

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES FROM LEMON PEEL EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

This research project aimed to study the extraction of flavonoids from lemon peels using different solvents, namely deionized water (DI water), isopropanol, ethanol, and methanol. Among these, DI water yielded the highest amount of flavonoids. The extracted compounds were then utilized for the synthesis of zinc oxide nanoparticles (ZnO NPs) through a green chemical method. Various amounts of lemon peel extract (10, 15, 20, 25, and 30 ml) were mixed with $\text{Zn}(\text{NO}_3)_2$ during the synthesis process. The ZnO NPs were characterized using UV-VIS spectrophotometry, X-ray diffractometry (XRD), Fourier transform-infrared spectrometry (FT-IR), and Raman spectrometry. The results revealed successful synthesis of ZnO NPs, except for those produced using DI water extract, which exhibited a different structure (wurtzite). Subsequently, the antibacterial properties of the ZnO NPs were tested against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) using the disc diffusion method. The ZnO NPs synthesized from different solvent extracts demonstrated effective inhibition zones against both bacterial strains, indicating their potential as antibacterial agents.

Keywords: Antibacterial activity; green synthesis; lemon peel extract; zinc oxide nanoparticles

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Introduction

Green synthesis, also known as sustainable synthesis or eco-friendly synthesis, is a concept in chemistry and nanotechnology that refers to the development of chemical compounds, materials, or nanoparticles using environmentally friendly and sustainable methods. The goal of green synthesis is to minimize or eliminate the use of hazardous chemicals, reduce energy consumption, and generate minimal waste, thus contributing to the preservation of the environment and promoting sustainability. Green synthesis methods often utilize natural and renewable resources, such as plant extracts, microbes, or enzymes, as well as non-toxic solvents and eco-friendly reaction conditions. By adopting green synthesis approaches, researchers aim to produce products with less environmental impact, lower toxicity, and enhanced biocompatibility, making them suitable for various applications in fields like medicine, agriculture, energy, and materials science. Overall, green synthesis is a crucial area of research that aligns with the principles of green chemistry and sustainable development, aiming to create innovative solutions while minimizing harm to the environment and human health (Elumalai and Velmurugan, 2015; Saif *et al.*, 2016).

Lemon peels are a byproduct of the citrus industry, and their disposal can lead to environmental concerns. By utilizing lemon peels for green synthesis, waste is effectively converted into a valuable resource, reducing environmental burden and promoting sustainable practices. The bioactive compounds in lemon peels, such as flavonoids and polyphenols, have reducing capabilities, which means they can effectively convert metal ions into nanoparticles under mild reaction conditions. Additionally, these compounds can stabilize the formed nanoparticles, preventing agglomeration and enhancing their stability. Lemon peels are non-toxic and pose no harm to the environment or human health, making them ideal candidates for green synthesis. Unlike some conventional chemical reducing agents, the use of lemon peel extracts eliminates the need for hazardous chemicals and promotes safer practices. Lemon peels are readily available and cost-effective compared to many other chemical reducing agents. Their sustainable sourcing and ease of extraction contribute to the economic feasibility of green synthesis. Nanoparticles synthesized using lemon peel extracts are more likely to exhibit biocompatibility, which is essential for various biomedical applications. Green-synthesized nanoparticles can be utilized in drug delivery,

diagnostics, and other medical fields with reduced risks of toxicity and side effects.

The extracted lemon peel is impactful on the development of green synthesis due to its rich content of bioactive compounds, particularly flavonoids, polyphenols, and terpenoids. These compounds possess antioxidant and reducing properties, making them valuable in reducing and stabilizing agents in the synthesis of nanoparticles and other chemical compounds using environmentally friendly methods (Kawaii *et al.*, 2000; Sabry *et al.*, 2022).

Flavonoids are natural polyphenolic compounds found in plants, responsible for their vibrant colors. They possess antioxidant properties that help neutralize harmful free radicals in the body, reducing oxidative stress and protecting cells from damage. Flavonoids are widely distributed in fruits, vegetables, herbs, and beverages like tea and wine. They are associated with various potential health benefits, including anti-inflammatory, anticancer, cardioprotective, and neuroprotective effects, making them valuable for human health and wellness (Pietta, 2000; Duthie and Crozier, 2000).

