

RESEARCH PAPER

# Chemical Synthesis of Zinc Oxide Nanoparticles and Their Antimicrobial Potential Against Opportunistic Hospital Pathogens

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## ABSTRACT

Bacterial resistance to antibiotics is a growing global challenge. This study investigates the antimicrobial effects of zinc oxide nanoparticles (ZnO-NPs) on clinical strains of *Acinetobacter baumannii* and *Staphylococcus aureus*, focusing on their synergy with the antibiotic ciprofloxacin. ZnO-NPs were synthesized and characterized using XRD, FT-IR, and SEM techniques. Their antimicrobial activity was evaluated against both bacteria, and the minimum inhibitory concentration (MIC) was determined. The results demonstrated that ZnO-NPs effectively inhibited bacterial growth, with enhanced effects when combined with ciprofloxacin, particularly against *A. baumannii*. These findings suggest that ZnO-NPs could reduce antibiotic resistance and offer a promising approach to combating multidrug-resistant bacteria.

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## INTRODUCTION

Hospital-acquired infections, also known as nosocomial infections, represent a significant challenge to healthcare systems worldwide. These infections, which typically arise 48 hours after hospital admission or within three days post-discharge, are associated with high morbidity, mortality, and economic burden. Intensive care units (ICUs) are particularly prone to such infections, with Gram-negative and Gram-positive pathogens emerging as predominant culprits [1, 2]. Among Gram-negative bacteria, *Acinetobacter baumannii* has become a critical concern due to its remarkable resistance to almost all known antibiotics and its ability to persist in hospital environments [3, 4]. Similarly, methicillin-resistant

*Staphylococcus aureus* (MRSA) has long been recognized as a leading Gram-positive pathogen, responsible for severe infections and high mortality rates [5].

Antibiotic resistance, driven by the overuse and misuse of antibiotics, has escalated into a global public health crisis. Pathogens such as *A. baumannii* and MRSA not only exhibit resistance to multiple drugs but also possess virulence factors that enhance their pathogenicity. Traditional antibiotics have become increasingly ineffective, emphasizing the urgent need for alternative strategies to combat these multidrug-resistant pathogens [6].

Recent advancements in nanotechnology have introduced innovative approaches to

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antimicrobial therapy. Metal oxide nanoparticles, particularly zinc oxide nanoparticles (ZnO-NPs), have garnered significant attention for their potent antimicrobial properties. ZnO-NPs exhibit unique physicochemical characteristics, including a high surface-to-volume ratio, tunable shape and size, and the ability to generate reactive oxygen species (ROS). These features enable ZnO-NPs to disrupt bacterial membranes, increase cell permeability, and inhibit intracellular functions [7, 8]. Moreover, ZnO-NPs have shown promise in enhancing the efficacy of conventional antibiotics through synergistic effects, thereby addressing the limitations of monotherapy [9].

This study focuses on the antimicrobial activity of ZnO-NPs, both as standalone agents and in combination with ciprofloxacin, against clinical strains of *A. baumannii* and *S. aureus*. The research aims to investigate the synergistic effects of ZnO-NPs and ciprofloxacin, determine the mechanisms underlying their combined antimicrobial activity, and explore the potential of ZnO-NPs to counteract antibiotic resistance [10, 11]. By elucidating these mechanisms, the study seeks to contribute to the development of novel therapeutic strategies that can effectively manage multidrug-resistant infections in healthcare settings.

The results of this investigation could pave the way for the integration of nanotechnology in combating hospital-acquired infections, offering hope for addressing the pressing challenge of antibiotic resistance [7].

## MATERIALS AND METHODS

### *Biological Studies*

#### *Sterilization of Materials and Laboratory Equipment*

To maintain sterility during experiments, all tools, media, and buffers were sterilized using thermal methods and UV irradiation. Autoclaving was performed at 121°C and 103 kPa for 15–20 minutes to sterilize culture media, buffers, and other heat-resistant materials [12]. UV irradiation and 70% ethanol were employed to sterilize the working environment, including laminar flow hoods, ensuring a contamination-free setup.

