



Green Synthesis of ZnO–TiO₂ Nanoparticles Using *Allium ampeloprasum* (Kurrat) Extract and Their Antibacterial Activity

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Abstract: The potential of nanotechnology in medical applications, particularly for infection treatment, is increasingly recognized. This study synthesized zinc oxide nanoparticles (ZnO NPs) using a green method, using *Allium ampeloprasum* (kurrat) plant extract as a reducing agent. These ZnO NPs were subsequently used to fabricate ZnO–TiO₂ core–shell nanoparticles. The antibacterial activity of the synthesized nanoparticles was evaluated using the agar well diffusion method. Results revealed notable antibacterial effects: *Staphylococcus aureus* (Gram-positive) exhibited a 25 mm inhibition zone, and *Pseudomonas aeruginosa* (Gram-negative) showed a 20 mm inhibition zone at 100 µg/mL concentration. The nanoparticles were characterized using FTIR, UV–Vis spectroscopy, EDX, and AFM. FTIR spectra confirmed core–shell formation at 813.90–613.32 cm^{−1}. UV–Vis analysis indicated absorption peaks at 260 nm for ZnO NPs and 300 nm for ZnO–TiO₂ NPs. EDX analysis confirmed the presence of zinc, titanium, and oxygen with no detectable impurities. AFM revealed an average diameter of 46.74 nm for ZnO NPs and 148.9 nm for ZnO–TiO₂ core–shell NPs. These green-synthesized nanoparticles exhibit promising antibacterial properties, supporting their potential in biomedical applications.

Keywords: Green synthesis; ZnO–TiO₂ (core-shell); ZnO nanoparticles; TiO₂ nanoparticles; Antimicrobial activity

1. Introduction

The management of pathogenic microorganisms remains a critical challenge in modern life. While many microbes coexist harmlessly with humans, the uncontrolled growth of certain pathogenic strains can lead to serious health threats [1]. As antibiotic resistance continues to rise, there is growing interest in nanotechnology as a novel strategy to combat microbial infections [2–4]. Unlike conventional antibiotics, nanoparticles (NPs) interact with multiple bacterial components, making it difficult for pathogens to develop resistance [5]. Nanoparticles possess a high surface-area-to-volume ratio and exhibit unique physicochemical properties compared to their bulk counterparts, enabling them to interact with biological systems [6–8] effectively. Among various nanomaterials, zinc oxide (ZnO) and titanium dioxide (TiO₂) have been extensively studied for their antibacterial activity and biocompatibility [9–11]. These nanoparticles operate at the atomic or molecular scale (1–100 nm), and their reduced size enhances properties such as surface reactivity, optical absorption, and bioavailability [12].

Recent advances in green synthesis offer eco-friendly alternatives to conventional chemical and physical nanoparticle fabrication methods. Plant extracts, rich in natural reducing and stabilizing agents such as flavonoids, phenols, and sulfur-containing compounds, can replace toxic solvents and high-energy processes traditionally used in nanoparticle synthesis [13–15]. This sustainable approach minimizes environmental impact and may enhance the resulting nanoparticles' biological activity. Developing core–shell nanostructures, such as ZnO–TiO₂, has emerged as a further strategy to enhance nanoparticle stability, functionality, and safety. The core–shell architecture can protect the inner core from oxidation or degradation, reduce toxicity, and improve properties such as antibacterial activity, drug delivery efficiency, and catalytic performance [16–18]. Using *Allium ampeloprasum* (commonly known as kurrat, wild leek, or great-headed garlic) for this green synthesis is particularly advantageous due to its rich content of phytochemicals, including flavonoids, sulfur compounds, and antioxidants [19–20]. *Allium ampeloprasum* serves as a natural reducing agent and imparts bioactivity to the nanoparticles, potentially enhancing their antibacterial, antioxidant, and anticancer properties. Additionally, this plant-based synthesis eliminates the need for hazardous chemicals, making it a promising candidate for biomedical applications.

In this study, we first synthesized ZnO nanoparticles using *Allium ampeloprasum* extract, then fabricating ZnO–TiO₂ core–shell nanoparticles by depositing a TiO₂ layer. The synthesized nanoparticles were then evaluated for their antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria using agar well diffusion. This work explores the potential of green-synthesized ZnO–TiO₂ nanoparticles in antimicrobial applications.

2. Materials and Methods

This experimental study was conducted in two stages. In the first stage, zinc oxide (ZnO) nanoparticles were synthesized. In the second stage, ZnO–TiO₂ core–shell nanoparticles were prepared by coating ZnO with titanium dioxide (TiO₂). The antibacterial activity of the synthesized nanoparticles was then evaluated.

2.1 Materials and chemicals

Zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O) was obtained from Sigma-Aldrich (Germany), and titanium tetrachloride (TiCl₄) was purchased from ACROS Organics (France). Other materials used include Mueller-Hinton Agar (MHA) from HI-Media (India) and deionized and distilled water supplied by local Iraqi manufacturers.

2.2 Bacterial Strains and Culture Conditions

Two clinical bacterial strains were used *Staphylococcus aureus* (Gram-positive, standard strain) and *Pseudomonas aeruginosa* (Gram-negative), obtained from the Department of Biotechnology, College of Science, University of Baghdad. Each strain was cultured on Mueller-Hinton Agar using a sterile bacterial spreader. Plates were incubated at 37°C for 24 hours to assess bacterial growth and activity. Confluent bacterial lawns were observed after incubation.

2.3 Preparing *Allium ampeloprasum* (kurrat) extract plant

Fresh leaves of *Allium ampeloprasum* (kurrat) were collected from farms in Iraq. The leaves were washed thoroughly with double-distilled water and blended using an electric blender until a juice-like consistency was achieved. The resulting mixture was filtered through Whatman filter paper (0.2 mm pore size) to remove plant residues. The filtrate was then centrifuged at 8000 rpm for 30 minutes, and the supernatant was filtered again to obtain a clear extract. The extract was stored at 4°C and used for nanoparticle synthesis [21]. The procedure must be done many times to gather sufficient nanoparticles. This exploratory research began on January 3, 2024, and ended on January 6, 2024. (100) g were used, a liter of water (tap water) was added and mixed in the electric blender and continue mixing until become like juice, Afterward, filtered via a piece of cloth to get rid of plant residues, then filtered it another time with filter papers with diameter (0.2) mm, and filtered again until we have clear solution without of any impurities, subsequently transferred the solution into tubes holding ten milliliters and centrifuged it for thirty minutes at a speed of at speed 60 cycle/ second.

Then, it was filtered with filter papers to have a clear solution, and it was saved for use in the manufacture of nanoparticles [22].