This research project holds significant importance as it focuses on the study of extracting flavonoids from lemon peels using various solvents, including deionized water (DI water), isopropanol, ethanol, and methanol. The exploration of flavonoid extraction is of great interest due to their diverse pharmacological properties and their role as key biological regulators in plants. The use of lemon peels as a primary source for this extraction is intriguing and presents opportunities for laboratory research and industrial applications. The genesis of this research stems from the interest in investigating the diverse properties of flavonoids and their potential biological applications. Extracting flavonoids from lemon peels provided valuable insights into the obtained extracts, which were subsequently utilized in synthesizing zinc oxide 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. To comprehend the significance of this research, further studies may involve in-depth chemical and biological analyses of the flavonoid extracts and the synthesized zinc oxide nanoparticles. Additionally, investigations into the antibacterial capabilities of the nanoparticles could be crucial, potentially playing a pivotal role in medical and industrial applications related to disease control. Understanding the

comprehensive aspects of this study will contribute to advancing knowledge in the field and offer potential avenues for practical applications in various domains.

Materials and Method

Extraction of Compounds from Lemon Peels

The extraction of compounds from lemon peels through the process of deionized water cleaning and subsequent solvent extraction is a significant research endeavor. The primary focus is on the extraction of flavonoids, which are bioactive compounds present in the peels of citrus fruits. Lemon peels were cleaned using deionized water to remove impurities, and then they were peeled, sliced into small pieces, and subjected to heat treatment at 100°C for 12 h to reduce brittleness and enhance cleanliness.

Following the heat treatment, the lemon peel samples were ground into a fine powder using a grinder. Two grams of the finely powdered lemon peel were placed into a 250 ml pink bottle, to which 100 ml of various solvents (deionized water, ethyl alcohol, methyl alcohol, and isopropanol) were added as extractants. The mixture was then

subjected to agitation for three hours to enhance the extraction process. After agitation, the pink bottle containing the lemon peel extract was heated at 60°C for an additional hour inside a temperature-controlled bath to maintain the stability of the extract and improve extraction efficiency. Finally, the extract was filtered to remove unwanted residues as shown in Figure 1.

Synthesis of ZnO Nanoparticles Using Green Chemical Process

In this step, the appropriate ratio between the starting material, zinc nitrate ($\text{Zn}(\text{NO}_3)_2$), and the extract from lemon peels will be studied. The process involves placing 2 g of zinc nitrate in a 1,000-ml beaker, then add lemon peel extract (10, 15, 20, 25, and 30 ml) into the same beaker. The mixture is stirred with a glass rod for 60 min. Afterward, the resulting mixture of zinc nitrate and lemon peel extract is heated to 60°C for 1 h in a temperature-controlled water bath.

Once the time is completed, the mixture is then taken for drying in an oven at 100°C and subsequently calcined at 400°C for 4 h, as shown in Figure 2.