#### *Preparation of Culture Media*

Bacterial cultures were grown using nutrient agar (NA) and nutrient broth (NB). These media are commonly used for cultivating bacteria with minimal selectivity:

**Nutrient Agar (NA):** To prepare the NA medium, 13 g of powdered medium was dissolved in 1 L of deionized water, followed by autoclaving for 15 minutes. After cooling to 50°C, the medium was poured into sterile petri dishes under aseptic conditions and stored at 4°C [13].

**Nutrient Broth (NB):** For NB preparation, 2.8 g of powdered medium was dissolved in 100 mL of deionized water. The solution was autoclaved at 121°C, allowed to cool, and stored under sterile conditions until use [13].

#### *Preparation of 0.02 M NaOH Solution*

A 0.02 M sodium hydroxide solution was prepared by dissolving 1 g of NaOH in 2 mL of deionized water. A 2 mL aliquot of this solution was diluted to 100 mL with deionized water, ensuring precise molarity for subsequent synthesis steps [18].

#### *Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs)*

The synthesis of ZnO-NPs was performed using a co-precipitation method with zinc acetate ( $C_4H_{10}O_6Zn$ ) as the precursor and sodium hydroxide (NaOH) as the precipitating agent [3,7,14].

**Step 1:** A 0.21195 g sample of zinc acetate was dissolved in 90 mL of deionized water and cooled to 4°C [3,16].

**Step 2:** While maintaining the temperature between 0–4°C, 80 mL of 0.02 M NaOH solution was added dropwise using a burette under constant stirring [3,16].

**Step 3:** The reaction mixture was heated in a water bath at 65°C for 2 hours, forming a white precipitate [3,17].

**Step 4:** The precipitate was allowed to age at room temperature (25°C) for 3 days, then centrifuged at 12,000 rpm for 20 minutes [3,14].

**Step 5:** The precipitate was washed four times with a 1:1 mixture of deionized water and ethanol to remove impurities [3,15].

**Step 6:** Finally, the washed precipitate was dried in a vacuum oven at 10–40°C for 10 hours, yielding a fine white ZnO-NP powder [3,17].

#### *Preparation of ZnO Nanofluid*

To prepare a nanofluid, 4 mg of ZnO-NPs was autoclaved for 15 minutes. One milliliter of autoclaved deionized water was added, and the mixture was sonicated for 15 minutes [15,18]. This stock solution was stirred magnetically for 48 hours to ensure uniform dispersion, resulting in a

4 mg/mL nanofluid concentration [14] [19].

#### Characterization of ZnO-NPs

##### X-ray Diffraction (XRD)

XRD analysis was performed to determine the crystalline structure of the ZnO-NPs. The nanoparticle size was calculated using the Scherrer equation:  $D = \beta \cos \theta / k \lambda$

Where D = nanoparticle size, k = Scherrer constant (0.9),  $\lambda$  = X-ray wavelength (1.54 Å),  $\beta$  = peak width, and  $\theta$  = Bragg angle [20,21].

##### Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis identified functional groups in the synthesized nanoparticles. The spectra were recorded in the range of 4000–400  $\text{cm}^{-1}$  using a Thermo Nicolet AVATAR-370-FT-IR spectrometer [20,22].

##### Dynamic Light Scattering (DLS)

DLS measurements were conducted to determine the hydrodynamic size and zeta potential of ZnO-NPs in suspension. A VASCO3 DLS device was used for precise measurements [23,24].

##### Scanning Electron Microscopy (SEM)

The morphology and size of ZnO-NPs were analyzed using an LEO 1450 VP SEM. Samples were prepared by dispersing 10 mg of ZnO-NPs in 20 mL ethanol, followed by ultrasonication for 1 hour [23,25].

