Research Article

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Use (ZnO -TiO2) Nanoparticles as (core-shell) to eliminate both gram (positive and negative) bacteria.

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ABSTRACT

The field of medical science has begun to acknowledge the potential importance of nanotechnology, particularly in the development of innovative applications related to the treatment of infections. In this study zinc oxide nanoparticles (ZnO NPs) were green synthesized from the Allium ampeloprasum (kurrat) extracte plant after that, the ZnO-TiO2 core-shell nanoparticles were synthesized by using plant extract that acts as a reducing agent. The antibacterial activity was examined using the agar well diffusion method, the results showed that ZnO-TiO2 NPs have an antibacterial effect on both strains of bacteria, the of staphylococcus aureus (gram-positive) reached 25mm at 100µg/ml concentration while the Pseudomonas aeruginosa (gram-negative) showed inhibition zone reached to 20mm at 100µg/ml. Characteristics of the ZnO NPs and ZnO-TiO2 core-shell NPs included FTIR, UV-Vis, EDX, and AFM, The FTIR result indicates the formation of the core-shell around 813.90-613.32 cm-1 frequency. Furthermore, the Zinc nanoparticles' UV-Vis absorption peak was detected at 260 nm, whereas the ZnO-TiO2 nanoparticles' absorption peak was detected at 300 nm. The result of EDX revealed the synthesis of ZnO NPs and synthesized the core shell containing zinc, titanium, and oxygen elements chiefly and absent the impurities. according to the AFM investigation, the average diameter measurement of ZnO NPs is 46.74nm and the average diameter size of ZnO-TiO2 NPs is 148.9nm, the green-produced nanoparticles have robust biological activity and are used in a variety of biological applications and tests.

Keywords : Green synthesis, ZnO-TiO_2 (core-shell), ZnO nanoparticles, TiO_2 nanoparticles, Antimicrobial activity, Pseudomonas aeruginosa.

1. INTRODUCTION

The management of pathogenic microorganisms is an inescapable result of the rapid evolution of human life. Numerous microbes coexist peacefully with humans in their surroundings, but their unchecked and fast growth can cause major issues [1]. The desire to use nanotechnology as an incredibly potent nano weapon to address this specific problem is growing daily in the modern world. Nanoparticles (NPs) target a variety of distinct bacterial components, making it extremely difficult for microbes to survive against the[2], [3]. This is in stark contrast to the mechanism of action of antibiotics [4]. Generally speaking, nanoparticles (NPs) have comparatively larger surface areas than macroparticles due to their nanoscale size [5], [6], [7].

Since the former are controlled or manipulated, exhibiting considerably distinct characteristics from bulk materials [8]. The growing resistance to antibiotics has sparked interest in nanomaterials with antibacterial activity [9], [10], [11]. Significant toxicity and/or antibacterial activity has been documented for some nanomaterials, including ZnO and TiO2 [12], [13]. at the level of one atom (1 to 100 nm). The primary characteristics of metal nanoparticles (NPs) such as silver (Ag), zinc oxide (ZnO), and Titanium dioxide (TiO2) are their morphological, crystallographic, and compositional characteristics. The material's chemistry, mechanics, electrics, structure, morphology, and optics may all be impacted by the reduction to nanoscale size. Eventually, this will enable NPs to interact specifically with bio-cell molecules and make it easier for an NP to physically transmit inside inner cellular structures [14], [15].

Enzymes, sugars, and triodes are examples of natural substances and biological agents found in plant extracts that are substituted for solvents and harmful chemical substances employed in the chemical methodology to create green nanoparticles. As a result, the production of nanoparticles

through the utilization of natural resources decreases the amount of energy and environmentally harmful chemical solvents used in the synthesis process [16].

The aim of my work that we use plant extracts for green synthesis of nanoparticle ZnO second stage, synthesized core-shell nanoparticle ZnO-TiO_2 by coating a thin layer of TiO_2 on the ZnO. Following that, discover if nanoparticles are effective against both gram-positive and gram-negative bacteria in terms of antimicrobial activity.

