

Synthesis, Characterization, and Antibacterial Activity of Manganese Doped, Zinc Oxide Nanoparticles against Multidrug-Resistant *Acinetobacter baumannii*

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Abstract

Background: The rapid emergence of multidrug resistant (MDR) *Acinetobacter baumannii* has become a critical global healthcare challenge, necessitating the development of alternative antimicrobial strategies. Metal oxide nanoparticles have attracted considerable attention because of their broad-spectrum antibacterial activity. Manganese (Mn)-doped zinc oxide (ZnO) nanoparticles exhibit enhanced physicochemical properties that may improve antimicrobial performance. This study aimed to synthesize and characterize Mn doped ZnO nanoparticles and evaluate their antibacterial activity against a clinical MDR *A. baumannii* isolate. **Methods:** Mn-doped ZnO nanoparticles were synthesized using a co-precipitation method and characterized by UV Visible Spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and

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Atomic Absorption spectroscopy (AAS) to determine their optical, structural, morphological, and elemental properties. Antibacterial activity was assessed against a clinical MDR *A. baumannii* isolate using the broth microdilution assay at nanoparticle concentrations of 10, 20, and 30 µg/mL. **Results:** Characterization analyses confirmed the successful synthesis of crystalline Mn-doped ZnO nanoparticles with a hexagonal wurtzite structure and an average crystallite size of approximately 28 nm. The nanoparticles exhibited concentration-dependent antibacterial activity, producing growth inhibition of 45.6%, 62.7%, and 91.7% at concentrations of 10, 20 and 30 µg/mL respectively. The highest concentration resulted in near-complete suppression of bacterial growth, demonstrating the strong antibacterial efficacy of Mn-doped ZnO nanoparticles against MDR *A. baumannii*. **Conclusions:** Mn-doped ZnO nanoparticles demonstrated potent *in vitro* antibacterial activity against multidrug-resistant *A. baumannii*, highlighting their potential as a promising nanomaterial for combating antibiotic-resistant bacterial infections. These findings provide a foundation for further investigations into their antibacterial mechanisms, biocompatibility, and *in vivo* therapeutic applications.

Keywords

Acinetobacter baumannii, Manganese, Zinc Oxide, Antimicrobial Activity, Multi-Drug Resistance, Reactive Oxygen Species

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most significant threats to global public health, compromising the effectiveness of antibiotics and increasing the burden of infectious diseases worldwide [1]. The World Health Organization (WHO) recognizes AMR as one of the top ten global health challenges, with projections suggesting that antimicrobial-resistant infections could cause millions of deaths annually by 2050 if effective interventions are not implemented [2]. The rapid emergence and dissemination of multidrug-resistant (MDR) extensively drug-resistant (XDR) have substantially reduced the therapeutic efficacy of conventional antibiotics, resulting in prolonged hospital stays, increased healthcare costs, and higher mortality rates [3]. The continued decline in the discovery of new antibiotics, coupled with the remarkable adaptability of bacterial pathogens, has intensified the need for alternative antimicrobial strategies capable of overcoming conventional resistance mechanisms. *Acinetobacter baumannii* has become one of the most clinically significant opportunistic microorganisms due to its remarkable capacity to survive under adverse environmental conditions and rapidly acquire resistance to multiple classes of antibiotics [4]. *A. baumannii* is a Gram-negative, non-fermentative, aerobic, non-motile coccobacillus belonging to the family Moraxellaceae. Although it was historically regarded as a low-virulence environmental organism, it has evolved into one of the leading causes of hospital-acquired infections, particularly among critically ill and immunocompromised

patients admitted to intensive care units (ICUs) [5]. The organism is frequently associated with ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound and burn infections, meningitis, and catheter-associated infections, all of which contribute to considerable morbidity and mortality in hospitalized patients [6]. *A. baumannii* accounts for the majority of clinically [4] significant isolates and is responsible for approximately 70% - 80% of reported *Acinetobacter* associated infections worldwide [7]. The organism's exceptional environmental adaptability and genetic plasticity have facilitated its emergence as a dominant nosocomial pathogen in healthcare settings across both developed and developing countries [8]. The incidence of *A. baumannii* infections has increased substantially over the past two decades, largely driven by the widespread use of broad-spectrum antibiotics and the growing population of critically ill patients requiring intensive medical care [9]. Pakistan has experienced a substantial increase in the prevalence of multidrug-resistant. Clinical isolates recovered from tertiary-care hospitals have demonstrated alarming levels of multidrug resistance, highlighting the urgent need for effective infection control measures and innovative therapeutic interventions [10]. The increasing prevalence of carbapenem-resistant isolates within Pakistani healthcare institutions reflects a broader global trend and underscores the necessity for developing alternative antimicrobial approaches that are less susceptible to conventional resistance mechanisms [11]. Nanotechnology has emerged as a promising approach for combating antimicrobial resistance by exploiting the unique physicochemical properties of nanomaterials. Owing to their nanoscale dimensions, high surface-area-to-volume ratio, and tunable surface chemistry, nanoparticles interact efficiently with bacterial cells and exert antimicrobial activity through multiple simultaneous mechanisms [12]. These include disruption of the bacterial membrane, release of metal ions, generation of reactive oxygen species (ROS), interference with protein synthesis, and damage to nucleic acids. Because these effects target multiple cellular structures simultaneously, nanoparticles are considered less susceptible to the development of bacterial resistance than conventional antibiotics [13]. Among metal oxide nanomaterials, Manganese doped zinc oxide (Mn-ZnO) nanoparticles have attracted considerable attention because of their broad-spectrum antimicrobial activity, chemical stability, biocompatibility, and low toxicity [14]. Their antibacterial activity is primarily mediated through reactive oxygen species (ROS) generation, Zn^{2+} ion release, and direct interactions with bacterial membranes, resulting in oxidative stress, membrane disruption, and bacterial cell death [15]. Despite these promising properties, the antibacterial potential of Mn-ZnO nanoparticles against multidrug-resistant (MDR), *Acinetobacter baumannii* remains inadequately explored. Most previous studies have focused on nanoparticle synthesis, characterization, or activity against common bacterial species, while evidence regarding their efficacy against clinically relevant MDR *A. baumannii* is limited [16]. In this study, Mn-ZnO nanoparticles were synthesized using a simple and cost-effective co-precipitation method and characterized to determine their structural, morphological, optical, and elemental properties. Their antibacterial activity was evalu-