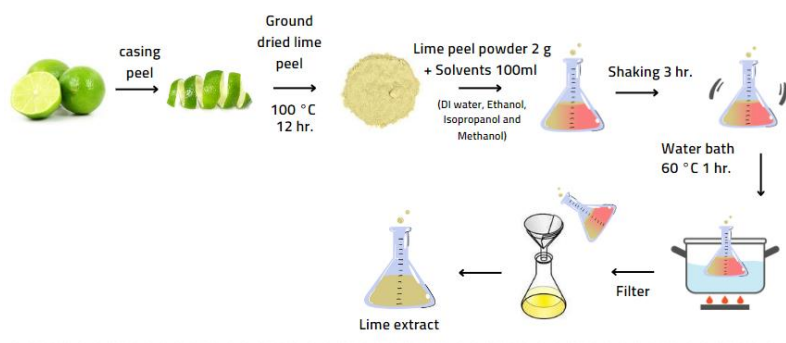


Figure 1. Diagram of extraction of compounds from lemon peels

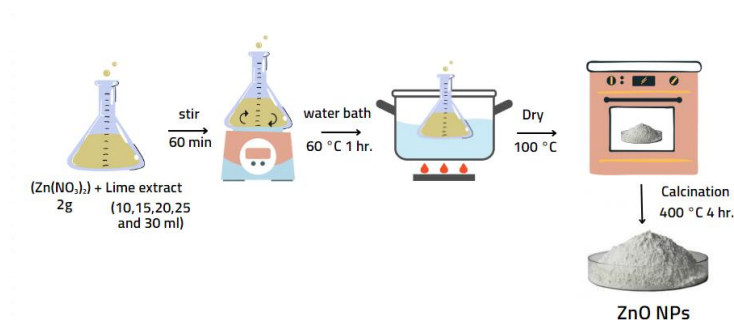


Figure 2. The synthesis of ZnO nanoparticles by green chemical process

Characterization and Measurements

The flavonoid extracted compounds were measured the absorbance from a UV-VIS spectrophotometer PG Instrument with model T92+ Spectrophotometer with mono-chromatic at the wavelength of 374 nm. To determine the phase, composition, and structural characteristics of zinc oxide nanoparticles were analyzed from X-ray diffraction (XRD) patterns obtained from scanning 2-theta from 10-80° at a fixed incident angle of 0.4° (Rigaku Smartlab) using an X-ray diffractometer with CuK α radiation ($\lambda = 0.15406$ nm). Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of the. The FT-IR spectra by Perkin Elmer UATR 2 spectrophotometers and Raman Spectroscopy were the techniques used for the identification of flavonoid extracted and possible interactions between their materials. It can provide valuable information on the synthesized ZnO nanoparticles purity and molecular structure.

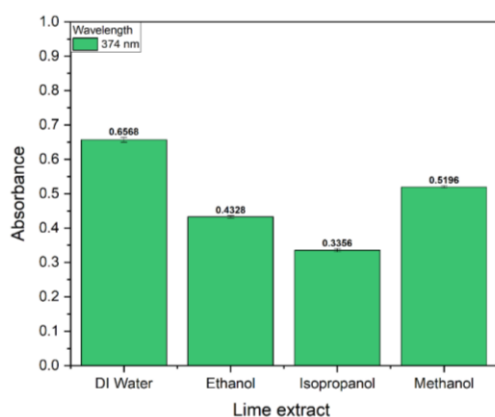


Figure 3. The absorbance of different solvents

The Process for Bacterial Inhibition

Start by swabbing the bacterial culture all over the surface of an agar plate. Spread the bacteria evenly and leave it on the plate. Place a filter paper disc with a diameter of 7 ml, which has been sterilized to kill any bacteria, on the agar plate. Apply the synthesized zinc oxide nanoparticles onto the filter paper disc by placing a drop of the nanoparticle dispersion, prepared in deionized water at a concentration of 0.5 mg/1 mL, at a designated position on the disc, approximately 30 μ l in volume. The drop of deionized water without nanoparticles should also be placed on another part of the disc as a control reference. Take the agar plate with the bacterial culture and nanoparticle-loaded filter paper disc and incubate it at the appropriate

temperature and time for both bacterial strains. After incubation, measure the diameter of the inhibition zone (clear zone where bacterial growth is inhibited) around the filter paper disc, using millimeters as the unit of measurement. Measure from one edge of the zone to the other.

This process allows for the assessment of bacterial inhibition using the synthesized zinc oxide nanoparticles. The presence and effectiveness of the nanoparticles in inhibiting bacterial growth will be indicated by the size of the inhibition zone around the nanoparticle-loaded filter paper disc.

Results and Discussion

Flavonoid Compounds from Lime Peel Extract by UV-VIS Spectrophotometer

We have synthesized zinc oxide nanoparticles using the green chemical process, where flavonoids from lemon peel extracts acted as reducing agents in the reaction. In this experiment, we measured the absorption by the mono-chromatic wavelength of the flavonoid components to determine which solvent type had the highest concentration of flavonoids. The measurements were taken at a wavelength of 374 nm (Pedro *et al.*, 2015).

Based on Figure 3, the absorption at a wavelength of 374 nm shows that the flavonoid component with the highest light absorption is found in the extract dissolved in DI water. Following that, the order of decreasing light absorption for the flavonoid components in different solvents is as follows: Methanol, Ethanol, and Isopropanol.

In this experiment, the type of flavonoid used is Flavanone, as it belongs to the group of flavonoids known for their antioxidant properties and is commonly found in plants of the Citrus genus (Ullah *et al.*, 2020). The results indicate that the extract dissolved in DI water has the highest concentration of flavonoid components based on the measured light absorption values. The chemical reaction between extracted flavanone and Zinc nitrate to become ZnO NPs is presented in Figure 4.

Figure 4. shows the mechanism for the formation of ZnO nanoparticles through flavonoid extracts and zinc nitrate. Hesperidin is a type of flavonoid compound in the peels of plants in the citrus family (Garg *et al.*, 2001). Hesperidin acts as a ligand to ambient Zinc ions (Zn^{2+}), which act as central metal ions to form zinc complexes (Zn-complex). Then calcine zinc complexes ion changed to ZnO nanoparticles (Zn-NPs) (Çolak and Karaköse, 2017; Luque *et al.*, 2018).