2. MATERIALS AND METHODS

This experimental work was conducted in two stages: first, zinc oxide nanoparticles were prepared. second, prepared (Zinc-Titanium) oxide nanoparticles (core-shell), and tests were conducted to ascertain the nanoparticles' (core-shell) antibacterial ability.

2.1. Collection and Identification of clinical Specimens

Bacteria were identified staphylococcus aureus was grown as a standard strain of the gram-positive type. Also, Pseudomonas aeruginosa strains were selected from the gram-negative type and were obtained from the Biotechnology Department Laboratories/ College of Science/ University of Baghdad. A bacterial cell spreader was used for distributing the bacterial inoculum on a sterile Petri dish Mueller Hinton Agar (MHA), and they were then incubated for 24 hours at 37˚C to determine their bacteria activity. Confluent bacterial growth was seen following incubation.

2.2. preparing Allium ampeloprasum (kurrat) extract plant

The leaves of the kurrat Plant were harvested from Iraqi farms, To prepare the Kurrat extract for leaf processing, they were washed in double-distilled water, put in the electric blender, and continued mixing until it became juice, the mixture was then filtered by filter paper with a diameter (0.2) mm to get rid of plant residues, subsequently transferred the solution into tubes holding ten milliliters and centrifuged it for thirty minutes at a speed of 8000 rpm. then filtered with filter papers to have a clear solution, and was saved for use in the manufacture of nanoparticles (Ghadban and Ali, 2021).

2.3. Green Synthesis of ZnO Nanoparticles from Kurrat Extract

In the first step, each (10) ml of the extracted kurrat plant was incorporated with 1 gm of zinc acetate (CH3COO)2Zn.2H2O) in a flask, and the resultant mixture was shaken overnight at room temperature in a darkroom. The mixture was centrifuged at 8000 rpm for 30 minutes. To get rid of any residual extracted plant material, the precipitate of a solution containing all of the zinc oxide (ZnO) nanoparticles was twice rinsed with deionized distilled water. Overnight at 40°C, the precipitated nanoparticles were dried in an oven. The powder was finally placed within a dark container [18].

2.4. Green Synthesis of ZnO-TiO2 (core-shell) Nanoparticles from Kurrat extract

In the second stage, (150) ml of the Kurrat extract was added to (4.5) gm from ZnO NPs that had been prepared previously, and then (3) ml of Titanium tetrachloride was added to the solution under continuous stirring. After that, the mixture was shaken overnight in a darkroom at room temperature. After that, the mixture was centrifuged at 8000 rpm for 30 minutes. To get rid of any residual extracted material from plants, the precipitate of a solution containing all of the zinc and titanium ZnO-TiO2 (core-shell) nanoparticles was rinsed two times with deionized distilled water. Overnight at 40°C, the precipitated nanoparticles were dried in an oven. To prevent the powder from evaporating, it was finally placed in a dark container (Hameed and Yaaqoob, 2022).

2.5. Estimation of the antibacterial effect of ZnO-TiO2 (core-shell) nanoparticles

The agar well diffusion technique was employed for verification the minimal inhibition concentration (MIC) of biologically generated ZnO-TiO2 (core-shell) NPs for their antibacterial properties against Gram-positive staphylococcus aureus and Gram-negative Pseudomonas aeruginosa. Employing the sterile cotton swab procedure, the grown test species were propagated on the Müller Hinton agar sterilized medium. As a consequence, various amounts of ZnO-TiO2 NPs (25, 50, 75, and 100) µg/ml were added to the pre-made wells. after that, the newly created plates were infected for twenty-four hours at 37 °C. After that, the pre-made wells' zone of inhibition was measured (Hameed and Yaaqoob, 2022).