ated against a clinical MDR *A. baumannii* isolate using an *in vitro* broth microdilution assay. By investigating the effect of manganese doped ZnO nanoparticles, this study provides insights into their potential antimicrobial strategy against drug-resistant pathogens and supports the development of nanoparticle-based coatings, wound dressings, and other biomedical applications for preventing healthcare associated infections.

2. Materials and Methods

2.1. Ethical Consideration

We ensured that all aspects of this study complied with recognized ethical consideration. This research study has been reviewed and approved by CECOS University Institutional Review Board (IRB). Confirm that all procedures meet established ethical guidelines. Additionally, No Objection Certificate (NOC) was obtained from the Khyber Teaching Hospital (KTH), Peshawar, permitting the use of the clinical isolate of *Acinetobacter baumannii* solely for the purpose of this research.

2.2. Study Design

This was an experimental study, specifically a laboratory based *in vitro* investigation conducted under controlled laboratory condition.

2.3. Study Setting and Duration

A clinical isolate of *Acinetobacter baumannii* was obtained from the Microbiology Laboratory of Khyber Teaching Hospital (KTH), Peshawar. The isolate was transported to the Institute of Integrative Biosciences (IIB) and the Medical Laboratory Technology (MLT) Laboratory at CECOS University, Peshawar, for subsequent experimental work. Nanoparticle characterization was carried out at the University of Agriculture, Peshawar. The study was conducted over a period of six (6) months.

2.4. Materials

Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), Manganese(II) nitrate tetra hydrate ($\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), Potassium hydroxide (KOH) or ammonium hydroxide (NH_4OH), Deionized (DI) water, Ethanol (for washing and dispersion), Nutrient agar and nutrient broth. Magnetic stirrer with a hot plate, Beakers (100 mL, 250 mL, 500 mL), Falcon Tubes, Glass rods, pH meter, Centrifuge machine, Analytical Balance, Ultrasonicator, Drying oven, Furnace, Photo spectrometer, Autoclave, Biosafety cabinet, Flow hood, Pipette, 96 well plate, Incubator and light incubator, Flask.

2.5. Bacterial Sample Collection

The *Acinetobacter baumannii* isolate, obtained from Khyber Teaching Hospital (KTH), Peshawar, was identified as multidrug-resistant (MDR), exhibiting re-

sistance to ceftazidime (CAZ), ciprofloxacin (CIP), doxycycline (DO), imipenem (IPM), gentamicin (GEN) and amikacin (AK). The resistance profile was determined through antimicrobial sensitivity testing conducted at KTH using the Kirby-Bauer disc diffusion method. The sample was transported to the IIB Laboratory CECOS University under sterile conditions to prevent contamination.

2.6. Preparation of Media

Nutrient broth, Nutrient agar, and MacConkey agar were prepared according to the manufacturer's instructions for the cultivation, subculturing, and isolation of bacterial isolates. The required quantity of each dehydrated medium was weighed and dissolved in the appropriate volume of distilled water with continuous stirring until completely dissolved. The pH of the media was adjusted to the recommended value (approximately 7.2 - 7.4) where necessary. The prepared media were sterilized by autoclaving at 121°C and 15 psi for 15 - 20 minutes. After sterilization, nutrient broth was allowed to cool and was dispensed aseptically into sterile test tubes. Nutrient agar and MacConkey agar were cooled to approximately 45°C - 50°C before being poured into sterile Petri plates under aseptic conditions and allowed to solidify. The prepared media were stored at 4°C until use. Nutrient broth was used for bacterial enrichment, nutrient agar for routine subculturing and maintenance of bacterial isolates, and MacConkey agar for the selective isolation and differentiation of Gram-negative bacteria based on lactose fermentation.

2.7. Culturing of Bacteria and Confirmatory Tests

The known MDR *Acinetobacter baumannii* isolate obtained from Khyber Teaching Hospital (KTH), Peshawar, was sub cultured in the Institute of Integrative Biosciences (IIB) Laboratory, CECOS University Peshawar, to obtain fresh cultures for experimental use. The identity of the isolate was reconfirmed using standard microbiological methods, including Gram staining, catalase test and growth pattern observation on MacConkey agar, where the organism produced non-lactose fermenting colonies. Bacterial isolates were identified to the species level using the VITEK 2 automated microbial identification system (bioMérieux, France) according to the manufacturer's instructions. Isolates identified as *Acinetobacter baumannii* were included for subsequent analysis. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The antibiotics tested were cefepime (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), and minocycline (30 µg). Following incubation at 37°C for 18 - 24 h, inhibition-zone diameters were measured in millimeters and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2025 criteria. The isolate was used to evaluate the *in vitro* antibacterial activity of Mn-doped ZnO nanoparticles.