##### Bacterial Strains

Clinical isolates of *Acinetobacter baumannii* and *Staphylococcus aureus* were obtained from a Baghdad hospital. Bacteria were cultured on NA and NB media for experimental purposes.

##### Determination of Bacterial Concentration

Bacterial suspensions were standardized using McFarland 1 (equivalent to  $3 \times 10^8$  CFU/mL), prepared by mixing barium chloride and sulfuric acid to produce a turbid solution with known absorbance [24,25].

##### Bacterial Identification

###### Gram Staining

Gram staining was performed to classify bacteria based on cell wall properties. Gram-positive bacteria retained the violet crystal stain,

while Gram-negative bacteria appeared red after counterstaining with fuchsin [26,27].

##### Antibiotic Sensitivity Testing

Disc diffusion tests were conducted using tobramycin (30  $\mu\text{g}$ ), tetracycline (30  $\mu\text{g}$ ), and ciprofloxacin (5  $\mu\text{g}$ ) to evaluate bacterial susceptibility. Zones of inhibition were measured after incubation at 37°C for 18 hours [28,29].

##### Determination of Minimum Inhibitory Concentration (MIC)

MIC values were determined by exposing bacteria to ZnO-NPs in concentrations ranging from 0.015–5.0 mg/mL. Growth inhibition was monitored using an ELISA reader at 630 nm, and bacterial survival percentages were calculated [30,31].

##### Synergistic Effect of ZnO-NPs and Ciprofloxacin

The combined antimicrobial effects of ZnO-NPs and ciprofloxacin (16  $\mu\text{g}/\text{mL}$ ) were tested at 1/2 MIC of ZnO-NPs. Bacterial growth inhibition was assessed using optical density measurements [32,33].

##### Biofilm Inhibition Assay

Antioxidant activity of ZnO-NPs was evaluated by their ability to scavenge DPPH free radicals. Nanoparticles were incubated with DPPH solution, and absorbance at 490 nm was recorded [36,37].

##### Antioxidant Activity (DPPH Assay)

Antioxidant activity of ZnO-NPs was evaluated by their ability to scavenge DPPH free radicals. Nanoparticles were incubated with DPPH solution, and absorbance at 490 nm was recorded [36,37].

##### Statistical Analysis

Data were analyzed using SPSS software. Experiments were repeated three times, and statistical significance was determined using ANOVA and Tukey's post hoc test for multiple comparisons [38,39].

## RESULTS AND DISCUSSION

### Characterization of Synthesized Nanoparticles

#### X-ray Diffraction (XRD) Spectrum

The structural characteristics, material composition, and crystalline properties of the synthesized zinc oxide (ZnO) nanoparticles were evaluated using X-ray diffraction (XRD). As shown

in the XRD spectrum (Fig. 1), diffraction peaks were observed at angles 32.9°, 34.2°, 36.1°, 47.3°, 56.4°, 62.6°, 67.9°, and 68.8°, corresponding to the crystal planes (112), (200), (103), (110), (102), (101),

(002), and (100), respectively. These peaks align with the Joint Committee on Powder Diffraction Standards (JCPDS), confirming the hexagonal wurtzite crystal structure of ZnO nanoparticles.