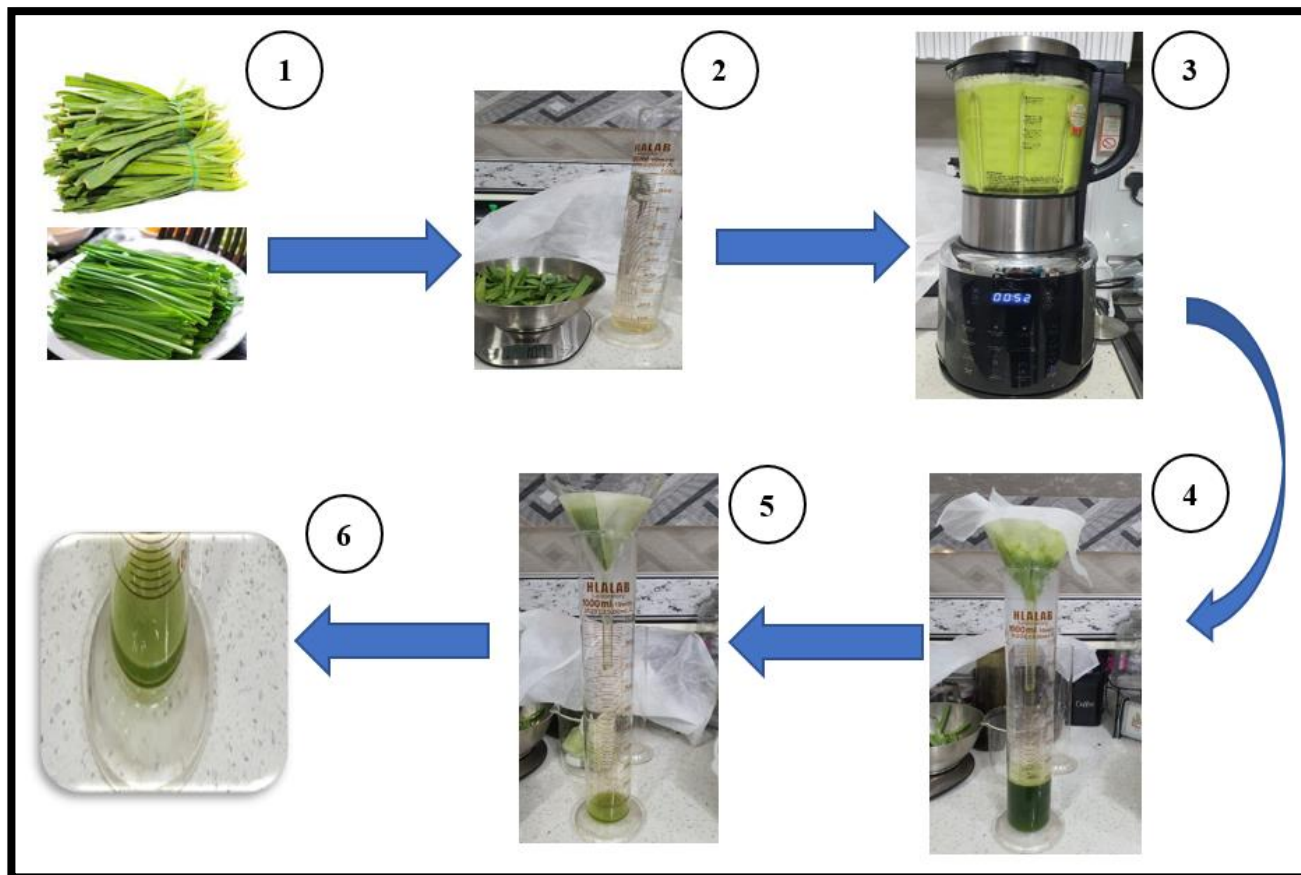


Figure 1. Kurrat leaves collection, 2. Kurrat leaves washing (100gm) with 1000 ml Distilled water, 3. Mixing in a blender, 4. Kurrat extract papers, 5. Filtration with a piece of paper, 6. Filtration with a piece of cloth.

2.4 Green Synthesis of ZnO Nanoparticles Using *Allium ampeloprasum* Extract

To synthesize zinc oxide (ZnO) nanoparticles, 200 mL of the prepared *Allium ampeloprasum* (kurrat) extract was mixed with 20 g of zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) in a flask. The mixture was stirred continuously on a magnetic stirrer at room temperature for 24 hours in the dark to prevent photochemical reactions and ensure complete dissolution. After incubation, the solution was centrifuged at 60 cycles/second for 30 minutes to collect the nanoparticle precipitate. The resulting pellet was washed twice with 5 mL of deionized distilled water to remove residual impurities. The cleaned precipitate was then transferred to sterile Petri dishes and incubated at 37 °C for 2–3 days to allow complete drying. Once dried, the solid product was gently scraped off using a spatula and stored in a dark, moisture-free incubator until further use [22].