2.8. Synthesis of Nanoparticles

Mn-doped ZnO nanoparticles were synthesized using the co-precipitation method.

Briefly, 3 g of zinc nitrate hexahydrate and 0.3 g of manganese nitrate tetrahydrate were dissolved in 50 mL of distilled water and stirred magnetically at room temperature for 10 - 15 minutes until a clear homogeneous solution was obtained. Separately, 9 g of potassium hydroxide (KOH) pellets were dissolved in 50 mL of distilled water to prepare the precipitating agent. The KOH solution was then added dropwise from a burette into the metal nitrate solution under continuous magnetic stirring at 600 - 800 rpm until a dirty brown precipitate formed, indicating complete precipitation. The resulting precipitate was collected by centrifugation at 9500 rpm for 15 minutes, washed three times with distilled water to remove residual nitrates, followed by a final wash with ethanol. After each washing step, the precipitate was resuspended and centrifuged again. The purified precipitate was dried in a hot air oven at 80°C for 12 - 16 hours (overnight). Finally, the dried powder was calcined in a muffle furnace at 200°C for 2 hours, 300°C for 1 hour, and 400°C for 1 hour using a heating rate of 5°C/min. After cooling naturally to room temperature, the calcined product was collected as Mn-doped ZnO nanoparticles.

2.9. Characterization of Nanoparticles

The synthesized manganese-doped zinc oxide nanoparticles were characterized at the Agriculture University, Peshawar, using multiple analytical techniques. X-ray Diffraction (XRD) analysis was carried out to determine the crystalline structure, phase composition, and average crystallite size of the nanoparticles. Scanning Electron Microscopy (SEM) was performed to examine the surface morphology and particle size distribution. Atomic Absorption Spectroscopy (AAS) was used to quantitatively determine the elemental composition of zinc and manganese. UV-Visible spectroscopy was employed to analyze the optical absorption properties and to estimate the band gap energy of the nanoparticles. FT-IR analysis was performed to identify the functional groups and chemical bonds associated with the synthesized Mn-doped ZnO nanoparticles. The FT-IR spectrum was recorded over the appropriate mid-infrared spectral range, and the characteristic absorption bands were analyzed and assigned based on their corresponding functional groups and previously reported ZnO-related vibrational modes.

2.10. Preparation and Dilution of Nanoparticles

The synthesized Mn-doped ZnO nanoparticles were accurately weighed using an analytical balance, and 10 mg of the nanoparticles was transferred into a sterile 15 mL centrifuge tube. Sterile distilled water was added to make a final volume of 10 mL, yielding a primary stock suspension with a concentration of 1 mg/mL (1000 µg/mL). The suspension was vortexed for approximately 60 seconds and subsequently sonicated in a bath sonicator for 10 - 15 minutes, with intermittent cooling intervals to prevent overheating and ensure uniform nanoparticle dispersion. Working suspensions of Mn-doped ZnO nanoparticles were prepared at concentrations of 10, 20, and 30 µg/mL. Equal volumes (100 µL) of each nanoparticle suspension and bacterial inoculum were added to the wells of a sterile 96-well mi-

cro-titer plate. Consequently, the final nanoparticle concentrations in the assay wells were 10, 20, and 30 µg/mL, respectively. Accordingly, 0.20 mL, 0.40 mL, and 0.60 mL of the stock suspension were transferred into separate sterile tubes and diluted with 9.80 mL, 9.60 mL, and 9.40 mL of sterile distilled water, respectively. Each working suspension was prepared under aseptic conditions, vortexed for 30 seconds, and briefly sonicated for 2 - 5 minutes before use to minimize nanoparticle aggregation and ensure a homogeneous dispersion. All stock and working solutions were freshly prepared under sterile conditions to maintain reproducibility and prevent contamination during subsequent microbiological assays.

2.11. Antibacterial Assessment of Mn-Doped ZnO Nanoparticles

The antibacterial activity of Mn-doped ZnO nanoparticles against *Acinetobacter baumannii* was evaluated by using the broth microdilution method in sterile 96-well flat-bottom microtiter plates. Three nanoparticle concentrations (20, 40, and 60 µg/mL) were used in the assay. A fresh culture of *A. baumannii* was obtained by streaking the bacterial sample on MacConkey agar to observe colony morphology, followed by Gram staining to confirm the Gram-negative nature of the isolate. Although the strain was already known, these confirmatory steps were performed for record purposes. The confirmed culture was then transferred into sterile nutrient broth and incubated in a shaking incubator at 37°C for 18 - 24 hours. The turbidity was adjusted to the 0.5 McFarland standard and diluted 1:100 in sterile nutrient broth to obtain an inoculum of approximately 1×10^6 CFU/mL. 100 µL of each nanoparticle concentration was dispensed into the designated wells of the 96-well plate, followed by 100 µL of the prepared bacterial inoculum, making a final volume of 200 µL per well and a bacterial density of approximately $5 - 7.5 \times 10^5$ CFU/mL. All experiments were performed in triplicate wells as technical replicates using the same bacterial inoculum. The results are presented as the mean $\pm$ standard deviation (SD) of the triplicate measurements. No inferential statistical test was performed to compare differences among the nanoparticle concentrations. As the primary objective of this study was to evaluate the antibacterial activity of Mn-doped ZnO nanoparticles against a clinical MDR *Acinetobacter baumannii* isolate.