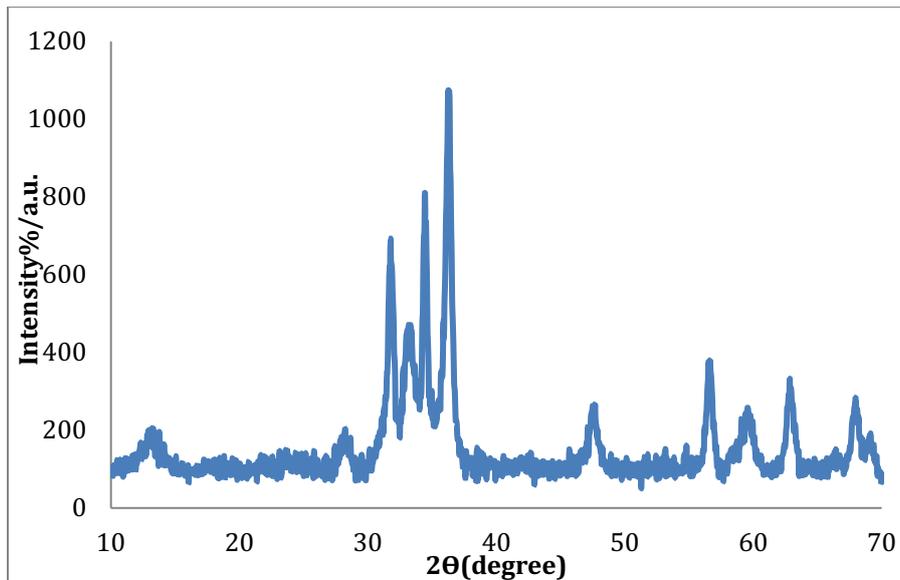


Fig. 1. XRD spectrum of zinc oxide nanoparticles, confirming their hexagonal wurtzite crystal structure and nanoscale size.

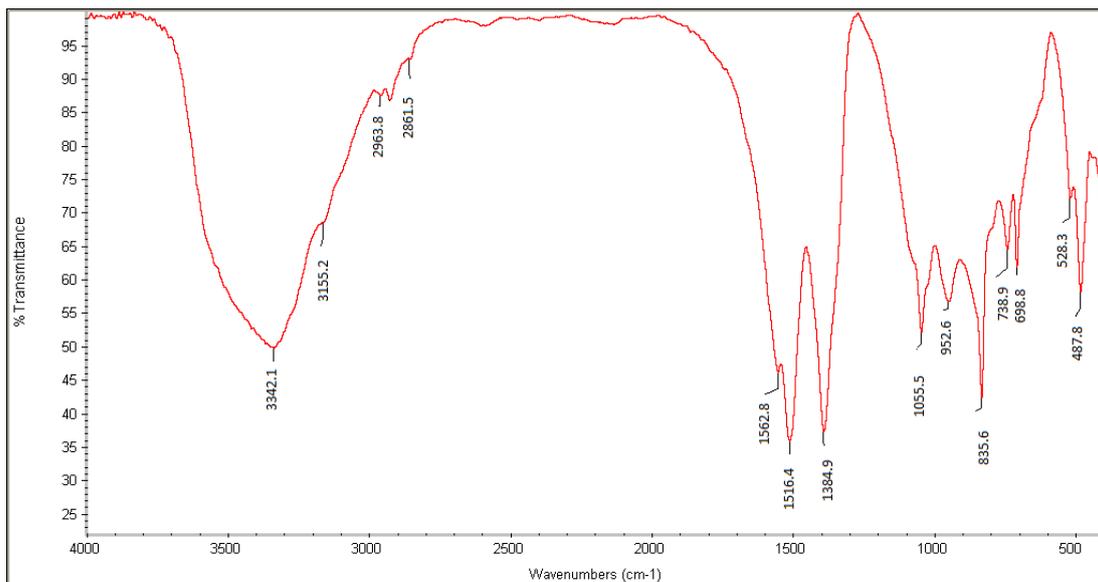


Fig. 2. FT-IR spectrum of zinc oxide nanoparticles, showing distinct peaks for O-H, C-H, and Zn-O functional groups.

No additional peaks were detected, indicating the high purity of the nanoparticles. Using the Debye-Scherrer equation, the average crystalline size was calculated to be approximately 87 nm.

*FT-IR Spectroscopy of ZnO Nanoparticles*

Fourier transform infrared (FT-IR) spectroscopy was used to identify the functional groups and chemical structure of the synthesized nanoparticles. The FT-IR spectrum (Fig. 2) exhibited characteristic absorption bands:

1. A strong absorption band at 3342 cm<sup>-1</sup> corresponds to O-H stretching vibrations.
2. Peaks at 3155 and 2963 cm<sup>-1</sup> are associated with C-H stretching vibrations.
3. A significant peak at 487 cm<sup>-1</sup> is attributed

to Zn-O stretching vibrations.