3. RESULTS

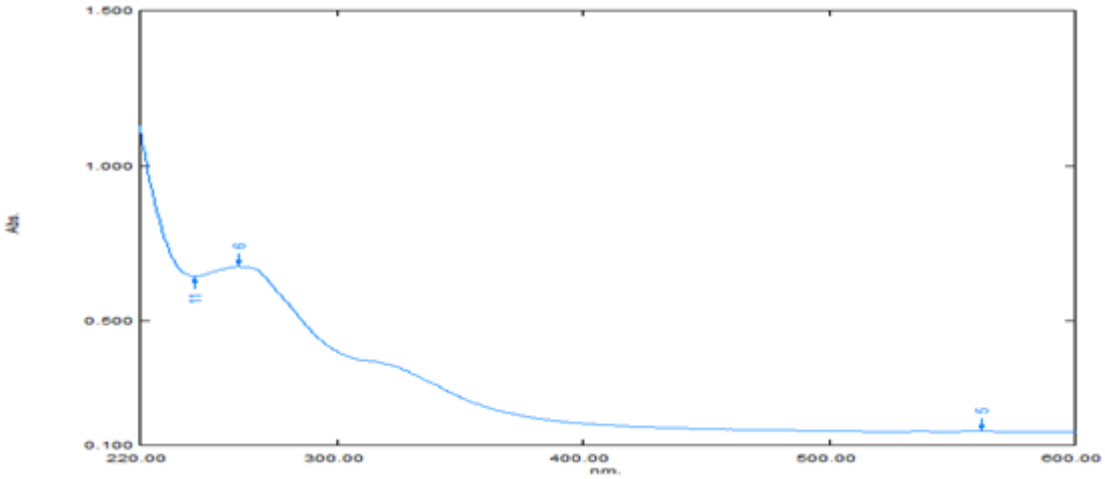
3.1. Characterization of ZnO-TiO2 (core-shell) NPs

3.1.1. Spectrophotometer (UV -Vis)

The absorption peak of ZnO nanoparticles was identified at 260 nm wavelengths in the zinc oxide nanoparticle investigation using a spectrophotometric UV-VIS, confirming the presence of zinc nanoparticles in the reaction solution. (**Fig. 1**).

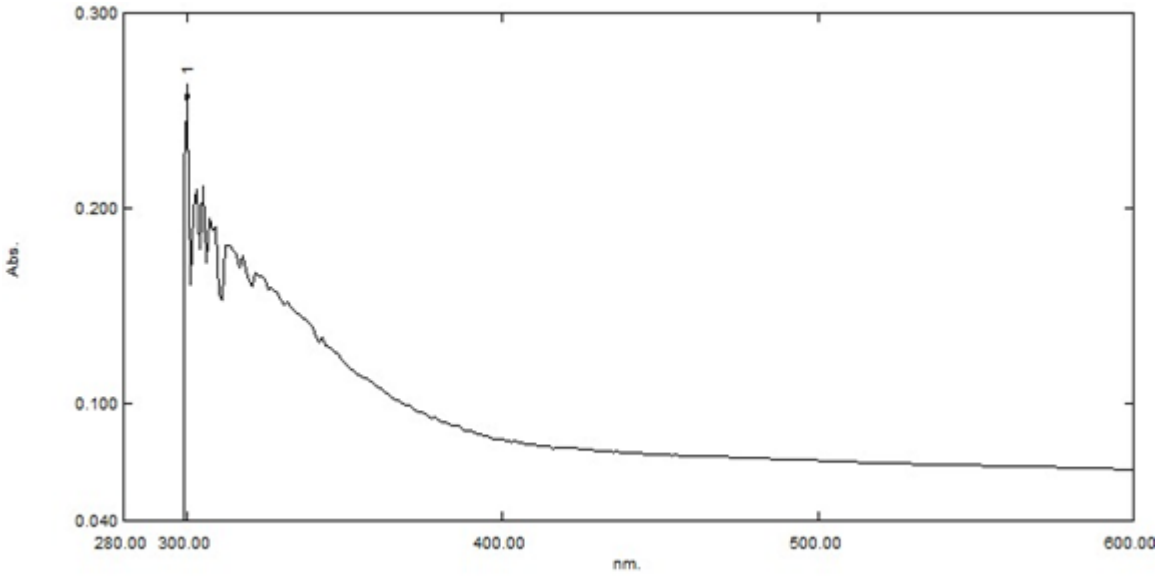
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Figure 1: The absorption spectrum of zinc nanoparticles in UV -Vis.



Whereas At wavelengths of 300 nm, the ZnO-TiO2 nanoparticles' absorption peak was identified. (**Fig. 2**). These values are agreed with the results obtained by (Zhang, An et al. 2010).