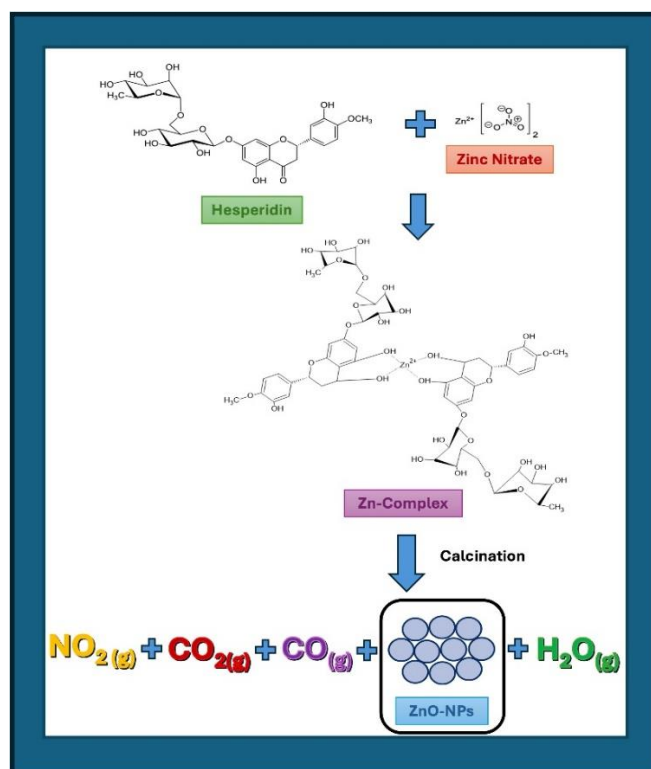


Figure 4. The chemical reaction between extracted flavanoid and Zinc nitrate to become ZnO NPs

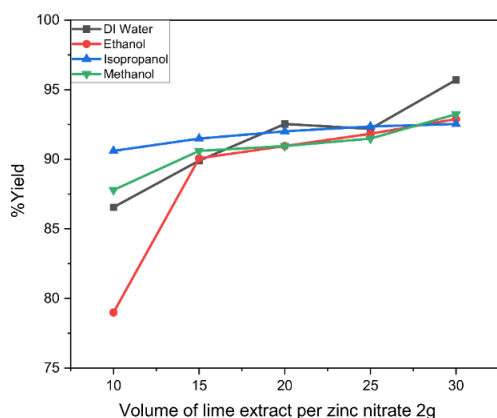


Figure 5. The relationship between the volume of lemon peel extracted per zinc nitrate and the percentage yield

Percentage Yield of Synthesized Zinc Oxide Nanoparticles

After the synthesis of zinc oxide nanoparticles, there was an analysis of the yield rates to compare the different types of solvents to find the one that resulted in the highest yield. The calculation was done using Equation 1 (Niranjan *et al.*, 2015).

$$\%Yield = \frac{\text{The actual weight of the synthesized ZnO}}{\text{Theoretical weight of ZnO}} \quad (1)$$

The graph in Figure 5, it is showing that the yield of the synthesized nanoparticles continuously increases with the increasing amount of reactants used. When considering the quantity of lemon peel extract, if a higher yield of synthesized nanoparticles is desired, it is recommended to use an amount of 30 ml.

Zinc Oxide Nanoparticles Analysis

Phase Analysis by X-ray Diffraction Technique

To determine the phase, composition, and structural characteristics of zinc oxide nanoparticles, we were subjected to X-ray diffraction (XRD). From the X-ray diffraction (XRD) analysis, the results shown in Figure 6(a-d) reveal the diffraction peaks for zinc oxide nanoparticles synthesized with different amounts of reactants. The diffraction peaks are observed at 2θ angles corresponding to the planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202).

Based on the XRD patterns, it can be deduced that the synthesized zinc oxide nanoparticles

possess a wurtzite crystal structure, which is consistent with the data available in the JCPDF file No.36-1451. The presence of these characteristic diffraction peaks confirms the formation of zinc oxide nanoparticles with a wurtzite crystal structure.