2.12. Controls Used

To ensure the accuracy and reliability of the antibacterial assay, appropriate experimental controls were included. A sterility control (negative control) consisting of nutrient broth only, without bacterial inoculum or nanoparticles, was used to confirm that the culture medium and experimental conditions were free from contamination. A growth control (positive control) containing nutrient broth inoculated with the test bacteria but without nanoparticles was included to verify normal bacterial growth under the experimental conditions. In addition, a nanoparticle control (NP blank) comprising nutrient broth supplemented with Mn-doped ZnO nanoparticles but without bacterial inoculation was prepared to account for any background turbidity or optical interference caused by the nano-

particles during absorbance measurements. These controls ensured the validity and accurate interpretation of the antibacterial activity results.

2.13. Assessment of Antibacterial Activity and Growth Inhibition

Following inoculation, the 96-well microtiter plate was incubated at 37°C for 18 - 24 hours in a light incubator to allow bacterial growth. After incubation, bacterial growth was quantified by measuring the optical density at 600 nm (OD_{600}) using a microplate reader. To eliminate any interference caused by the intrinsic turbidity of the Mn-doped ZnO nanoparticles, the optical density values obtained from the nanoparticle control wells were used to correct the corresponding test wells. The antibacterial activity of the nanoparticles was then determined by calculating the percentage inhibition of bacterial growth using the corrected optical density values. This calculation enabled the quantitative assessment of the inhibitory effect of Mn-doped ZnO nanoparticles on bacterial growth.

3. Results

In this study, manganese-doped zinc oxide (Mn-ZnO) nanoparticles were successfully synthesized using the co-precipitation method. The resulting nanoparticles appeared as a light brown precipitate, which was thoroughly washed, dried, and ground into a fine powder for further analysis. This investigation proceeded with the characterization of the synthesized nanoparticles to evaluate their morphology, structure, and elemental composition. Scanning Electron Microscopy (SEM) was employed to observe the surface morphology and particle size distribution, while Atomic Absorption Spectroscopy (AAS) was used to quantify the manganese content within the nanoparticles. The SEM analysis revealed detailed surface features and particle size uniformity, whereas the AAS confirmed the successful doping of manganese into the ZnO matrix.

3.1. Spectroscopy of UV-Visible

To confirm either Mn doped zinc oxide nanoparticles was synthesized or not the UV-Vis Spectroscopy analysis was performed. A range of 300 - 600 nm was used to scan the nanoparticles. The UV-visible absorption spectra of Mn-doped zinc oxide NPs at room temperature are shown in **Figure 1**. A peak in the spectra at 359 nm indicated that manganese doped zinc oxide nanoparticles had formed.

3.2. XRD (X-Ray Diffraction)

Effective production of Mn-doped zinc oxide nanoparticles with a hexagonal wurtzite crystal structure was confirmed by the XRD analysis. Manganese doping does not produce any additional phases, suggesting that the material may find use in various optoelectronic and photocatalytic applications. The high degree of purity of the produced nanoparticles indicated by the lack of peaks corresponding to impurity phases. The Mn-doped zinc oxide nanoparticles' average size was found to be 28 nm using the Scherer equation. Details were shown in **Figure 2**.

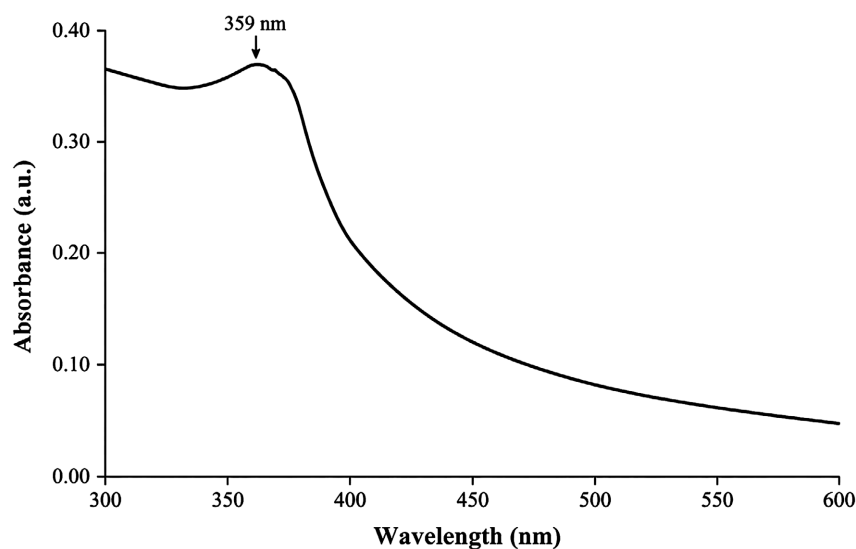


Figure 1. The above figure shows spectroscopy of Mn-doped ZnO NPs.