Figure 2: The absorption spectrum of ZnO-TiO2 nanoparticles in UV -Vis



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

The presence of 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 plant indicate the groups found in the plant (Devi and Battu 2019). Metal oxide is indicated by the ZnO peak, which was detected at the frequency of 617.18-559.32 cm-1 It pointed to the zinc oxide nanoparticles' fingerprint location (Rajendran and Sengodan 2017), (**Fig. 3,4**).

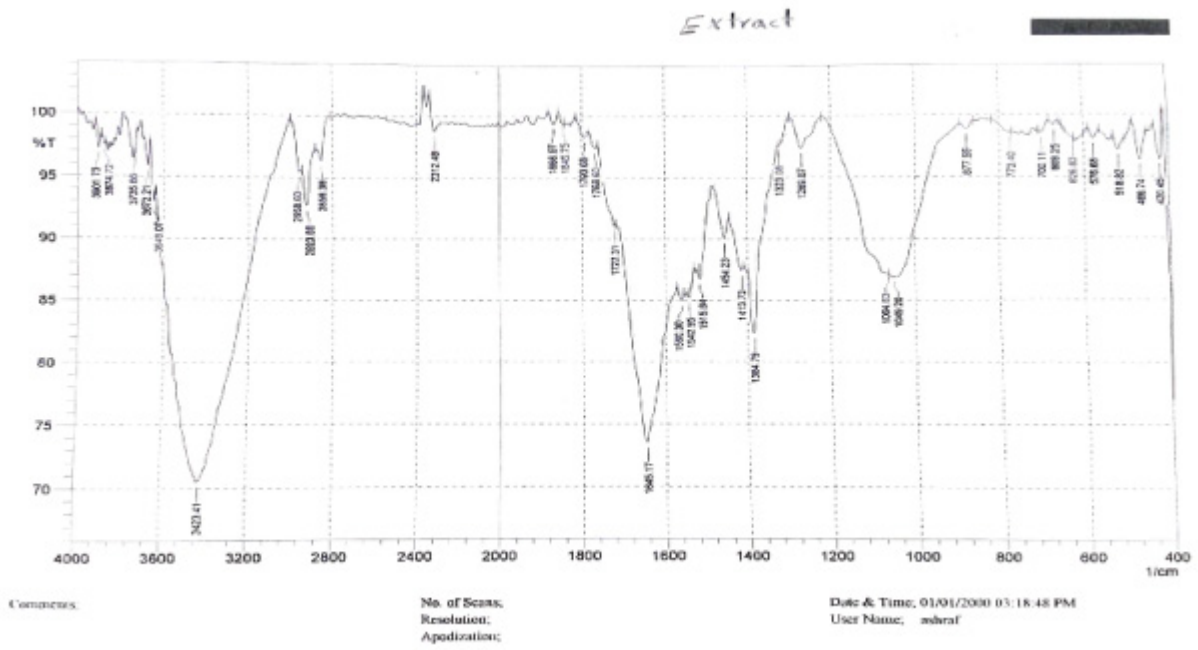
The metal oxide of the ZnO-TiO2 functional group could be detected around 813.90-613.32 cm-1 which indicates the formation of the core-shell (**Fig. 5**), (**Table 1**).

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Table 1: FTIR of extract and nanoparticle.

	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
Plant Extract	3423.41	N-H stretching	aliphatic primary amine
	1645.17	C=C stretching	monosubstituted
	1384.79	C-H bending	gem dimethyl
	1064.63-1049.20	C-O stretching	Primary Alcohol
ZnO NPs	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-TiO2 (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 – TiO2

Figure 3: FTIR for extract prepared from kurrat plant.