From the X-ray diffraction (XRD) pattern, the crystallite size of the synthesized zinc oxide nanoparticles can be calculated using Scherrer's equation (Hamrayev and Shameli, 2021). The crystallite size of the synthesized zinc oxide nanoparticles using different solvents was found to be smallest with DI water at 25.87 nm, followed by Ethanol at 28.74 nm, Methanol at 29.51 nm, and

isopropanol solutions have a higher boiling point than other solvents. The higher boiling points tend to have lower evaporation rates than those with lower boiling points. This is because a high boiling point indicates that the substance is more concentrated and possesses higher molecular energy. Consequently, the molecules of the substance do not easily escape from the solution, leading to a reduction in the evaporation rate. Solutions with low evaporation rates tend to form larger particles, while solutions with high evaporation rates tend to produce smaller particles. This phenomenon results from slow evaporation, allowing the substance's

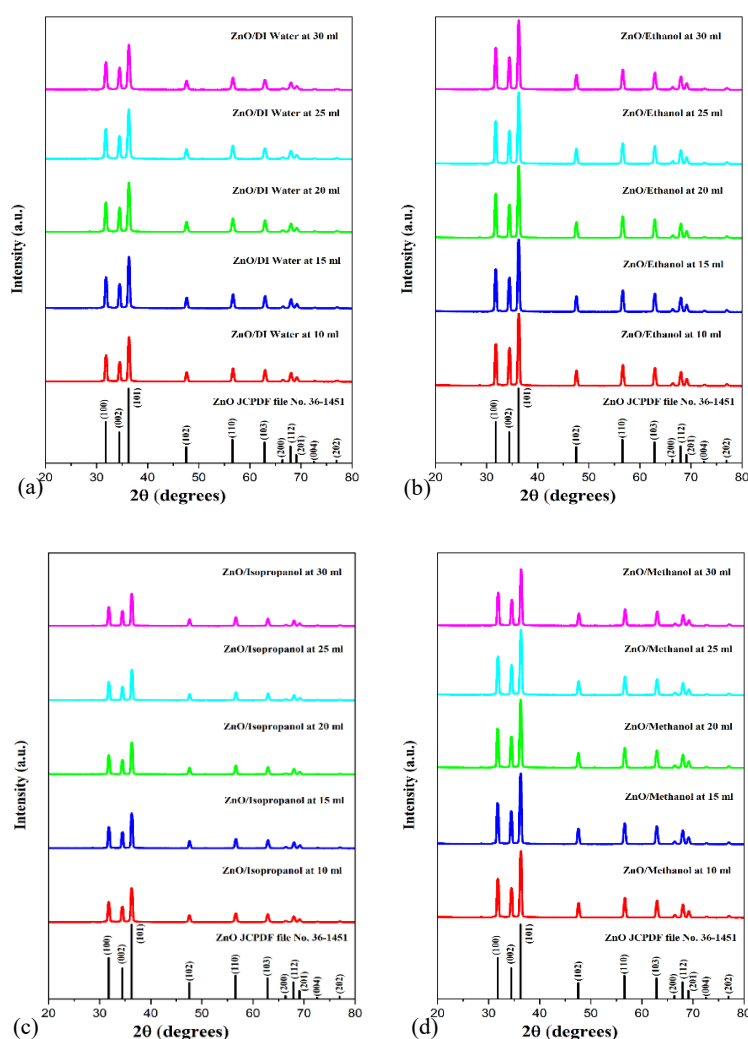


Figure 6. X-ray diffraction patterns of zinc oxide nanoparticles synthesized with various solvents: (a) DI Water (b) Ethanol (c) Isopropanol, and (d) Methanol

Isopropanol, which exhibited the largest crystallite size among the solvents, measuring 31.07 nm. These results are opposite to Scherrer's theory because

molecules more time to remain dissolved, potentially facilitating particle formation and growth.

The purity of the Zinc Oxide Nanoparticle by Fourier Transform Infrared Spectrometer (FT-IR)

The purity of the zinc oxide nanoparticles phase was studied by Fourier Transform Infrared Spectrometer (FT-IR) as shown in Figure 7. It was observed that all solvents used exhibited consistent phase purity characteristics. The graph shows the percentage of transmittance of zinc oxide, and it can be noticed that the transmittance decreases in the wavelength range of $580\text{--}400\text{ cm}^{-1}$ (Abdulkhair *et al.*, 2019). This range corresponds to the absorption region of the synthesized zinc oxide, resulting from the vibration of the Zn-O bond between zinc and oxygen atoms. Therefore, it can be inferred that the observed phases are pure phases of zinc oxide.

The Purity of Zinc Oxide Nanoparticles by a Raman Spectrometer

The technique used to verify the vibrations of bonds in the molecules of compounds is known as Raman spectroscopy. This technique was employed by Silambarasan *et al.* (2015) in their research to examine the vibrations of bonds in ZnO with the Raman spectroscopy technique. The aim was to confirm the purity of the ZnO nanoparticles, as depicted in Figure 8. It has been observed that the vibrational patterns of bonds in ZnO nanoparticles correspond to the vibrational patterns of bonds in ZnO compounds, except when synthesized using DI water as a solvent. These particles showed vibrations in the wavelength range of $1,030\text{--}1,080\text{ cm}^{-1}$, possibly due to incomplete decomposition of organic materials during the calcination process.