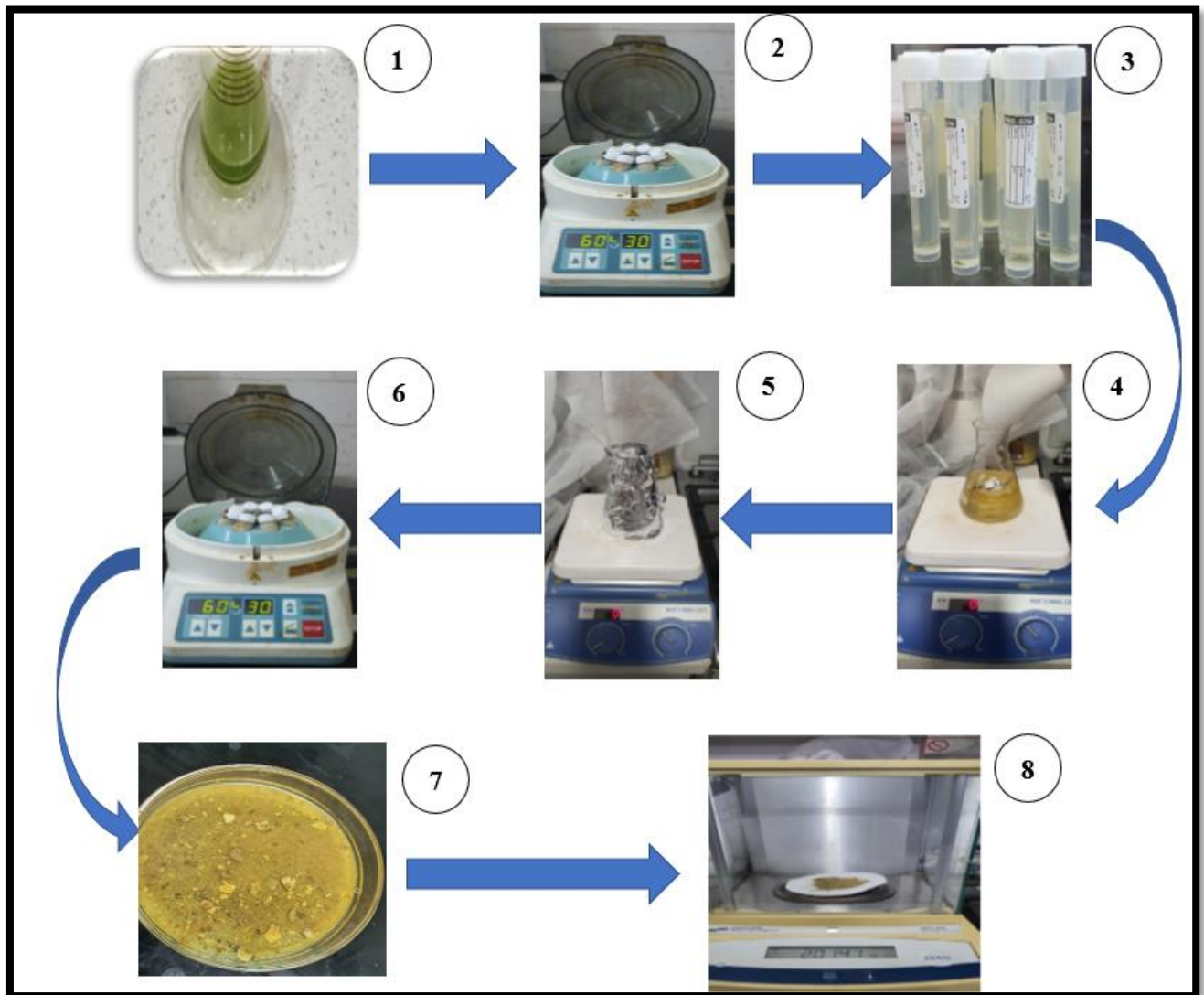


Figure 2. Kurrat extract papers, 2. A centrifuge is used to remove plant extract after centrifugation, as well as residue papers. 3. Extract after centrifugation papers, 4. Add zinc acetate salts, 5. stirring for 24 hours, 6. use the centrifuge twice, 7. Green Synthesis of ZnO Nanoparticles, 8. Net weight of ZnO Nanoparticles.

2.5 Green Synthesis of ZnO–TiO₂ Core–Shell Nanoparticles Using *Allium ampeloprasum* Extract

In the second stage, 150 mL of *Allium ampeloprasum* (kurrat) extract was mixed with 4.5 g of previously synthesized ZnO nanoparticles. Under continuous stirring, 3 mL of titanium tetrachloride (TiCl₄) was gradually added to the mixture, as illustrated in Figure 3. The resulting solution was kept under magnetic stirring at room temperature in the dark for 24 hours to ensure proper reaction and shell formation. The mixture was then centrifuged at 60 cycles/second for 30 minutes to collect the precipitate. The precipitate containing ZnO–TiO₂ core–shell nanoparticles was washed twice with deionized distilled water to eliminate residual plant material or unreacted components. The purified nanoparticles were dried overnight in a hot-air oven at 40 °C, then carefully collected and stored in a light-protected, airtight container to avoid degradation or moisture exposure [23].

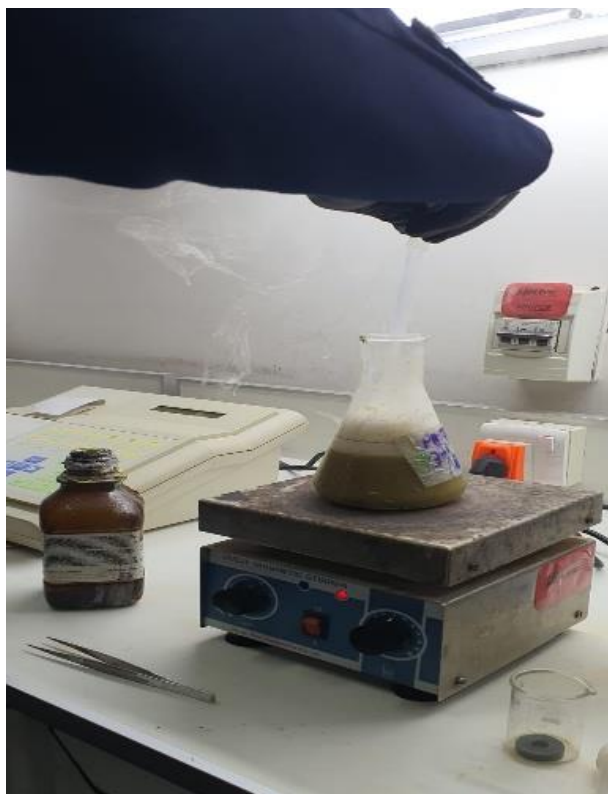


Figure 3. Synthesis of ZnO–TiO₂ Core–Shell Nanoparticles

2.6 Evaluation of Antibacterial Activity of ZnO–TiO₂ Core–Shell Nanoparticles

The agar well diffusion method assessed the antibacterial activity of green-synthesized ZnO–TiO₂ core–shell nanoparticles. The assay targeted two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Bacterial suspensions were uniformly spread on **Mueller-Hinton Agar (MHA)** plates using a sterile cotton swab. Wells (6 mm diameter) were then created in the agar, and **various concentrations of ZnO–TiO₂ nanoparticles (25, 50, 75, and 100 µg/mL)** were introduced into the wells. The plates were incubated at **37 °C for 24 hours**, after which **inhibition zones** were measured around each well to determine antibacterial effectiveness [23].

2.7 Statistical Analysis of Inhibition Zones

For precision, the diameters of the inhibition zones were measured using Vernier calipers. All measurements were performed in **triplicate** to ensure reproducibility and accuracy. Data were interpreted following standardized protocols like those provided by the **EUCAST (European Committee on Antimicrobial Susceptibility Testing)** guidelines.

3. Results and Discussion

3.1 Characterization of ZnO–TiO₂ Core–Shell Nanoparticles

3.1.1 UV–Visible Spectroscopy (UV–Vis)

The UV–Vis spectrum of *Allium ampeloprasum* (kurrat) extract reveals the presence of various phytochemicals, particularly **polyphenols, flavonoids**, and other organic chromophores. As shown in **Figure 4**, prominent absorption peaks are observed in the **200–300 nm** region, which is typical of *Allium* species and confirms the presence of bioactive compounds that likely act as **natural reducing and stabilizing agents** in nanoparticle synthesis.

