY), b) raw mangoes (DIG), c) percentage of yield of ripe mangoes (DIY) and raw mangoes (DIG)

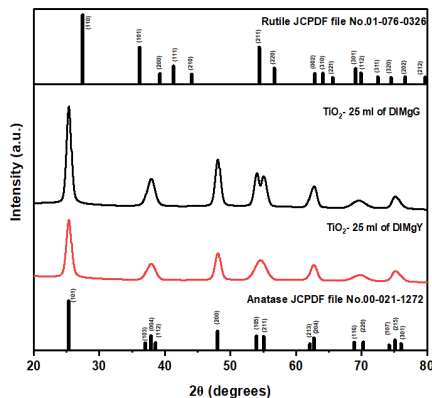


Figure 4. The X-ray diffraction pattern of ripe mangoes (DIMgY) (black) and raw mangoes (DIMgG) (red)

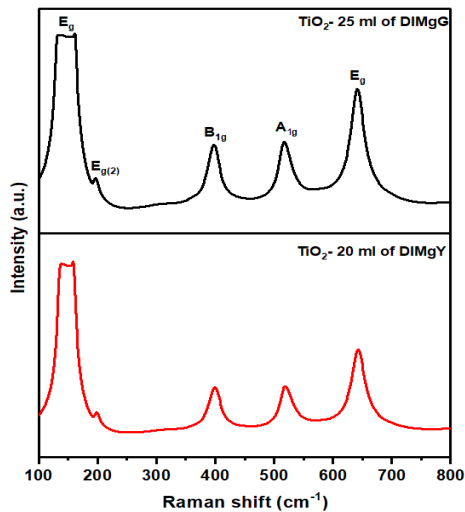


Figure 5. The Raman spectra of raw mangoes (DIMgG) (black) and ripe mangoes (DIMgY) (red)

stretching and bending vibrations of Ti-O bonds. Additionally, the A_{1g} mode at approximately 515 cm^{-1} indicates symmetric stretching of Ti-O bonds (Choudhury et al., 2013; Kernazhitsky et al., 2014; Khalid et al., 2021). These characteristic peaks in the Raman spectra of anatase TiO_2 provide crucial insights into its structural properties.

3.2.3 Fourier transform infrared spectroscopy (FTIR)

Figure 6 presents the Fourier transform infrared (FTIR) spectra of the synthesized titanium dioxide (TiO_2) from mango peels. The spectra display characteristic absorption bands that reveal essential vibrational modes within the crystal lattice and the surface features.

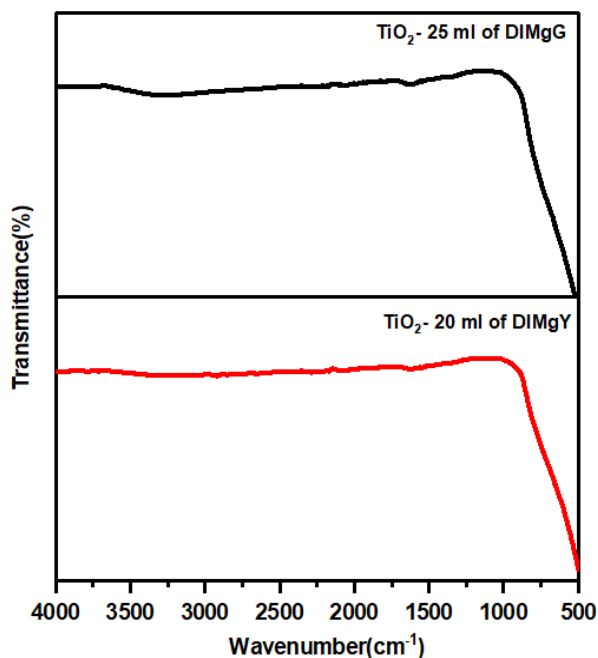


Figure 6. The FTIR spectra of raw mangoes (DIMgG) (black) and ripe mangoes (DIMgY) (red)

Common features include broad O-H stretching bands indicating adsorbed water or hydroxyl groups, sharp Ti-O stretching bands around 500-800 cm^{-1} , and bending modes indicative of O-Ti-O interactions (Chougala et al., 2017). Therefore, based on the results of XRD, Raman spectroscopy, and FTIR analyses, it can be confirmed that the titanium dioxide synthesized from flavonoid extracts exhibited an anatase phase structure.