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Figure 4: FTIR of nano zinc oxide NPs

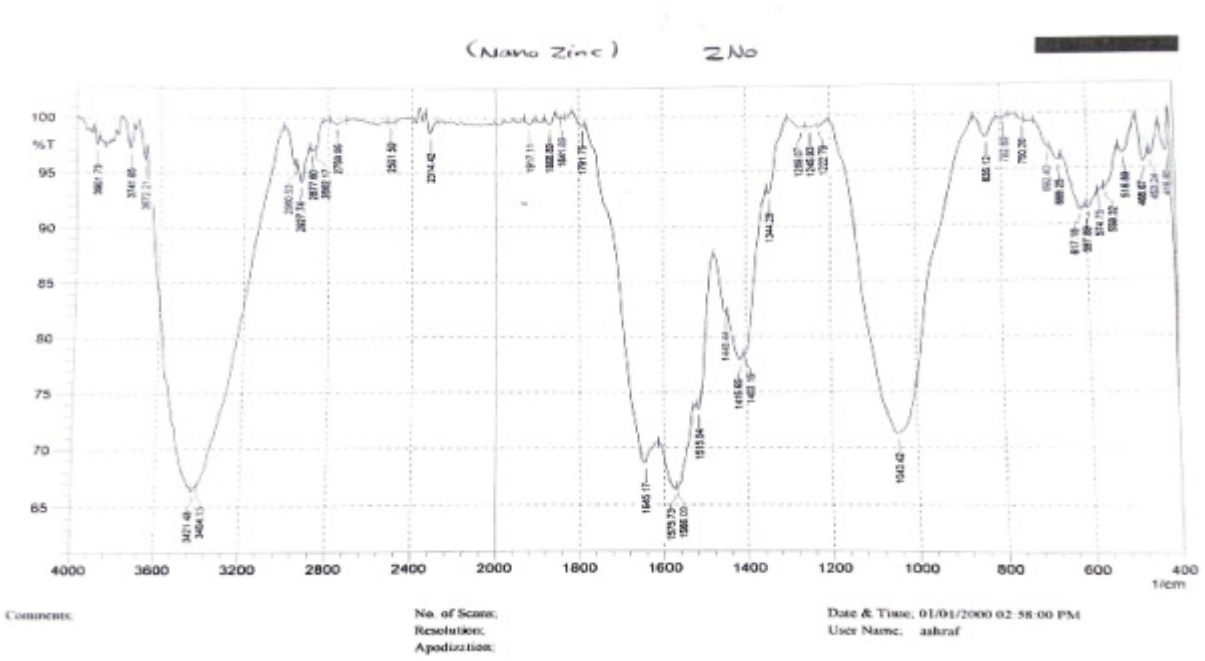
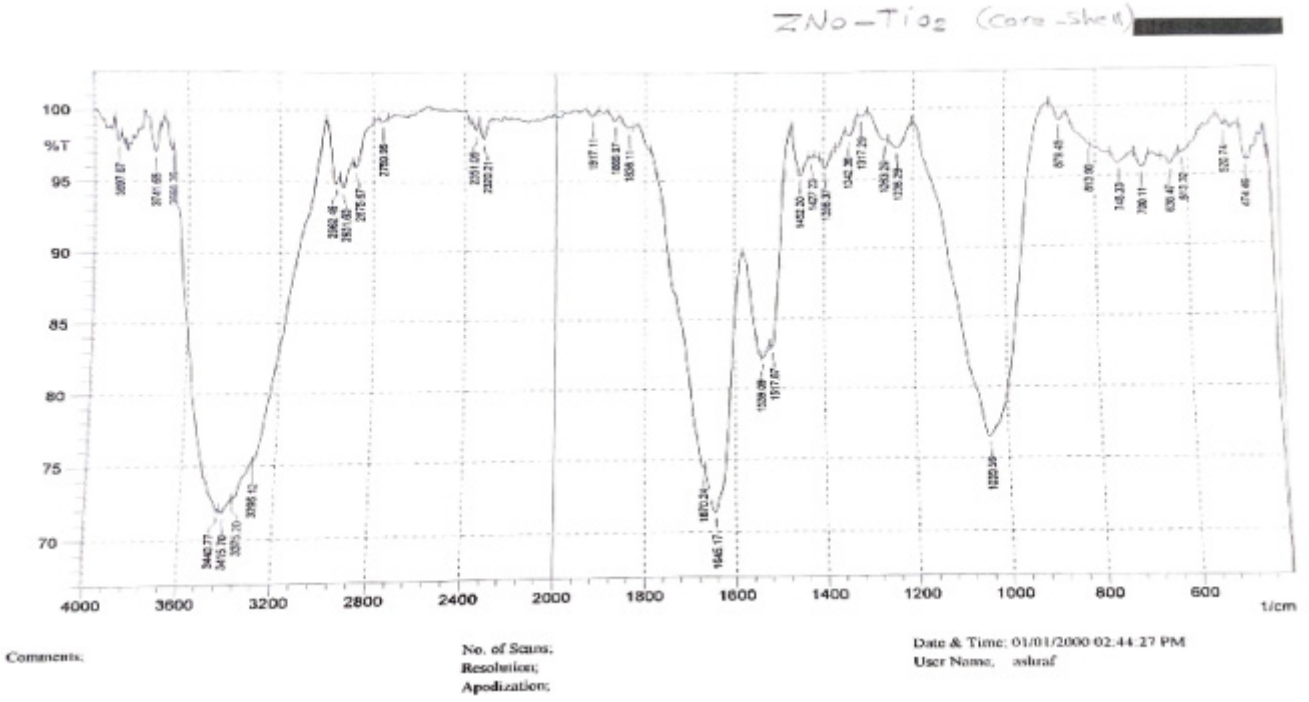