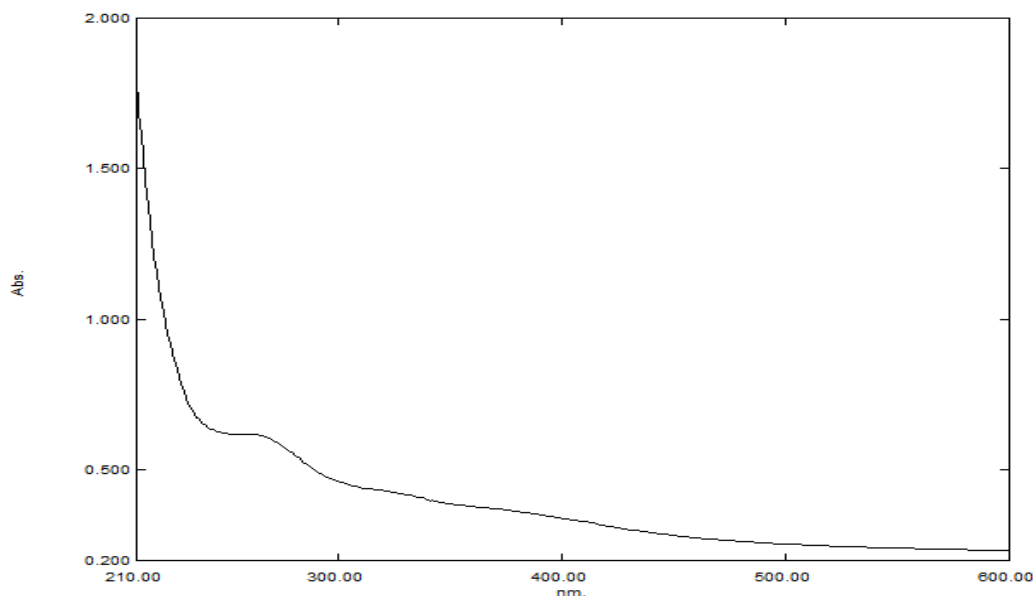


Figure 4. UV–Visible absorption spectrum of *Allium ampeloprasum* (kurrat) plant extract.

The synthesized ZnO nanoparticles' UV–Vis absorption spectrum (Figure 5) exhibits a characteristic peak in the **260–300 nm** range. This absorption band corresponds to the **intrinsic band-gap energy** of ZnO and serves as evidence of nanoparticle formation. The shift from the plant extract's profile confirms the successful synthesis of ZnO nanostructures.

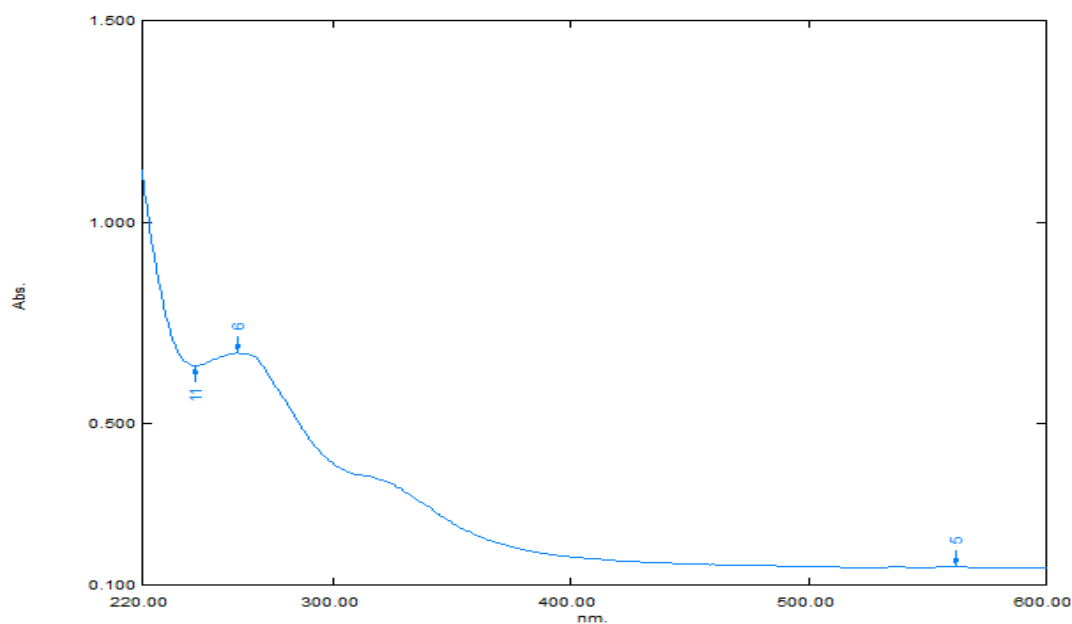


Figure 5. UV–visible absorption spectrum of green-synthesized ZnO nanoparticles.

Further, the **ZnO–TiO₂ core–shell nanoparticles** showed an absorption peak around **300 nm**, as illustrated in **Figure 6**. This slight shift in peak position compared to pure ZnO suggests the formation of the **TiO₂ outer shell**, which modifies the optical properties of the nanoparticles. These findings are consistent with previous literature reports on ZnO–TiO₂ hybrid structures [24].

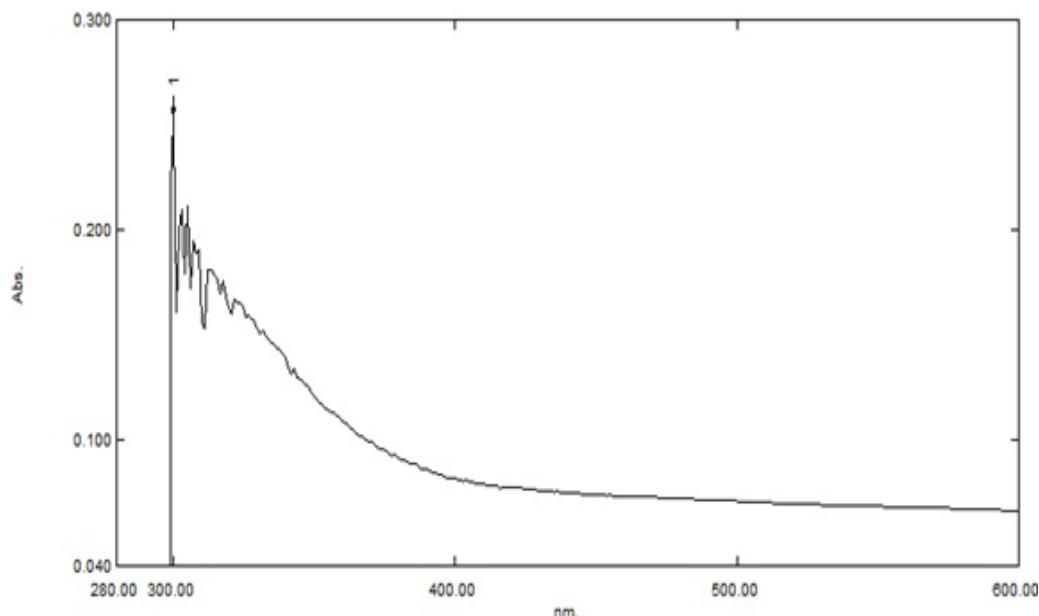


Figure 6. UV-visible absorption spectrum of ZnO-TiO₂ core-shell nanoparticles.