3.3 The morphology of TiO_2 from scanning electron microscopy (SEM)

Figure 7 shows the FE-SEM image of titanium dioxide nanoparticles synthesized using ripe and raw mango peel extracts. The synthesized nanoparticles exhibit even distribution and a smooth surface (Bruno et al., 2014). From Figure 7b, it is evident that the titanium dioxide nanoparticles have a smaller particle size (40 nm) compared to those in Figure 7a (208 nm). This was probably due to the effectiveness of the mango peel extracts as reducing agents can vary depending on their chemical composition and antioxidant properties. If ripe mango peel extracts have a higher reducing power or are more effective stabilizing agents for nanoparticles, they may promote the formation of larger nanoparticles due to slower nucleation and growth rates. Additionally, the extracts from ripe mango peels contain different chemical constituents of biomolecules compared to extracts from raw mango peels. These components can influence the nucleation and growth kinetics of titanium dioxide nanoparticles during synthesis, potentially resulting in different particle sizes.

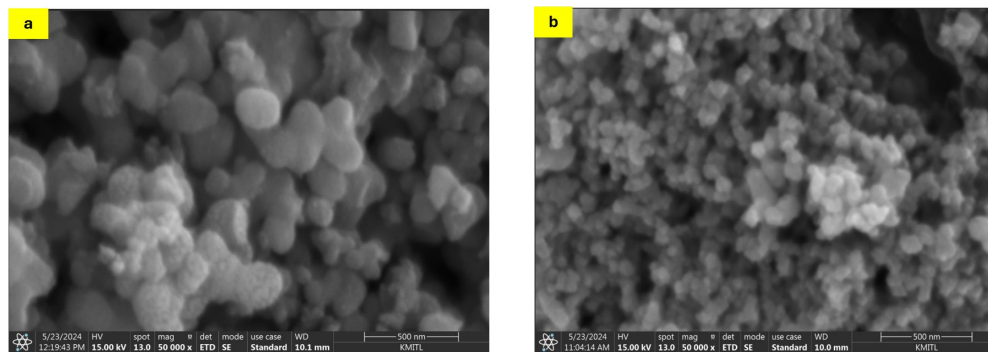


Figure 7. The morphology of synthesized TiO_2
a) ripe mangoes (DIY) and b) raw mangoes (DIG)

3.4 Antibacterial properties of titanium dioxide nanoparticles

The antibacterial properties of synthesized titanium dioxide nanoparticles in inhibiting bacterial growth were assessed. *Escherichia coli* representing gram-negative bacteria, and *Staphylococcus aureus* representing gram-positive bacteria were selected for the test.

According to Figure 8, it was observed that nanoparticles synthesized using various extracts demonstrated significant effectiveness in inhibiting bacterial growth. Specifically, titanium dioxide nanoparticles exhibited stronger inhibition against *E. coli* (Figure 8b) (with inhibition zones of DIG = 6.28 mm and DIY = 5.08 mm) compared to *S. aureus* (Figure 8a) (with inhibition zones of DIG = 5.01 mm and DIY = 5.25 mm). This difference might be because titanium dioxide nanoparticles often carry a positive charge, which interacts more strongly with the negatively charged surface of gram-negative bacteria. This interaction can disrupt the bacterial membrane, leading to increased permeability and cell damage. Additionally, gram-negative bacteria such as *E. coli* have an additional outer membrane composed of lipopolysaccharides. This outer membrane makes them more susceptible to disruption by nanoparticles like titanium dioxide due to its unique properties and charge interactions. The reason why gram-negative bacteria are less resistant to antimicrobial agents compared to gram-positive bacteria lies in the structural differences of their cell walls. The cell walls of gram-negative bacteria are much thinner than gram-positive bacteria and are surrounded by an outer membrane. While the cell walls of gram-positive bacteria compose of up to 90% peptidoglycan, along with teichoic acids that are covalently linked to the peptidoglycan. These teichoic acids contribute to the overall strength and rigidity of the cell wall. This structural difference makes gram-positive bacteria more resistant to the disruptive effects of titanium dioxide nanoparticles than gram-negative bacteria. Therefore, gram-positive bacteria are more resilient to the effects of titanium dioxide nanoparticles (da Silva et al., 2019; Yusof et al., 2019).