4. Additional peaks at 835 cm<sup>-1</sup> and 1055 cm<sup>-1</sup> correspond to C-N and C-O stretching vibrations, respectively.

These results confirm the presence of functional groups associated with the ZnO nanoparticles, indicating their successful synthesis.

*Size and Zeta Potential of ZnO Nanoparticles Dynamic Light Scattering (DLS)*

Dynamic light scattering (DLS) was used to determine the size distribution of ZnO nanoparticles. The hydrodynamic diameter of the synthesized nanoparticles was measured to be 174 nm (Fig. 3), larger than the crystalline size due to hydration in aqueous suspension.

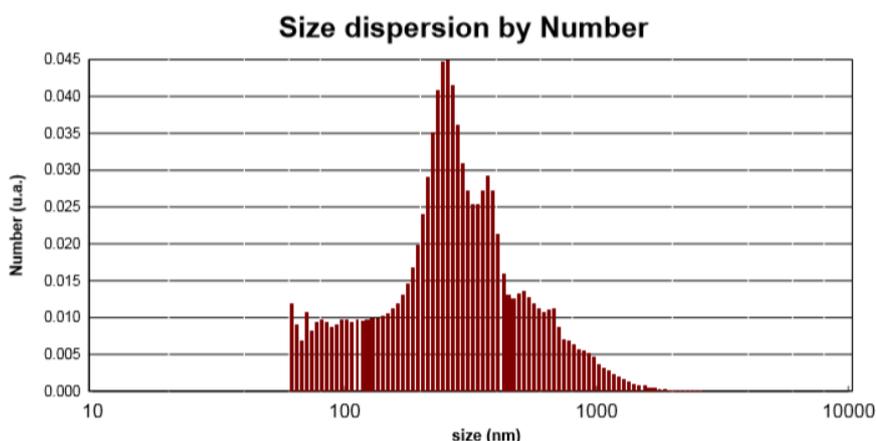


Fig. 3. Size distribution of zinc oxide nanoparticles.

Table 1. Size distribution and zeta potential of ZnO nanoparticles.

Nanoparticles	Particle size (nm)	PDI	Zeta potential (mV)
ZnO	174	≤0.3	-19 ± 1

Table 2. Resistance and sensitivity of bacteria to antibiotics based on CLSI standards.

Bacteria	Tobromycin (mm)	Ciprofloxacin (mm)	Tetracycline (mm)
Acinetobacter baumannii	19	7	15
Staphylococcus aureus	21	18	17



#### Zeta Potential

The zeta potential of the nanoparticles, measured using the Malvern Zeta Nano ZS, was determined to be -19 mV. This negative charge indicates that the nanoparticles possess a moderate colloidal stability. For optimal physical stability, the zeta potential should range between -30 mV and +30 mV.

#### Morphology of ZnO Nanoparticles

The morphology of ZnO nanoparticles was

analyzed using scanning electron microscopy (SEM). As shown in the SEM images (Fig. 5), the nanoparticles exhibit a predominantly spherical shape with some faceted structures. The average particle size was consistent with that obtained from the Debye-

#### Gram Staining

Gram staining of the bacterial samples showed that *Staphylococcus aureus* is Gram-positive, appearing in cluster or chain forms,