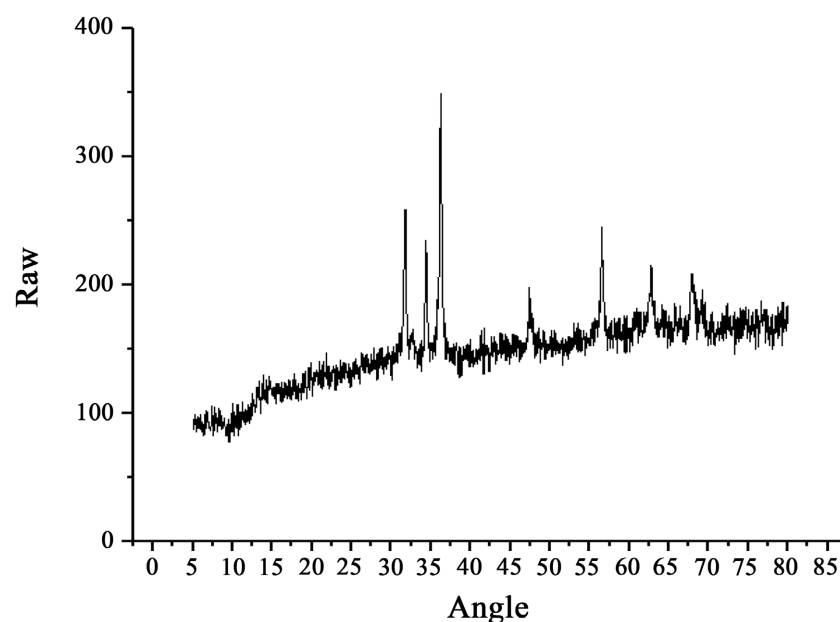


Figure 2. The above figure shows Mn-doped ZnO NPs XRD pattern.

3.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was employed to characterize the surface morphology of the synthesized manganese-doped zinc oxide (Mn-doped ZnO) nanoparticles. The SEM micrographs acquired at magnifications of $\times 30,000$ and $\times 60,000$ revealed irregularly shaped, agglomerated nanoparticles with non-uniform morphology. The observed aggregation is typical of metal oxide nanoparticles and is attributed to their high surface energy. The SEM analysis confirmed the successful synthesis of Mn-doped ZnO nanoparticles and provided detailed information regarding their surface morphology and structural characteristics, as shown in **Figure 3**.

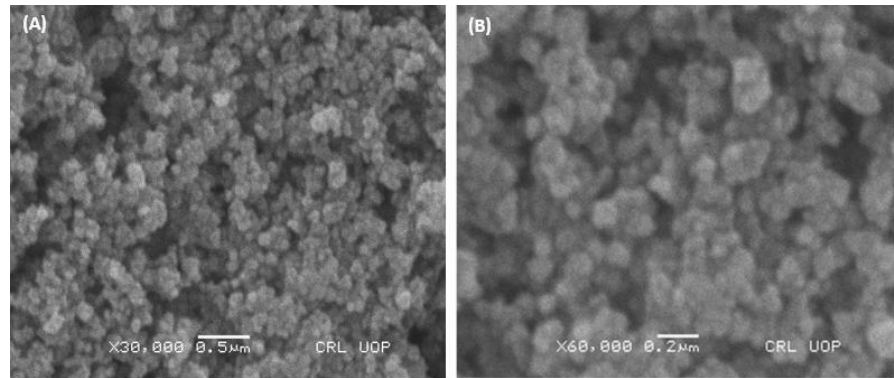


Figure 3. SEM photographs of manganese doped zinc oxide nanoparticles.

3.4. Atomic Absorption Spectroscopy (AAS)

The findings of Atomic Absorption Spectroscopy (AAS), with an emphasis on the concentrations of zinc and manganese in the generated nanoparticles, gave quantitative information on the elemental composition. This information offers a crucial foundation for understanding the overall composition and doping efficiency of the nanoparticles. The quantities of zinc (Zn) and manganese (Mn) in the synthesized manganese-doped zinc oxide nanoparticles were measured using Atomic Absorption Spectrometry (AAS). The average results are displayed below in **Table 1**.

Table 1. Shows the AAS result of the manganese-doped zinc oxide nanoparticles.

Analysis/Test	Parameter	Value
Manganese (Mn) Analysis	Concentration	0.197 mg/L
	Absorbance	0.0995
	% Recovery	50.42%
	Wavelength	279.5 nm
Zinc (Zn) Analysis	Concentration	21.10 mg/L
	Absorbance	0.3002
	Wavelength	213.9 nm

3.5. FT-IR Spectroscopy of the NPs

FT-IR spectroscopy was employed to identify the functional groups associated with the synthesized Mn-doped ZnO nanoparticles. The FT-IR spectrum exhibited a broad absorption band at 3437.8 cm^{-1} , corresponding to the O-H stretching vibration of surface hydroxyl groups and adsorbed water molecules. The absorption band at 2925.8 cm^{-1} was attributed to C-H stretching vibrations. Peaks observed at 1595.8 cm^{-1} , 1383.7 cm^{-1} , and 1355.5 cm^{-1} were assigned to O-H bending and residual nitrate or carbonate-related vibrations. Additional absorption bands at 1083.3 cm^{-1} , 863.4 cm^{-1} , and 770.6 cm^{-1} were associated with metal-

oxygen and lattice vibration modes. The characteristic absorption band at 575.9 cm^{-1} was assigned to the Zn-O stretching vibration, confirming the successful formation of Mn-doped ZnO nanoparticles. **Figure 4** showed the details of functional group.

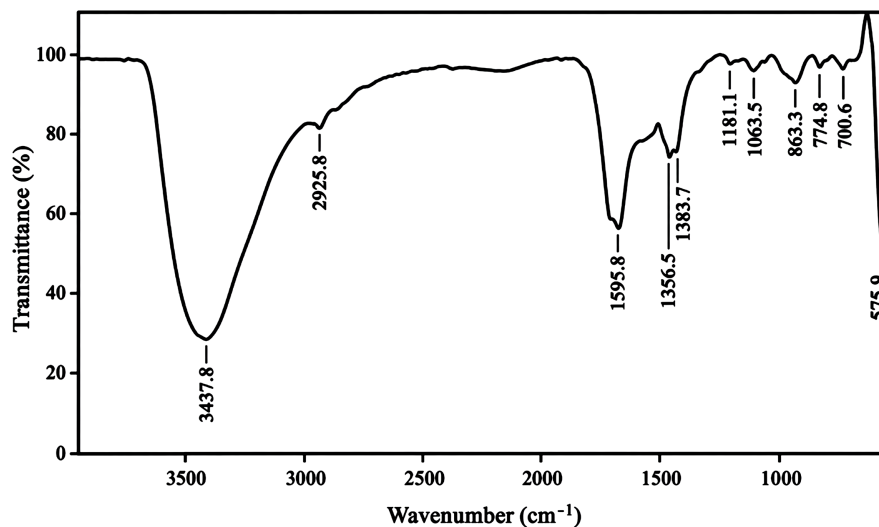


Figure 4. The above figure shows FTIR analysis of the Mn-doped ZnO NPs, different peaks indicate different functional groups.

