

Synthesis and antibacterial evaluation of Ag–ZnO nanocomposites against *Pseudomonas* and *Staphylococcus* species: A combined experimental and molecular docking study

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Abstract

Due to increasing antibacterial resistance, new antimicrobial materials are required. Metal-based nanocomposites are of interest because they may exert antibacterial effects through multiple mechanisms of action. In this study, an Ag–ZnO nanocomposite was synthesized via a chemical reduction method and characterized using XRD, FTIR, SEM, and EDX techniques. XRD results revealed the coexistence of hexagonal wurtzite ZnO and face-centered cubic (FCC) Ag phases, with an average crystallite size of approximately 15.6 nm. SEM images showed quasi-spherical agglomerated nanoparticles, while EDX analysis confirmed the presence of Ag, Zn, and O. The Ag–ZnO nanocomposite exhibited antibacterial activity against *P. aeruginosa*, *P. chengduensis*, *S. aureus*, and *S. haemolyticus*, with inhibition zones increasing with the applied volume. The differences among mean inhibition zones at various tested volumes were significant for all four bacteria by one-way ANOVA ($p < 0.001$; $\eta^2 = 0.962–0.976$). The minimum inhibitory concentration (MIC) values ranged from 0.125 to 0.50 mg/mL, with *P. chengduensis* showing the lowest MIC value of 0.125 mg/mL. To investigate possible interactions between the Ag–ZnO nanocluster and bacterial protein targets, molecular docking studies were performed against MurC, Sortase A and DNA gyrase B. The Ag–ZnO nanocluster showed favorable predicted interactions with all three proteins, with the most favorable docking score obtained for MurC, followed by DNA gyrase B and Sortase A. Overall, the Ag–ZnO nanocomposite showed promising antibacterial activity, while molecular docking provided exploratory insights into potential protein interactions that require further experimental validation.

Keywords: Ag–ZnO nanocomposite, Antibacterial activity, Minimum inhibitory concentration, Molecular docking, MurC

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Introduction

Nanotechnology is rapidly emerging and gained much significance in medical field for both diagnostic and therapeutic applications (Haleem et al., 2023). The increasing emergence of antibiotic-resistant pathogens has created an urgent need for development of alternative strategies to combat antimicrobial resistance. Nanoparticles are of particular interest because they can act through different mechanisms and target multiple sites in bacterial cells, potentially making the development of resistance more complex as multiple genetic changes may be required (Mondal et al., 2024; Wang et al., 2017). This multi-target activity is particularly valuable in addressing pathogenic bacteria that can form biofilms as nanoparticles have also been reported to interfere with biofilm formation and disrupt established biofilms (Hoseinzadeh et al., 2017; Tungare et al., 2024).

The various inorganic metal-based nanoparticles have numerous advantages over other conventional antibacterial materials because of their extremely small size, large surface area, surface charge, etc. which help in greater interaction with microorganisms (Yang et al., 2022). Among various inorganic metal-based nanoparticles, the bimetallic nanoparticles (BNPs) have gained considerable interest due to their varied physical and chemical properties and distinct mechanisms of action. BNPs are generally composed of two different metal components, and interactions between these components have been reported to enhance antibacterial activity compared with their individual monometallic counterparts (Arora et al., 2020; Malik et al., 2023; Padilla-Cruz et al., 2021). This enhanced activity has been associated with interactions between the constituent metals, with antibacterial effects involving membrane degradation, disruption of bacterial homeostasis, oxidative stress and generation of reactive oxygen species (Singh et al., 2022).

Silver nanoparticle generally ranging from 1 to 100 nm in size, have received considerable scientific interest due to their distinctive physicochemical properties and biological activities, particularly their antibacterial and antibiofilm effects (Almatroudi, 2020; Sati et al., 2025). The size- and shape-dependent physical and chemical properties of AgNPs render them capable of diverse biomedical applications. Importantly, their physicochemical characteristics can be manipulated by using chemical, physical and biological approaches to endow them with required

properties for various biomedical applications (Lee and Jun, 2019). AgNPs exhibit antibacterial activity against both Gram-positive and negative bacterial strain, including multidrug-resistant strains, with activity reported against clinically relevant species such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Ahmed et al., 2021; Bruna et al., 2021). Their antibacterial action has been associated with the release of Ag⁺ ions, which can interact with cellular components such as proteins and DNA, together with induction of oxidative stress and subsequent cellular damage (Fahim et al., 2024; Karataş et al., 2026; Targhi et al., 2021).