3.1.2 Fourier transform infrared (FTIR) ZnO-TiO₂ (core-shell) NPs

Functional groups, aliphatic primary amine at frequency 3423.41 cm⁻¹ and primary alcohol at frequency 1064.63-1049.20 cm⁻¹ in an extract of the plant indicate the groups found in the plant [25] as shown in Figure 7. The FT-IR spectrum of ZnO NPs (Figure 8) shows the wavenumbers at 3375, 2349, 1375, 915, and 481 cm⁻¹, respectively. This was detected at 617.18-559.32 cm⁻¹ frequency, which pointed to the zinc oxide nanoparticles' fingerprint location [26]. The metal oxide of the ZnO-TiO₂ functional group could be detected around 813.90-613.32 cm⁻¹ in Table 1, which indicates the formation of the core-shell structure, as shown in Figure 9.

Table 1. FTIR of the extract and nanoparticle

Samples	Frequency of Absorption (cm ⁻¹)	Bonds	Compound class of Functional Groups
Plant Extract	3423.41	N-H stretching	of aliphatic primary amine
	1645.17	C=C stretching	monosubstituted
	1384.79	C-H bending	gem dimethyl
ZnO NPs	1064.63-1049.20	C-O stretching	Primary Alcohol
	3421.48-3404.13	N-H stretch	Primary Amine
	1645.17-1566.09	C=C stretching	cyclic alkene
	1448.44-1402.15	C-H	Alkane
	1043.42	CO-O-CO stretching	anhydride
	617.18-559.32	Metal oxide	ZnO
ZnO-TiO ₂ (Core-shell)	3440.77-3296.12	N-H stretch	aliphatic primary amine
	1670.24-1645.17	C=C stretching	alkene
	1539.09-1517.87	N-O stretching	nitro compound
	1039.56	O-H bending	phenol
	813.90-613.32	Metal oxide	ZnO – TiO ₂



Figure 7. FTIR spectrum of *Allium ampeloprasum* (kurrat) plant extract.

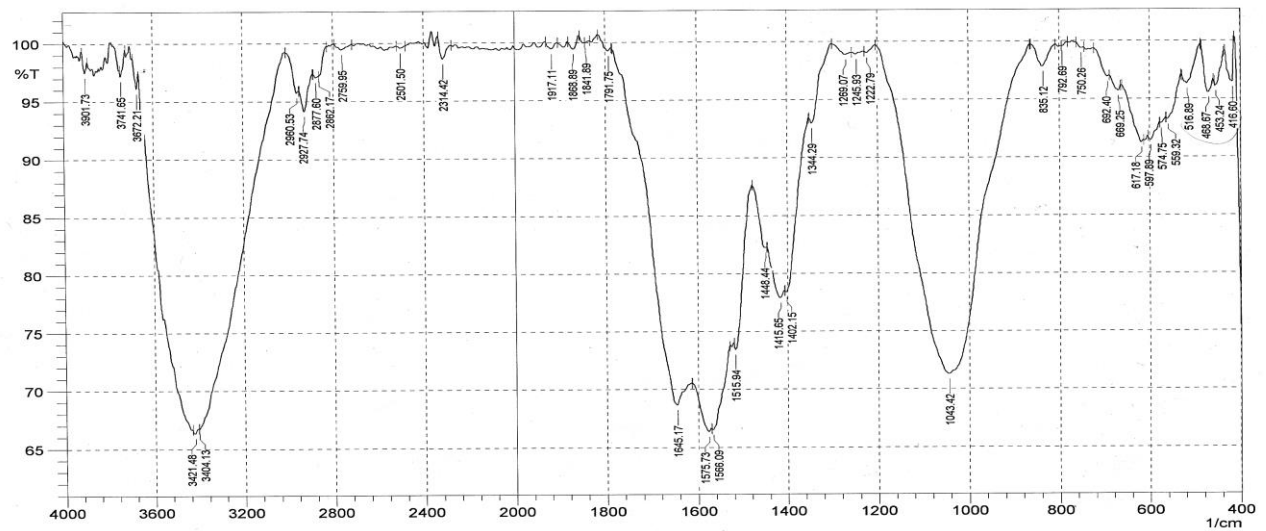


Figure 8. FTIR spectrum of green-synthesized ZnO nanoparticles.

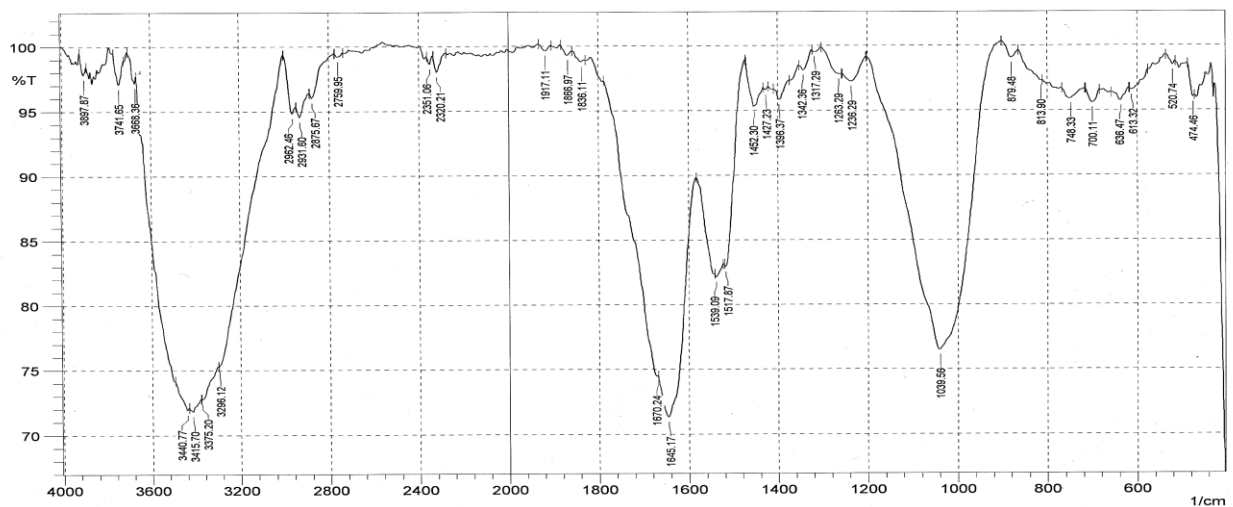


Figure 9. FTIR spectrum of ZnO-TiO₂ core-shell nanoparticles.

3.1.3 Energy-dispersive X-ray (EDX) analysis

EDX analysis was performed to determine the elemental composition of the synthesized nanoparticles. The EDX spectrum of ZnO nanoparticles (Figure 10 and Table 2) confirms the presence of zinc (Zn) as the dominant element, with a minor contribution from oxygen (O). The absence of other elements indicates the high purity of the green-synthesized ZnO nanoparticles [27].