3.6. Culture of *Acinetobacter baumannii*

On MacConkey agar, *Acinetobacter baumannii* produced small, round, smooth, and translucent to slightly opaque colonies that appeared pale or colorless due to its non-lactose-fermenting nature. The absence of pink coloration distinguished it from lactose fermenters such as *E. coli* and *Klebsiella*. Growth was observed after incubation at 37°C for 18 - 24 hours, confirming its characteristics as a Gram-negative, non-lactose-fermenting organism. As shown in **Figure 5**.



Figure 5. The above figure shows the colonies of *Acinetobacter baumannii*.

3.7. Gram Staining of *Acinetobacter baumannii*

Gram staining of the isolated colonies revealed *Acinetobacter baumannii* as Gram-negative *coccobacilli* as shown in **Figure 6**, appearing as short, plump, pink-colored rods under the microscope. The organism was observed as non-motile and arranged singly or in pairs. The absence of purple staining confirmed its Gram-negative nature, consistent with the typical morphology of *A. baumannii*.

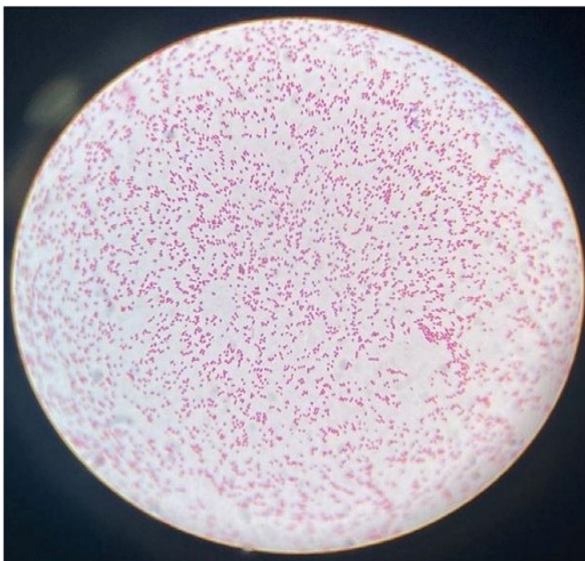


Figure 6. Gram stain of *Acinetobacter baumannii* showing gram negative coccobacilli.

3.8. Catalase Test

The catalase test for *Acinetobacter baumannii* was performed to determine its ability to produce the enzyme catalase. Upon the addition of hydrogen peroxide to a small portion of the bacterial colony, immediate effervescence (bubble formation) was observed, indicating a positive catalase reaction. This confirmed that *A. baumannii* possesses the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. Details were also shown in **Figure 7**.

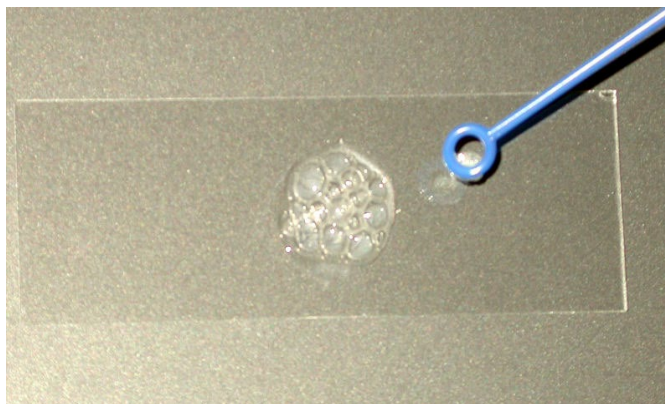


Figure 7. Catalase test of *Acinetobacter baumannii* showing bubble formation, indicating a positive result.

3.9. Oxidase Test

The oxidase test was performed by impregnating a sterile swab with oxidase reagent as shown in **Figure 8**, and then using it to pick a small portion of the bacterial colony. The swab was observed for any color change. No purple coloration developed on the swab within 30 seconds, indicating a negative oxidase reaction. This result confirmed that *Acinetobacter baumannii* was oxidase-negative, which is consistent with its known biochemical characteristics.



Figure 8. Oxidase test showing swab with no color change, indicating a negative result for *Acinetobacter baumannii*.

3.10. Antibiotic Susceptibility Test

The antibiotic susceptibility test was performed to reconfirm the multidrug-resistant (MDR) nature of the *Acinetobacter baumannii* isolate, although it was previously identified as MDR. The test was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. After incubation at 37°C for 18 - 24 hours, the isolate exhibited resistance to multiple antibiotic classes, including β -lactams, aminoglycosides, fluoroquinolones, and carbapenems. *A. baumannii* isolate demonstrated resistance to cefepime (30 μ g), ciprofloxacin (5 μ g), and meropenem (10 μ g), with no measurable inhibition zones (0 mm), and to amikacin (30 μ g), which produced a 10-mm inhibition zone. In contrast, minocycline (30 μ g) produced an 18-mm inhibition zone and was interpreted as susceptible according to CLSI 2025 criteria. The isolate therefore demonstrated nonsusceptibility to antimicrobial agents from multiple antimicrobial classes, supporting its classification as multidrug-resistant (MDR) *A. baumannii*. The isolate is sensitive only to Minocycline and is therefore confirmed as multidrug-resistant (MDR) *Acinetobacter baumannii*. As shown in **Figure 9** and **Table 2**.