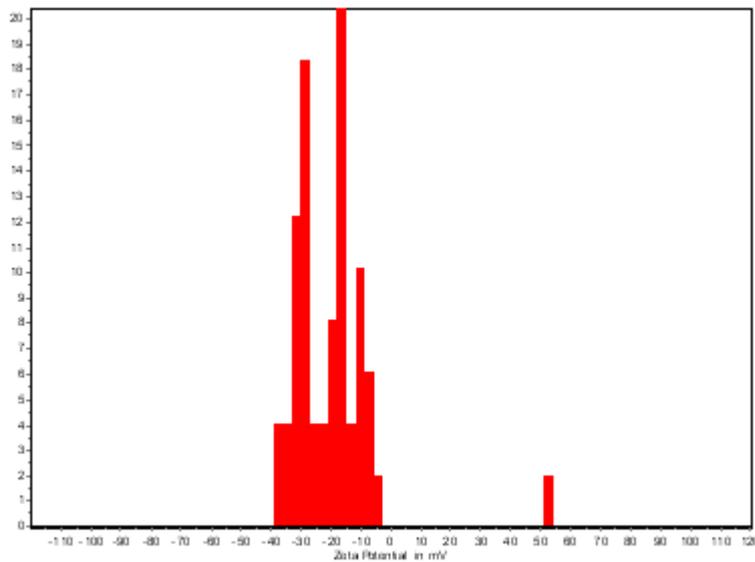


Fig. 4. Zeta potential of zinc oxide nanoparticles, showing a net negative charge.

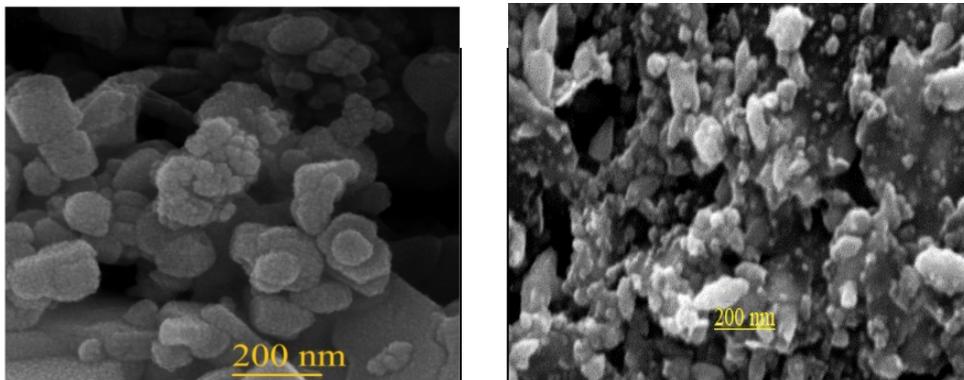


Fig. 5. SEM images of zinc oxide nanoparticles, illustrating their spherical morphology and nanoscale dimensions.

while *Acinetobacter baumannii* is Gram-negative, primarily appearing in coccobacillus forms but occasionally as rods or spheres.

**Antibiotic Sensitivity Test Results**

The antibiotic sensitivity tests revealed varying resistance patterns for the bacterial strains.

1. *Staphylococcus aureus* exhibited resistance to tetracycline, ciprofloxacin, and

tobramycin, with inhibition zones of 17 mm, 18 mm, and 21 mm, respectively.

2. *Acinetobacter baumannii* showed the highest resistance to ciprofloxacin, with an inhibition zone of only 7 mm. Tobramycin and tetracycline inhibition zones were 19 mm and 15 mm, respectively.

These findings highlight the need for alternative strategies to combat *Acinetobacter baumannii* due

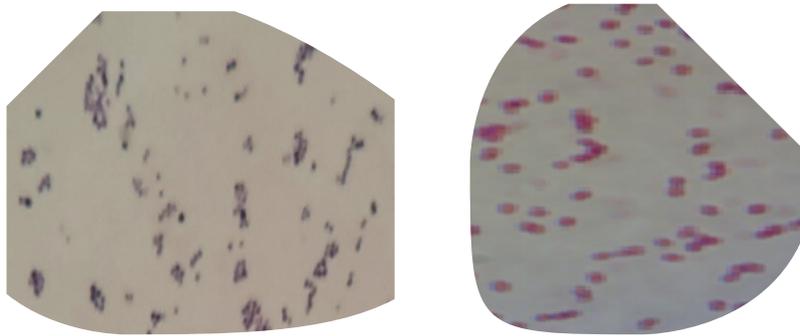


Fig. 6. (A) Gram-positive bacteria (*Staphylococcus aureus*). (B) Gram-negative bacteria (*Acinetobacter baumannii*).