Table 2. EDX elemental composition of ZnO nanoparticles.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
Zn	96.9	1.0	88.6	0.9
O	3.1	0.3	11.4	1.2

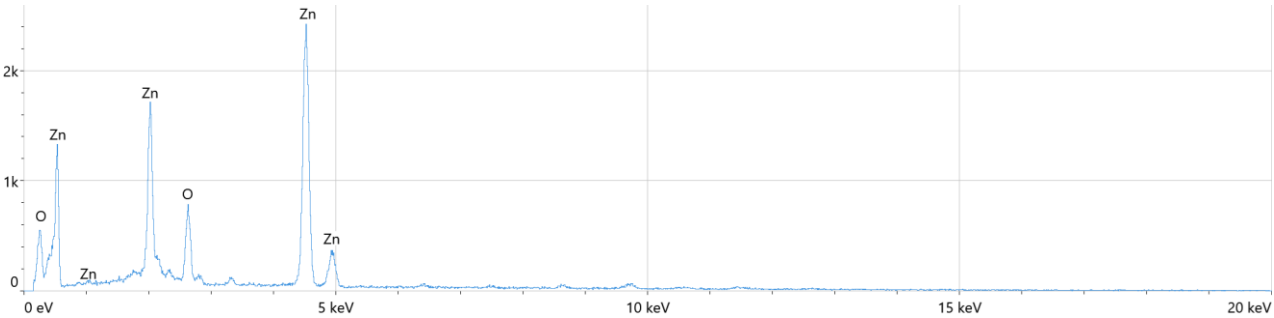


Figure 10. EDX spectrum of green-synthesized ZnO nanoparticles.

The EDX spectrum of ZnO–TiO₂ core–shell nanoparticles (Figure 11 and Table 3) reveals the presence of oxygen (O), titanium (Ti), and zinc (Zn) as the primary elements. This confirms the **successful coating of ZnO with TiO₂**, and the absence of any foreign elements suggests **no significant contamination** during synthesis [24].

Table 3. EDX elemental composition of ZnO–TiO₂ core–shell nanoparticles.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	90.5	1.0	74.7	0.8
Ti	7.5	0.1	18.7	0.2
Zn	2.0	0.1	6.7	0.4

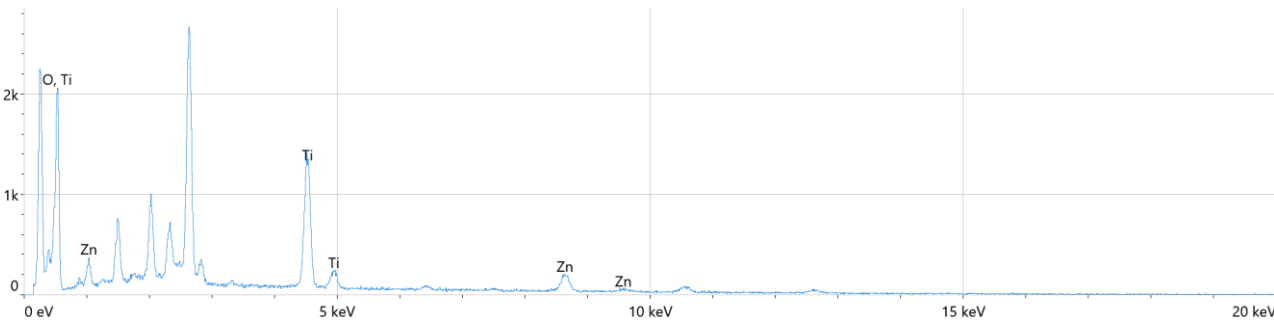


Figure 11. EDX spectrum of ZnO–TiO₂ core–shell nanoparticles.

3.1.4 Atomic Force Microscopy (AFM) Analysis

AFM was used to examine the surface morphology and grain size of ZnO and ZnO–TiO₂ core–shell nanoparticles. The AFM images (Figures 12 and 13) show the 2D and 3D surface topographies. The average grain size of ZnO nanoparticles was 46.76 nm, while the ZnO–TiO₂ core–shell nanoparticles exhibited an increased average size of 76.32 nm, consistent with shell layer formation. Furthermore, surface roughness (Ra) and root mean square (RMS) values indicate slightly increased surface irregularity for the core–shell structure compared to pure ZnO nanoparticles.

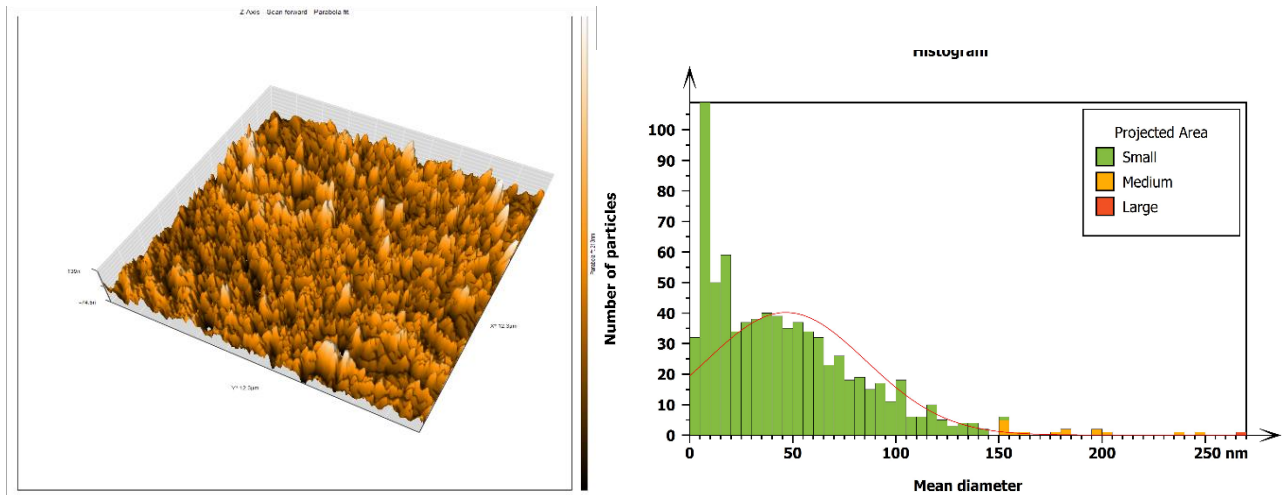


Figure 12. AFM image of ZnO nanoparticles (2D and 3D views).