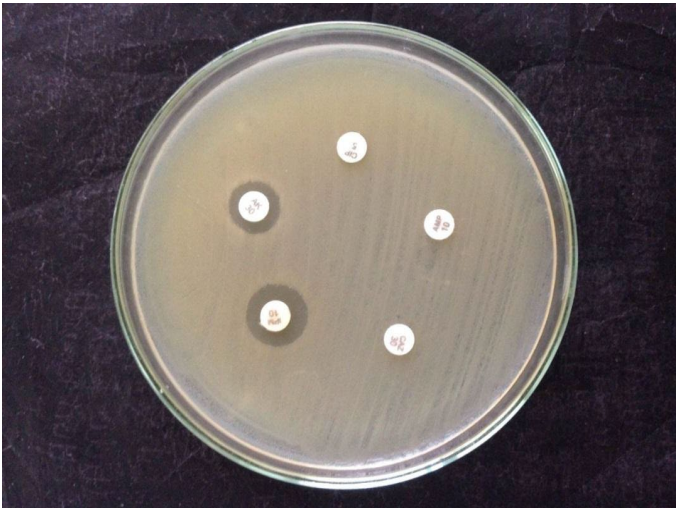


Figure 9. Antibiotic Susceptibility test confirming *Acinetobacter baumannii* is MDR.

Table 2. Antimicrobial susceptibility profile of the multidrug-resistant *Acinetobacter baumannii* isolate determined by the Kirby-Bauer disk diffusion method, showing antibiotic class, disk potency, inhibition-zone diameter, and susceptibility interpretation according to CLSI 2025 criteria.

Antibiotic	Disk potency	Zone	Interpretation
Cefepime	30 µg	0 mm	Resistant
Amikacin	30 µg	10 mm	Resistant
Ciprofloxacin	5 µg	0 mm	Resistant
Meropenem	10 µg	0 mm	Resistant
Minocycline	30 µg	18 mm	Susceptible

3.11. Antibacterial Assessment of Mn-Doped ZnO Nanoparticles

The antibacterial activity of Mn-doped ZnO nanoparticles against *Acinetobacter baumannii* was assessed using OD₆₀₀ readings to calculate percentage inhibition and growth. The results showed a concentration-dependent inhibitory effect, where higher nanoparticle concentrations led to greater bacterial growth suppression. At 10 µg/mL, inhibition was moderate, with noticeable bacterial growth still present. Increasing the concentration to 20 µg/mL resulted in a substantial reduction in bacterial proliferation, while 30 µg/mL exhibited the highest antibacterial activity, with minimal bacterial growth observed. The growth control showed maximum OD, confirming normal bacterial growth in the absence of nanoparticles, while the sterility control showed no detectable growth, ensuring assay validity.

The percentage inhibition was calculated using the formula:

The percentage growth was calculated as: % Growth = 100 – % Inhibition. Details were also shown in Table 3. Figure 10 illustrated the effect of different con-

centrations of Mn-doped ZnO nanoparticles on the growth and inhibition of *Acinetobacter baumannii*. At 10 µg/mL, the bacterial growth was relatively higher compared to inhibition, indicating a weaker antibacterial effect at lower concentration. However, as the concentration increased to 20 µg/mL, inhibition rose notably while growth decreased, showing a concentration-dependent response. At the highest tested concentration of 30 µg/mL, inhibition reached its peak with minimal bacterial growth observed, reflecting a strong antibacterial activity of the nanoparticles. Overall, the results showed that Mn-doped ZnO nanoparticles exhibited a clear dose-dependent inhibitory effect against *A. baumannii*.

Table 3. OD₆₀₀ values, percentage inhibition, and percentage growth of *A. baumannii* at different Mn-doped ZnO nanoparticle concentrations.

Final Concentration (µg/mL)	Mean OD ₆₀₀ ± SD	% Inhibition ± SD	% Growth ± SD
Blank control	0.050 ± 0.000	-	-
10	0.412 ± 0.012	45.6 ± 1.8	54.4 ± 1.8
20	0.298 ± 0.009	62.7 ± 1.4	37.3 ± 1.4
30	0.105 ± 0.006	91.7 ± 0.9	8.3 ± 0.9
Growth control (Positive)	0.715 ± 0.015	-	100
Sterility control (Negative)	0.000 ± 0.000	-	-