Figure 5: FTIR of ZnO-TiO2 core-shell NPs.



3.1.3. Energy-dispersive X-ray (EDX) analysis

The EDX spectrum of ZnO nanoparticle samples is displayed in **Figure 6** and **Table 2**. For the ZnO sample, The ZnO is the sample's primary ingredient (Shah, Ali et al. 2016).

contains zinc, titanium, and oxygen elements chiefly as well as the absence of impurities (Vlazan, Ursu et al. 2015). **Figure 7** and **Table 3**.

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Table 2: The EDX of ZnO nanoparticles

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
Zn	99.8	1.0	99.8	1.0

Figure 6: Zinc oxide nanoparticle EDX spectra.

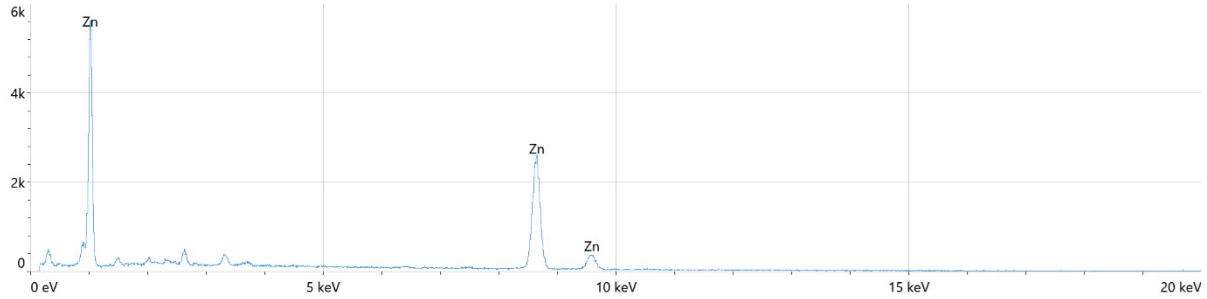
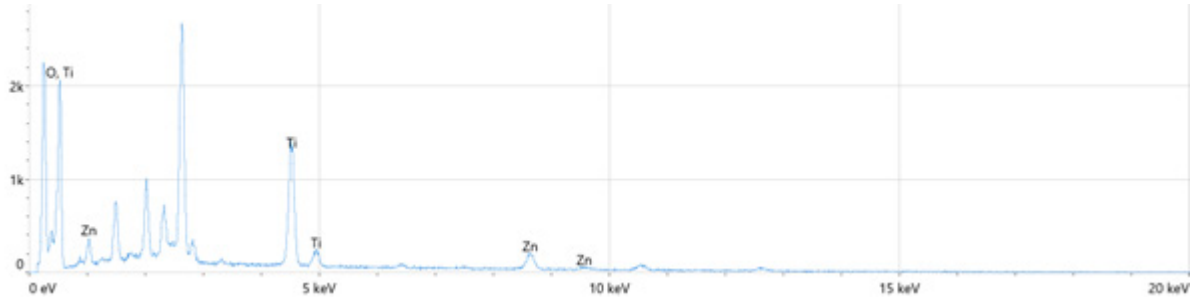


Table 3: The EDX of ZnO-TiO2 nanoparticles

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 7: ZnO-TiO2 nanoparticles EDX spectra.