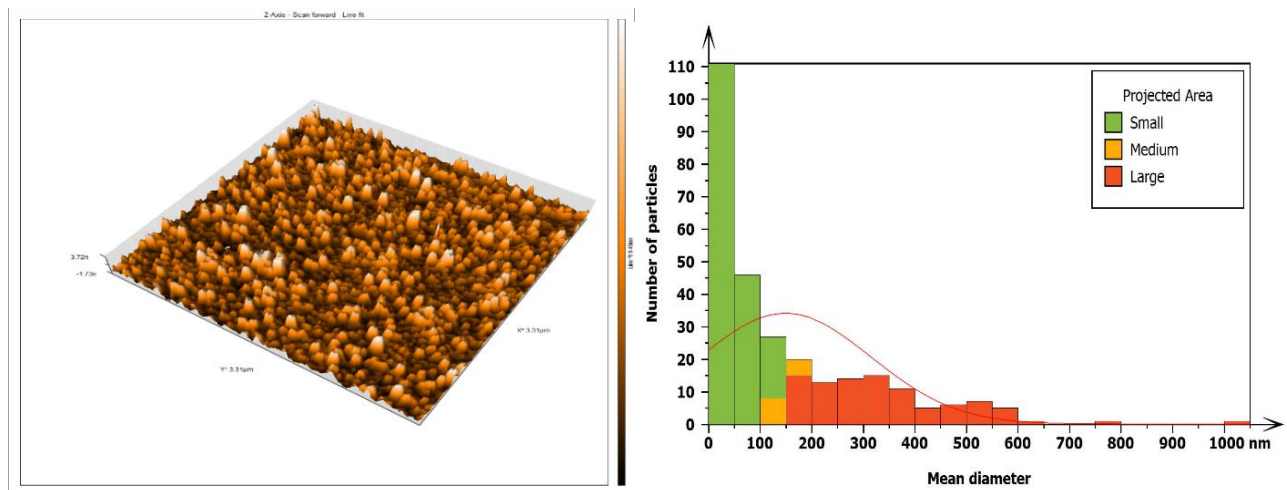


Figure 13. AFM image of ZnO-TiO₂ core-shell nanoparticles (2D and 3D views).

Table 4. AFM analysis: Average grain size, roughness, and RMS values..

Sample	Average grain size (nm)	Roughness (nm)	(RMS) (nm)
ZnO	46.76	0.84	50.79
ZnO-TiO ₂	76.32	0.92	56.99

Based on the results, it appears that the laser energy has a direct impact on the average particle size. As the laser energy increases, the nanoparticle size increases as well. As the laser energy increases, it provides higher thermal and kinetic energy to the nanoparticles. This high energy increases the speed at which the ablated nanoparticles hit the quartz substrate, causing nanoparticles to obtain high surface mobility at the surface. Large clusters are formed to reduce the system's high surface energy. Hence, aggregations occur with an overall increase in the nanoparticle's grain size.

The etched surface consists of pores distributed over the whole surface. These pores lead to increased surface roughness, which is attributed to the etching parameters affecting the surface characterization.

3.2 Antibacterial Evaluation and Discussion:

Table 5 shows the outcomes of ZnO-TiO₂ nanoparticles' antibacterial activity against *Pseudomonas aeruginosa* at various doses. Demonstrate that the diameter of the growth inhibition zone has shrunk as ZnO-

TiO₂ nanoparticle concentration has reduced and that the antibacterial efficacy of ZnO-TiO₂ nanoparticles has significantly diminished at a concentration of 25 µg/ml, as shown in Figure 14.

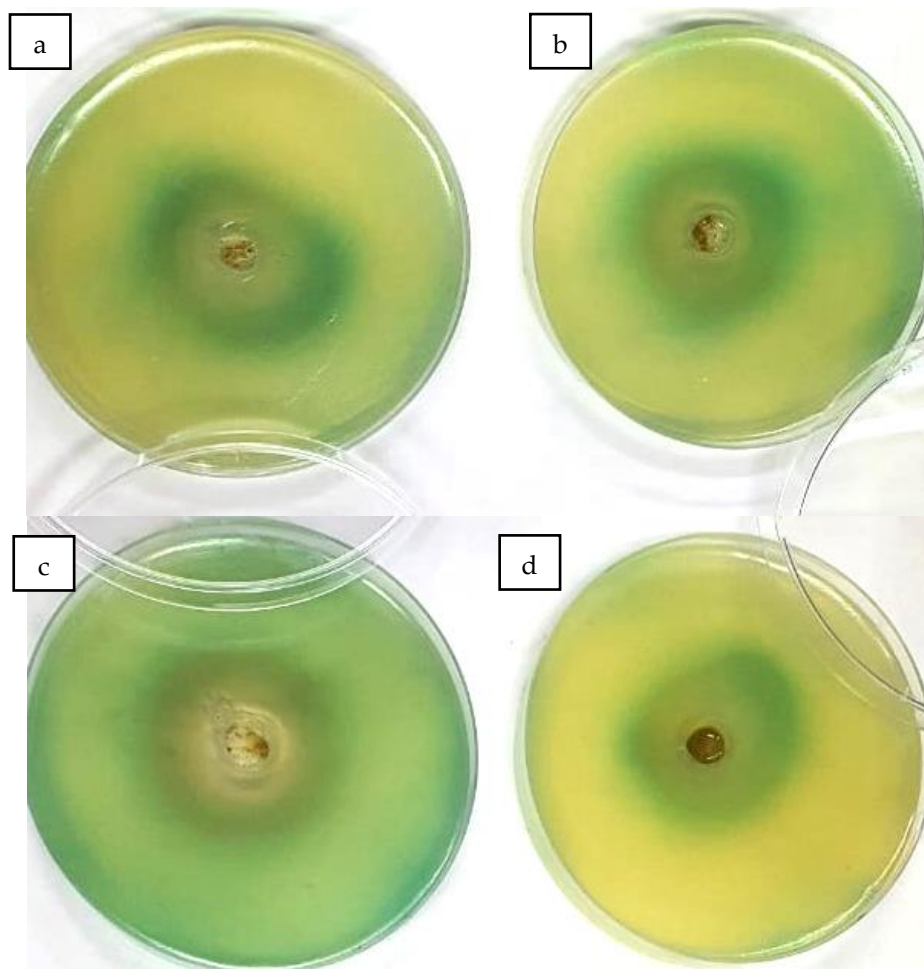


Figure 14. Inhibition zone of *Pseudomonas aeruginosa* bacteria at a) 25 (µg/ml), b) 50 (µg/ml), c) 75 (µg/ml), and d) 100 (µg/ml) concentrations.

Table 5. Zones where ZnO-TiO₂ nanoparticles inhibit *Pseudomonas aeruginosa*.

ZnO-TiO ₂ NPs concentration (µg/ml)	Diameter of the inhibitory zone (mm)
25	8
50	12
75	18
100	20

Whereas the *Staphylococcus aureus* showed more antibacterial activity against ZnO-TiO₂ with increasing the concentration of NPs, as shown in Figure 15, reaching (25 mm) at 100 µg/ml concentration, are listed in Table 6.

Table 6. Zones where ZnO-TiO₂ nanoparticles inhibit *Staphylococcus aureus*.