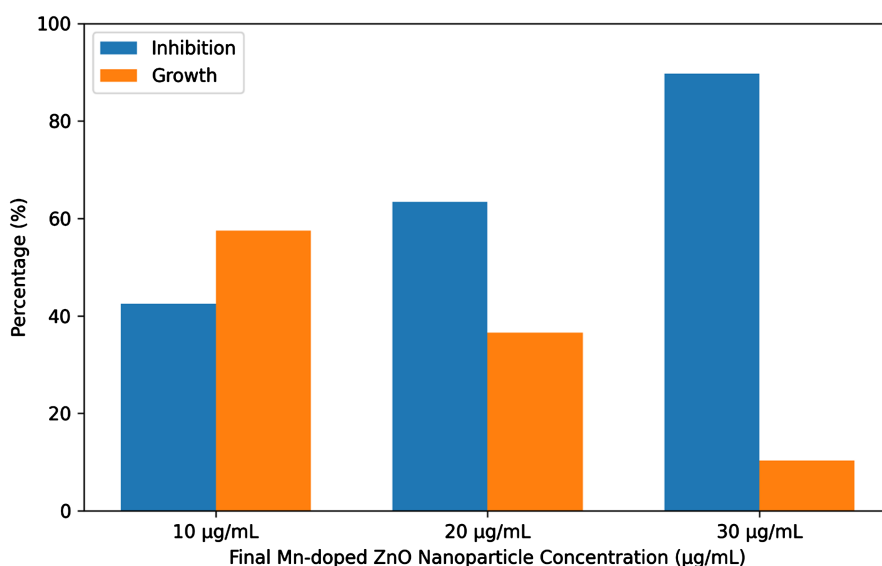


Figure 10. The above bar graph shows concentration-dependent antibacterial activity of Mn-doped ZnO nanoparticles against multidrug-resistant *Acinetobacter baumannii*.

4. Discussion

Antimicrobial resistance (AMR) has emerged as one of the most significant threats to global public health, compromising the effectiveness of antibiotics and

increasing the burden of infectious diseases worldwide [1]. This study demonstrated that manganese-doped zinc oxide (Mn-ZnO) nanoparticles effectively suppressed the growth of *A. baumannii* at concentrations of 10, 20, and 30 µg/mL, as measured by optical density. Growth inhibition was evident at all tested concentrations, with the strongest effect observed at higher doses, confirming a clear concentration-dependent antibacterial activity. These findings establish that Mn-ZnO nanoparticles exert measurable inhibitory effects against this multidrug-resistant pathogen. The observed antibacterial activity aligns with previous reports showing that manganese doped ZnO nanoparticles antimicrobial potential [17]. Another study demonstrated that Mn-ZnO synthesized through green methods inhibited both Gram-positive and Gram-negative bacteria, attributing the effect to increased surface defects and oxidative stress [18]. Similarly, other studies reported Mn-ZnO nanomaterials to be effective against *A. baumannii*, supporting the consistency of the present findings with broader literature [19]. The antibacterial mechanisms of Mn-ZnO nanoparticles are well established and include generation of reactive oxygen species (ROS), membrane disruption, leakage of cytoplasmic proteins, and release of Zn^{2+} ions [15]. This multi-target mode of action reduces the likelihood of resistance development compared to conventional antibiotics [20]. The promising antibacterial effect observed suggests potential biomedical applications of Mn-ZnO NPs, such as their use in antimicrobial coatings for medical devices, incorporation into wound dressings, or as surface disinfectants in hospital environments [21]. Their ability to suppress growth of MDR *A. baumannii* highlights their relevance in clinical infection control. The present study was limited to *in vitro* assessment using optical density as a growth indicator. While these results are promising, further studies are required to confirm cytotoxicity profiles in mammalian cells, evaluate *in vivo* efficacy, and explore synergistic effects with existing antibiotics. Such investigations will be essential to translate these findings into practical clinical applications. A limitation of the present study is the absence of an undoped ZnO nanoparticle control. Therefore, although the synthesized Mn-doped ZnO nanoparticles demonstrated antibacterial activity against MDR *Acinetobacter baumannii*, the specific contribution of manganese doping relative to undoped ZnO nanoparticles could not be determined. Future studies should include direct comparative evaluations of undoped and Mn-doped ZnO nanoparticles under identical experimental conditions to better elucidate the role of manganese incorporation in enhancing antibacterial performance.

5. Conclusion

This study successfully synthesized manganese-doped zinc oxide (Mn-ZnO) nanoparticles using a chemical precipitation method and demonstrated their antibacterial activity against *A. baumannii*, a multidrug-resistant pathogen of major clinical concern. The nanoparticles inhibited bacterial growth in a concentration-dependent manner, with measurable effects observed at 10 µg/mL, stronger inhi-

bition at 20 µg/mL, and the highest bacteriostatic/growth-inhibitory activity at 30 µg/mL. These results highlight the effectiveness of Mn-ZnO nanoparticles at relatively low concentrations and confirm their potential as promising antimicrobial agents. The findings contribute to the growing body of evidence supporting the application of nanotechnology in combating antimicrobial resistance. While the results are encouraging, further investigations are needed to advance these nanoparticles toward clinical application. Future studies should include cytotoxicity testing on mammalian cell lines, *in vivo* efficacy evaluation, and exploration of synergistic effects with conventional antibiotics. Such investigations will be essential to ensure both the safety and therapeutic effectiveness of Mn-ZnO nanoparticles in biomedical applications.

Author Contributions

Dr. Sami Ullah and Dr. Kamran supervised the experimental work, contributed to the study methodology, conceived and designed the study, Wajid Nawaz performed the experiments, analyzed the data, and drafted the initial manuscript. Muhammad Jawad, Mehwish, Yasin Khan, Abu Huraira, Kamran Khan, Mehmood Khan, Shakir Ullah, Uzma Khan, Suneel Gill, Farman Ullah Khan, Asim Ullah, Nasir Shah and Salman Nawaz contributed to the laboratory experiments, data collection, and validation of the results. Gulzar Ahmad and Muhammad Ijaz performed the formal data analysis, statistical interpretation, and contributed to manuscript revision and editing. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Data Availability Statement

The data supporting the findings of this study are included within the manuscript.

Conflicts of Interest

The authors declare that they have no competing financial or non-financial interests related to this manuscript.

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