Table 3. Growth inhibition of *S. aureus* and *A. baumannii* in the presence of ZnO nanoparticles.

Growth Inhibition (%)	Antibacterial Factor
ZnO NPs (0. 25 mg/ml)	92±4
CP (8 µg/ml)	3±0
ZnO NPs + CP	100

Table 4. Growth inhibition of *A. baumannii* in the presence of ZnO nanoparticles and ciprofloxacin.

Growth Inhibition (%)	Antibacterial Factor
ZnO NPs (0. 25 mg/ml)	92±4
CP (8 µg/ml)	3±0
ZnO NPs + CP	100

to its high antibiotic resistance.

**Antimicrobial Activity of ZnO Nanoparticles**

The antimicrobial activity of ZnO nanoparticles was evaluated using the minimum inhibitory concentration (MIC) method. Results showed a concentration-dependent inhibition of bacterial growth. At 0.5 mg/mL, ZnO nanoparticles completely inhibited the growth of *Acinetobacter*

*baumannii* and achieved a 91% growth inhibition of *Staphylococcus aureus*.

**Combined Effect of ZnO Nanoparticles and Ciprofloxacin**

The combined antimicrobial effect of ZnO nanoparticles (0.25 mg/mL) and ciprofloxacin (8 µg/mL) was significantly greater than either agent alone. The combination achieved a 100%

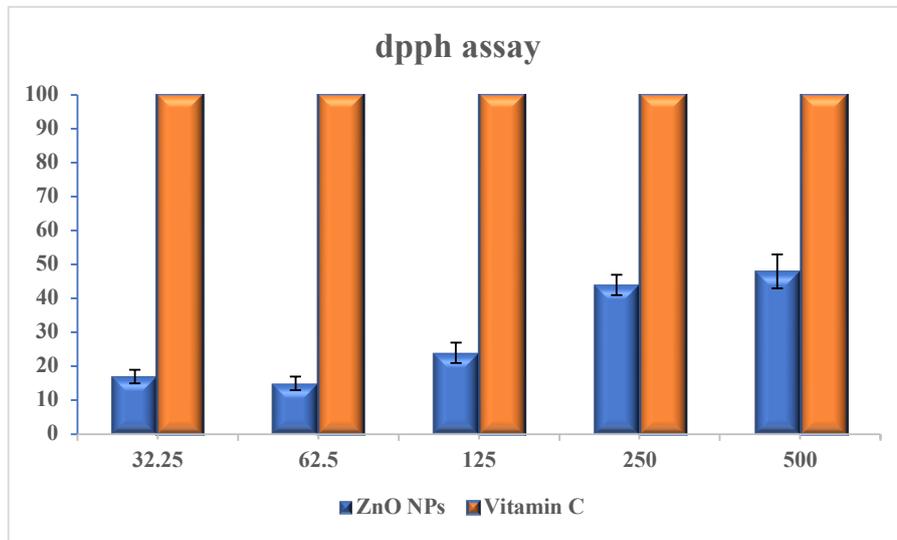


Fig. 7. Antioxidant activity of ZnO nanoparticles in DPPH radical removal.