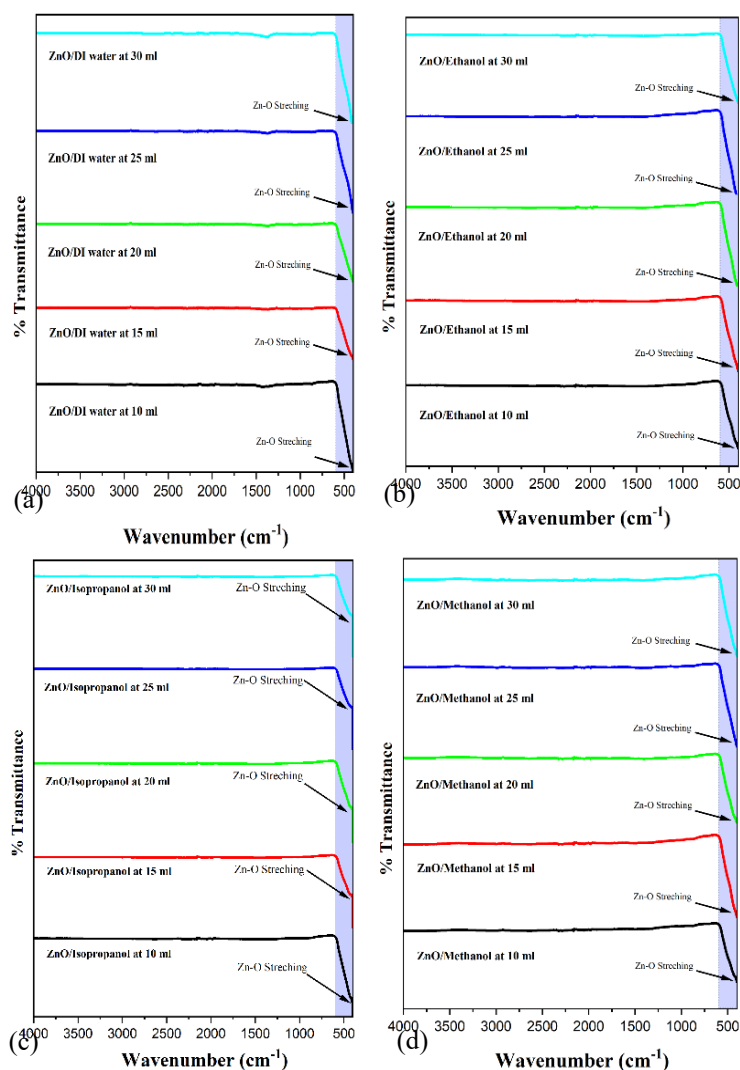
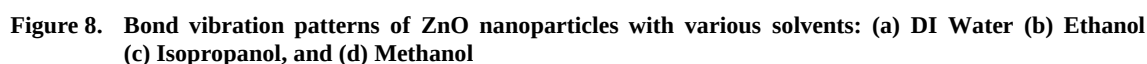


Figure 7. Purity of zinc oxide nanoparticles synthesized with various solvents. (a) DI Water (b) Ethanol (c) Isopropanol, and (d) Methanol



The Biological Properties of Zinc Oxide Nanoparticles in Antibacterial Activity

Figure 1 consists of two panels, (a) and (b), showing the inhibition of bacterial growth by ZnO nanoparticles. Panel (a) is an agar diffusion assay showing four wells containing ZnO/DI Water, ZnO/Methanol, ZnO/Ethanol, and ZnO/Isopropanol. Each well shows a clear zone of inhibition. Panel (b) is a microdilution assay showing the minimum inhibitory concentration (MIC) for the same ZnO formulations. The MIC is determined by the first well showing no visible growth.

Figure 9. The inhibition of *E. coli* and *S. aureus* bacteria by ZnO nanoparticles synthesized using different solvents. (a) *E. coli* (b) *S. aureus*

From the study of the antibacterial properties of zinc oxide nanoparticles, it was found that nanoscale zinc oxide particles synthesized using various solvents could effectively inhibit bacterial growth. Among them, zinc oxide nanoparticles showed better inhibition against *E. coli* compared to *S. aureus*, as seen in Figure 9.