Figure 8c shows a histogram comparing the inhibition zone during flavonoid extraction from raw and ripe mangoes. It demonstrates that raw mangoes are more effective at inhibiting bacterial growth compared to ripe mangoes because the higher concentration of mangiferin typically found in raw mango peel. Mangiferin is a polyphenolic compound present in mangoes, particularly in the peel, skin, and seeds. It possesses potent antioxidant, anti-inflammatory, and antimicrobial properties, which contribute to the enhanced antibacterial effectiveness of the raw mango peel extract. These bioactive

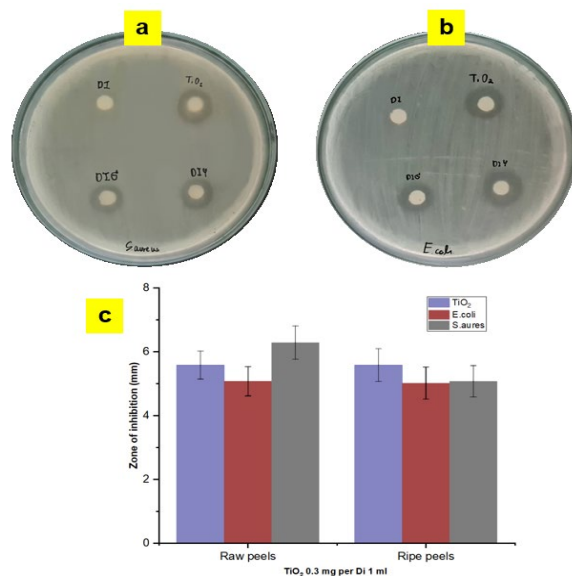


Figure 8. Antibacterial activities a) *S.aureus*, b) *E.coli*, and c) graph of inhibition zone

compounds are likely responsible for the increased antibacterial activity observed in titanium dioxide nanoparticles synthesized with raw mango peel extract (Kuçük, N., et al., 2024). Additionally, raw mangoes are more acidic than ripe mangoes. The lower pH can create an environment that is less favorable for bacterial growth. However, the TiO_2 nanoparticles synthesized from ripe mango peel extract exhibited lower antibacterial activity compared to those synthesized from raw mango peel extract. This difference in antibacterial efficacy is likely due to the smaller particle size of the nanoparticles prepared from raw mango peel extract, which results in a larger surface area. The increased surface area enhances the interaction between the nanoparticles and bacterial cells, leading to stronger antibacterial effects (Santhoshkumar et al., 2014; Serov et al., 2024).

4. Conclusions

This research was focused on the green synthesis of titanium dioxide (TiO_2) nanoparticles using mango peel extracts with different solvents, emphasizing eco-friendly methods. Among the extracts, the one obtained with deionized water showed the highest flavonoid content, indicating it may serve as a potent reducing and stabilizing agent in the synthesis. The synthesized TiO_2 nanoparticles were confirmed to have an anatase crystal structure through various characterization techniques, including X-ray Diffraction (XRD), Raman spectroscopy, and Fourier transform Infrared (FTIR) spectroscopy. Scanning electron microscopy (SEM) images revealed that the nanoparticles were evenly distributed with smooth surfaces, reflecting a high-quality synthesis. In terms of antibacterial efficacy, the TiO_2 nanoparticles demonstrated stronger inhibition against *E. coli* compared to *S. aureus*, highlighting their potential as antibacterial agents. Interestingly, it was also found that the extracts from raw mango peels were more effective in inhibiting bacterial growth than those from ripe mangoes, suggesting that the stage of ripeness impacts the antibacterial properties of the peel extracts.

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6. Conflicts of Interest

The authors declare no conflict of interest with respect to the research, authorships, and/or publication of this article.

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