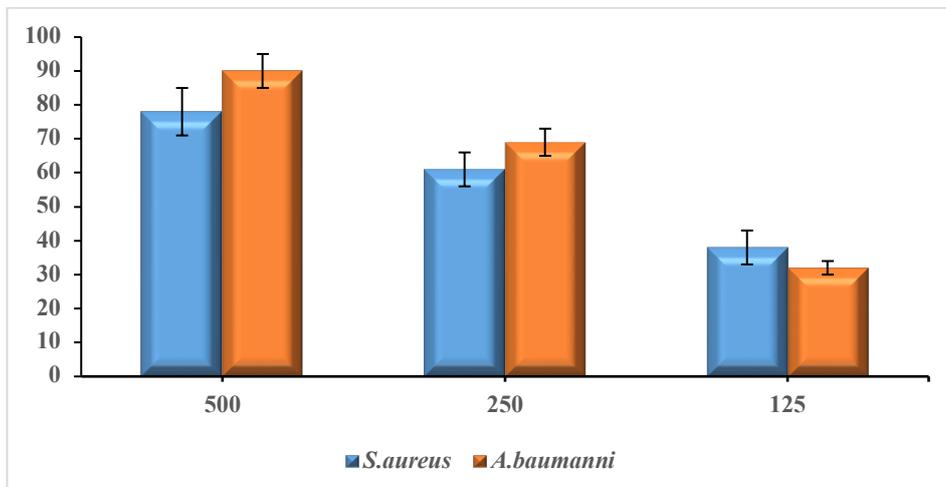


Fig. 8. Biofilm inhibition percentage of ZnO nanoparticles.

Table 6. Biofilm inhibition percentages.

Concentration ( $\mu\text{g/ml}$ ) sample	32.25	62.5	125	250	500
ZnO NPs	%17 $\pm$ 2	%15 $\pm$ 2	%24 $\pm$ 3	%44 $\pm$ 3	%48 $\pm$ 5
Vitamin C	%10	%100	%100	%100	%100

Table 6. Biofilm inhibition percentages.

Standard strain	500 ( $\mu\text{g/ml}$ )	250 ( $\mu\text{g/ml}$ )	125 ( $\mu\text{g/ml}$ )
<i>S.aureus</i>	78 $\pm$ 7	61 $\pm$ 5	38 $\pm$ 5
<i>A.baumannii</i>	90 $\pm$ 5	69 $\pm$ 4	32 $\pm$ 2

inhibition of *Acinetobacter baumannii*, compared to 92% with ZnO nanoparticles alone and 3% with ciprofloxacin alone.

#### Antioxidant Activity of ZnO Nanoparticles

The antioxidant activity of ZnO nanoparticles was assessed using the DPPH radical scavenging assay. The highest activity (48%) was observed at a concentration of 500  $\mu\text{g/ml}$ .

#### Anti-Biofilm Activity of ZnO Nanoparticles

ZnO nanoparticles inhibited biofilm formation in both *Staphylococcus aureus* and *Acinetobacter baumannii*. At 500  $\mu\text{g/ml}$ , biofilm inhibition was 78% for *S. aureus* and 90% for *A. baumannii*.

#### CONCLUSION

This study highlights the potential of zinc oxide nanoparticles (ZnO-NPs) as a promising antimicrobial agent against drug-resistant pathogens, particularly *Acinetobacter baumannii* and *Staphylococcus aureus*. The synthesized ZnO-NPs exhibited significant antibacterial activity, with their efficacy being enhanced in combination with ciprofloxacin, demonstrating a synergistic effect that could mitigate bacterial resistance. The nanoparticles also displayed potent anti-biofilm and antioxidant properties, further underlining their multifunctional utility in combating bacterial infections.

The characterization of ZnO-NPs revealed a hexagonal wurtzite structure with nanoscale dimensions and favorable colloidal stability. The antimicrobial activity of ZnO-NPs was dose-dependent, with complete inhibition of *A.*

*baumannii* observed at a concentration of 0.5 mg/mL. Moreover, their ability to inhibit biofilm formation and scavenge free radicals suggests their potential application in various biomedical fields, including infection control and wound healing.

This work underscores the importance of integrating nanotechnology with conventional antimicrobial strategies to address the growing challenge of antibiotic resistance. Future research should focus on exploring the mechanisms of action of ZnO-NPs in greater depth, assessing their biocompatibility in clinical settings, and investigating their efficacy in vivo to facilitate their translation into therapeutic applications. The promising findings of this study pave the way for the development of nanoparticle-based therapies as a complementary approach to traditional antibiotics.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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