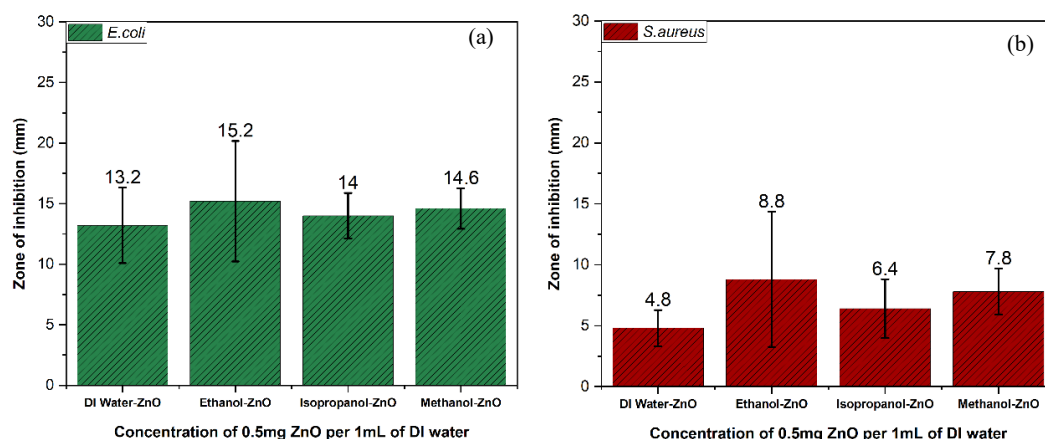


Figure 10. Size of the clear zone formed by ZnO nanoparticles synthesized with different solvents (a) *E.coli* and (b) *S.aureus*

Furthermore, Figure 10 demonstrated that nanoscale zinc oxide particles synthesized using ethanol as a solvent exhibited the highest inhibition of bacterial growth, followed by methanol, isopropanol, and DI water, respectively. This conclusion was based on the size of the clear zone observed, as shown in Figure 9.

Fluorescence spectra of ZnO nanoparticles

Figure 11 shows the fluorescence spectra of ZnO nanoparticles using different solvents with an emission wavelength of 606 nm which is a green light. The green emission results from zinc interstitials and oxygen vacancy (Yang *et al.*, 2006). An increased presence of oxygen vacancies can lead to the formation of defects in the material, affecting its optical properties (Radjehi *et al.*, 2019). In photocatalysis phenomenon plays a crucial role in influencing the mechanism of bacterial resistance. Specifically, it enhances the generation of ROS (Reactive Oxygen Species), pivotal in causing damage to cell walls. It exists both inside and on the surface of the particle. The intensity of the fluorescence depends on the surface defects. Consequently, changing the solvent may result in different particle sizes and oxygen vacancies. Therefore, a larger particle size results in a lower fluorescent intensity. Figure 11(b) shows ethanol solvent exhibits the highest intensity of released fluorescent. It indicated that within the ZnO structure employing ethanol solvent, oxygen vacancies are created. This observation aligns with the findings depicted in FE-SEM Figure 12(b, d). This indicates that the particle size of ethanol is smaller than other solvents.

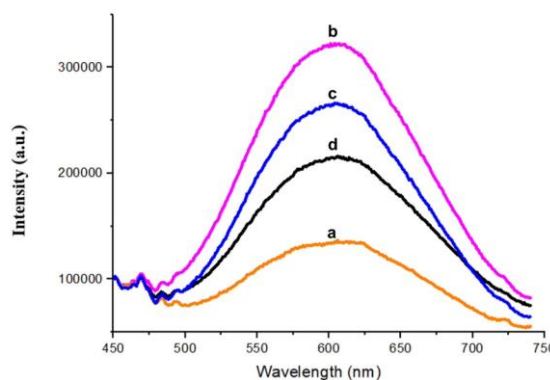


Figure 11. Fluorescence spectra of ZnO nanoparticles at different solvents: (a) DI Water (b) Ethanol (c) Isopropanol, and (d) Methanol

The Morphology of ZnO Nanoparticles

Figure 12 presents the FE-SEM picture of synthesized ZnO nanoparticles by utilizing lemon peel extract. The SEM image of synthesized nanoparticles indicates that the particles are uniformly distributed and smooth. FE-SEM analysis confirms the synthesised of ZnO NPs. For used distilled water (b) can be observed shape nanorod-like are clustered nanocrystallines (Luque *et al.*, 2018). While (d) and (g) have similar shapes but image (g) has a larger particle size than image (d). It can be hexagonal prisms with rounded down corners and spherical (Saikia *et al.*, 2015; Berehu *et al.*, 2021), from (i) It has a small spherical shape that sticks to smooth surfaces (Rakhra *et al.*, 2020) by being decorated under different solvents. We determine the average size of the synthesized nanoparticles from 20 nanoparticles from FE-SEM