3.1.4. Atomic force microscopy (AFM)

The average grain size and roughness of ZnO NPs and ZnO-TiO2 core-shell were determined by AFM studies to be 46.74 nm and 148.9 nm, respectively (**figure 8,9**). The average diameter, surface roughness, and RMS values are listed in **Table (4)**.

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 ores lead to increased surface roughness attributed to the etching parameters affecting the surface characterization.

Table 4: Average diameter, roughness, and RMS values of the synthesized of ZnO and ZnO-TiO2.

Sample	Average grain size (nm)	Roughness (nm)	(RMS) (nm)
ZnO	46.76	0.84	50.79
ZnO-TiO2	76.32	0.92	56.99

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Figure 8: AFM for ZnO nanoparticles in 2D and 3D.

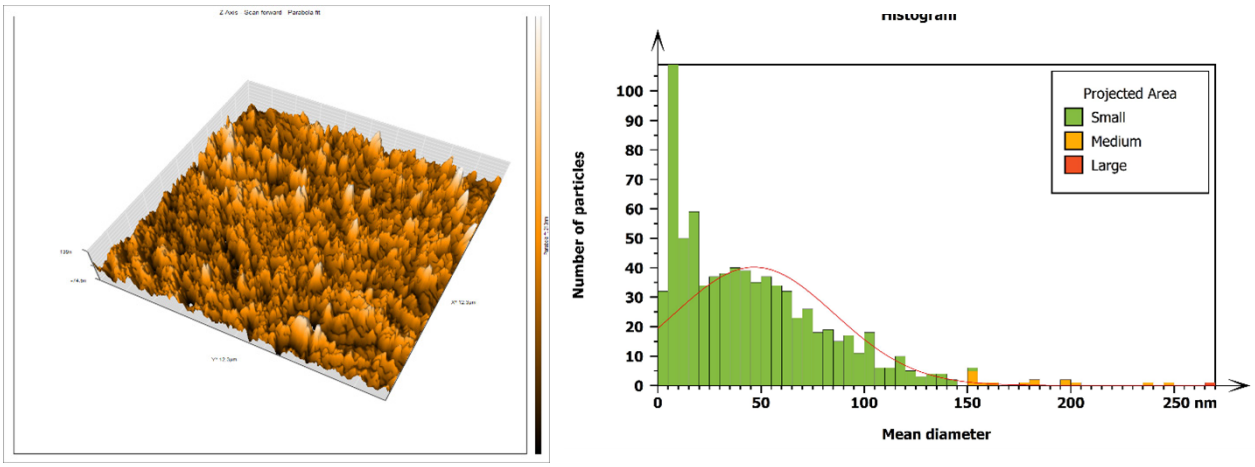
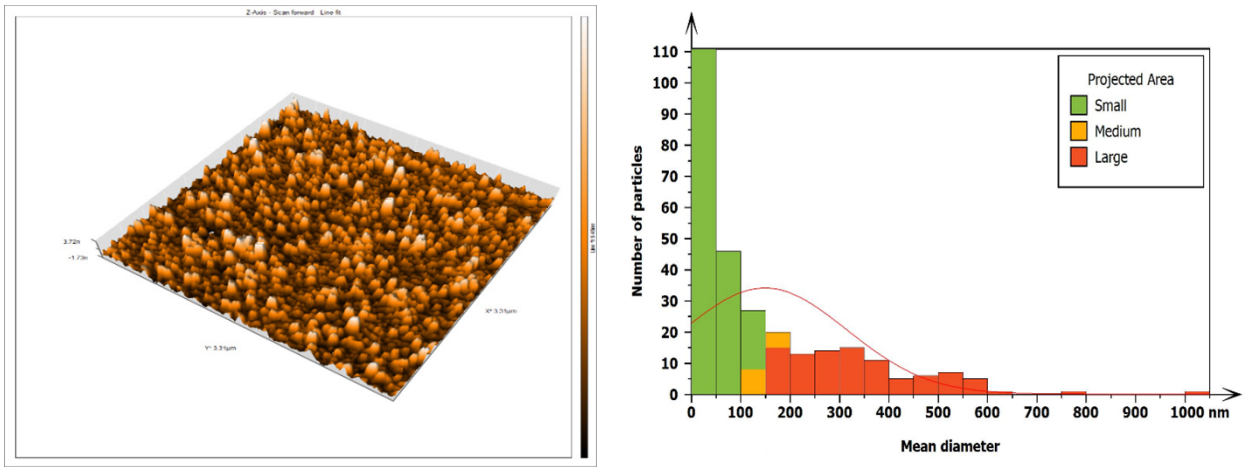