ZnO-TiO ₂ NPs concentration (µg/ml)	Diameter of the inhibitory zone (mm)
25	10
50	15
75	20
100	25

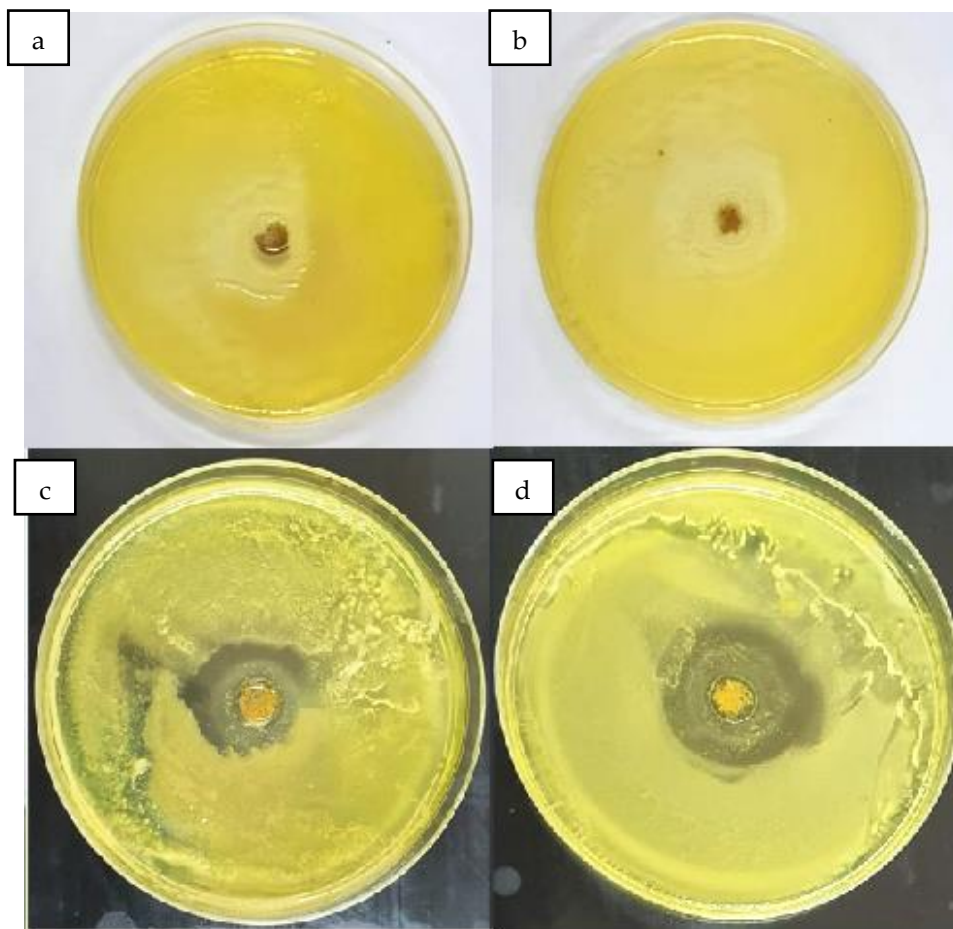


Figure 15. Inhibition zone of *Staphylococcus aureus* bacteria at a) 25 ($\mu\text{g/ml}$), b) 50 ($\mu\text{g/ml}$), c) 75 ($\mu\text{g/ml}$), and d) 100 ($\mu\text{g/ml}$) concentrations.

Effect of Laser Energy on Nanoparticle Morphology

The results indicate a clear relationship between laser energy and nanoparticle size. As laser energy increases, it imparts greater thermal and kinetic energy to the particles, enhancing their surface mobility upon impact with the quartz substrate. This increased mobility leads to the formation of larger clusters, as nanoparticles aggregate to minimize the system's high surface energy. Consequently, a direct increase in average particle size is observed with higher laser energy. In addition, surface etching produced a porous texture uniformly distributed across the substrate. These pores contribute to increased surface roughness, which is directly influenced by the etching conditions and parameters.

Antibacterial Susceptibility of ZnO–TiO₂ Nanoparticles

The agar well diffusion method assessed the antimicrobial activity of ZnO–TiO₂ core-shell nanoparticles against *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive).

The results showed that the diameter of the inhibition zones increased proportionally with nanoparticle concentration.

For *P. aeruginosa*, inhibition zones ranged from 8 mm at 25 $\mu\text{g/mL}$ to 20 mm at 100 $\mu\text{g/mL}$, indicating moderate sensitivity. *S. aureus*, on the other hand, displayed higher susceptibility, with inhibition zones increasing from 10 mm to 25 mm over the same concentration range [28]. This differential activity can be attributed to structural differences in the bacterial cell walls. The thicker peptidoglycan layer of Gram-positive bacteria like *S. aureus* may enhance interactions with ZnO–TiO₂ nanoparticles, leading to more effective antibacterial action. In contrast, the outer membrane in Gram-negative bacteria can act as a barrier, reducing nanoparticle penetration [28].

Nanoparticle Characterization Summary

The ZnO and ZnO–TiO₂ nanoparticles were thoroughly characterized using UV–Vis, FTIR, EDX, and AFM techniques. UV-Vis spectroscopy revealed a characteristic absorption peak for ZnO nanoparticles at 260 nm, consistent with previously reported values. For ZnO–TiO₂ core–shell particles, a red-shifted peak appeared at 300 nm, supporting the formation of the TiO₂ shell [30]. FTIR analysis confirmed Zn–O stretching vibrations at 617.18–559.32 cm⁻¹ and Ti–O–Zn bonds at 813.90–613.32 cm⁻¹, clearly indicating the formation of a core–shell structure [29]. EDX spectra demonstrated the presence of zinc, titanium, and oxygen in the core–shell nanoparticles, with no detectable impurities, confirming successful and clean synthesis [29]. AFM measurements indicated an increase in average grain size from 46.76 nm (ZnO) to 76.32 nm (ZnO–TiO₂), consistent with surface coating and shell development. A moderate increase in roughness and RMS values also reflected morphological changes [30].

4. Conclusions

This study demonstrates that ZnO–TiO₂ core–shell nanoparticles, synthesized via a green method using *Allium ampeloprasum* (kurrat) extract, possess significant antibacterial potential, particularly against Gram-positive *Staphylococcus aureus*. Characterization confirmed the successful synthesis and shell formation through multiple techniques, including UV–Vis, FTIR, EDX, and AFM. The ZnO–TiO₂ nanoparticles showed concentration-dependent antibacterial effects, with greater efficacy at higher concentrations. Their activity is attributed to mechanisms such as membrane disruption, oxidative stress, and interference with DNA and protein synthesis. These findings suggest that ZnO–TiO₂ core–shell nanoparticles may serve as promising antimicrobial agents, especially in the context of antibiotic-resistant bacteria, offering an effective alternative to conventional antibiotics. Plant-based synthesis also presents a sustainable, non-toxic, and scalable approach for nanoparticle production.

5. Acknowledgements

Perspectives for Future Research: The relationship between preparing nanomaterials in different ways and their effectiveness in killing bacterial groups, which can be used for sterilization in the future or preservation of food materials.

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Conflict of Interest Disclosure: The authors declare no conflict of interest.

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