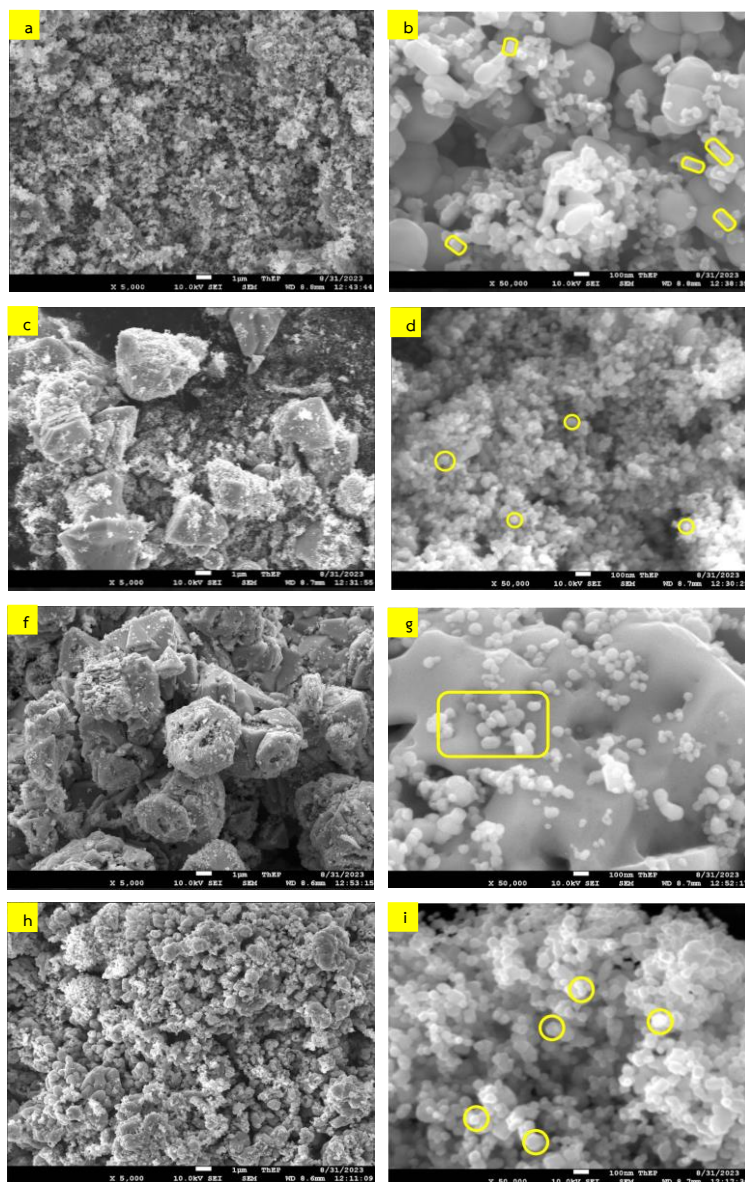


Figure 12. FE-SEM images of ZnO synthesized with various solvents at magnified 5,000 (right) 50,000 (left): (a, b) DI Water (c, d) Ethanol (f, g) Isopropanol, and (h, i) Methanol

images. From DI Water, ethanol, isopropanol, and methanol, the average particle size is 75.6, 29.2, 54.06, and 68.5 nm, respectively.

Conclusions

This research investigated the synthesis of zinc oxide nanoparticles using a green chemistry approach from lemon peel extracts with different solvents. The highest amount of flavonoids was found in the extract obtained with DI water. The extracted flavonoids were applied in the synthesis of zinc oxide nanoparticles and the yields

obtained from the synthesis of zinc oxide nanoparticles were consistent for all solvent types. The resulting zinc oxide nanoparticles exhibited a wurtzite crystal structure and the smallest crystallite size was observed for the DI water extract which measured approximately 25.871 nm. Regarding the antibacterial properties of the zinc oxide nanoparticles, those synthesized with the ethanol extract effectively inhibited both *E. coli* and *S. aureus*, representing gram-negative and gram-positive bacteria, respectively. For SEM images show the shape of ZnO nanoparticles that depend on the type of solvent. The fluorescence spectra of ZnO nanoparticles were observed using various

solvents, with an emission wavelength of 606 nm corresponding to green light. The green emission is attributed to zinc interstitials and oxygen vacancy.

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