Figure 9: AFM for ZnO-TiO2 core-shell in 2D and 3D.



3.2. Collection and identification of bacterial samples

Antimicrobial susceptibility examination:

Considering **Table 5**, the outcomes of ZnO-TiO2 nanoparticles' antibacterial activity against Pseudomonas aeruginos 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.

Table 5: Zones where ZnO-TiO_2 nanoparticles inhibit Pseudomonas aeruginos.

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-TiO_2 with increasing the concentration of NPs, reaching (25 mm) at 100 µg/ml concentration (**Table 6**).

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Table 6: Zones where ZnO-TiO_2 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

4. DISCUSSION

The study presented showed that ZnO NPs that were biosynthesized from Kurrat extract and prepared (Zinc-Titanium) oxide nanoparticles (core-shell) were identified by spectrophotometric Uv-vis, FTIR, EDX, and AFM. The results of Uv-vis showed The absorption peak of ZnO nanoparticles was identified at 260 nm wavelengths it was confirmed with other research, it was recorded in the wavelength range of 250–800 nm UV–visible absorption spectrum of the ZnO nanoparticles (Singh, Pandey et al. 2012), The UV absorption edge can be influenced by the size of the nanoparticles (Reinosa, Leret et al. 2016), therefore the absorption peak of ZnO-TiO2 nanoparticles' was identified at 300 nm wavelengths. the FTIR results indicate the characteristics functional group presenting the synthesized zinc oxide nanoparticles at the frequency of 617.18-559.32 cm-1 (Reinosa, Leret et al. 2016), the formation of the core-shell (ZnO-TiO2) detected around 813.90-613.32 cm-1. , The result of EDX revealed the synthesis of Zno and core-shell.

The use of plant extracts has been one of the cleanest and most biocompatible methods used for large-scale production of nanoparticles, then the effect of zinc oxide nanoparticles on gram-positive and gram-negative bacteria was investigated. The nanoparticles' antibacterial capability is more effective against the S. aureus (gram-positive) bacterium than the Pseudomonas aeruginosa (gram-negative) bacteria. The variance in inhibition diameter could result from several interactions between ZnO-TiO2 NPs and the microorganism, further to the bacteria's susceptibility employed in this investigation (Saleem, Ahmed et al. 2017) Many studies have shown that zinc oxide nanoparticles' antimicrobial activity (Hammadi, Habeeb et al. 2020) produces a major disruption to the cell wall and membrane's permeability, which has an additional impact on macromolecules like DNA and protein by impeding specific activities like protein synthesis and DNA replication.(Azizi-Lalabadi, Ehsani et al. 2019).

5. CONCLUSION

Scientists are currently interested in the noble metal-derived nanoparticles because of their antiviral and antibacterial properties, which enable them to effectively combat a wide range of antigens. The study results suggest that ZnO-TiO2 core-shell NPs could be used as effective antibacterial on gram-positive and gram-negative bacteria resistant to conventional antibiotics. The FTIR result indicates the formation of ZnO NPS, as well as the increased bond of frequency, indicating the formation of ZnO-TiO2 NPs core-shell. the Uv -vis, absorption peak of ZnO NPs and ZnO-TiO2 core-shell was observed. Furthermore, the result of EDX revealed the ZnO NP synthesis as well as synthesized the core-shell containing zinc, titanium, and oxygen elements chiefly and absent the impurities.

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