Nanoparticles containing zinc especially ZnO have recently gained considerable attention for biomedical applications, because of their antimicrobial activity, low toxicity under certain conditions and for their good biocompatibility (Aderibigbe, 2024; Asif et al., 2023). Their antibacterial activity is influenced by physicochemical characteristics such as particle size and morphology, as well as concentration (Jin and Jin, 2021; Mendes et al., 2024). Silver nanoparticles and zinc oxide nanoparticles have found applications in various environmental, biomedical, pharmaceutical and antibacterial applications. Their optical and catalytic properties are size and shape dependent (Hashem and El-Sayyad, 2024; Panwar and Yadav, 2022; Roy et al., 2026). Ag- and Zn-based nanostructures have shown antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Abdallah et al., 2022; Holubnycha et al., 2024). The combination of silver and zinc in bimetallic nanostructures has attracted interest for improving antibacterial activity by combining the complementary properties of the constituent metals. Recent studies have demonstrated that silver- and zinc-based nanostructures have potential to suppress, growth of both Gram-positive and Gram-negative bacteria (Dutta et al., 2023; Es-haghi et al., 2024; Madipoju et al., 2025). There is growing interest in Ag–ZnO nanomaterials as antibacterial agents. However, there is still a lack of studies on the antibacterial activity of chemically synthesized Ag–ZnO nanocomposites, particularly against new, rarely studied species of bacteria, for example *Pseudomonas chengduensis* and *Staphylococcus haemolyticus*. The objective of this study was to investigate the properties of Ag–ZnO nanocomposites, which were synthesized using a

chemical method, and their antibacterial activity against Gram-positive and Gram-negative human pathogens.

In this study, an Ag–ZnO nanocomposite was synthesized, characterized and investigated for its antibacterial activity against *Pseudomonas aeruginosa*, *Pseudomonas chengduensis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*. In addition, the study performed molecular docking of Ag–ZnO to investigate interactions with target proteins MurC, Sortase A and DNA gyrase B. Together, these experimental and computational approaches provide further insights into the antibacterial potential of Ag–ZnO nanocomposites and serve as a basis for further studies.

Material and Methods

Synthesis

Chemicals required

Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 98%, Cat. No. 228737) and silver nitrate (AgNO_3 , 99.8–100.5%, Cat. No. 10220) were used as metal precursors. Polyethylene glycol 6000 (PEG-6000, Cat. No. 81260) was used as a capping agent. L-Ascorbic acid (vitamin C, 99%, Cat. No. A92902) and anhydrous sodium carbonate (Na_2CO_3 , 99.5–100.5%, Cat. No. 13418) were used as the reducing and alkalinizing agents, respectively. All chemicals were obtained from Sigma-Aldrich, USA. Deionized water was used to prepare all solutions.

Synthesis of Ag–ZnO bimetallic nanoparticles

Ag–ZnO bimetallic nanoparticles were synthesized using a chemical reduction method. Based on preliminary optimization experiments, 30 mL of an aqueous solution containing L-ascorbic acid (0.057 M) and anhydrous Na_2CO_3 (0.094 M) was prepared in deionized water and stirred continuously at 800 rpm at room temperature under ambient atmospheric conditions. L-ascorbic acid served as the reducing agent, while Na_2CO_3 provided an alkaline reaction medium.

Following complete dissolution, 30 mL of 0.06 M AgNO_3 solution was slowly added dropwise under continuous stirring. Subsequently, 30 mL of 0.04 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution was added dropwise in the same manner, maintaining a nominal Ag:Zn molar ratio of 60:40. 0.60 g of PEG-6000 was added to reaction mixture after complete addition of both metal precursor solutions. The added PEG-6000 functions as a capping and stabilizing agent to prevent particle aggregation. The mixture was then further heated to 60 °C and vigorously stirred at 800 rpm for 30 minutes. The visible color change began following the addition of AgNO_3 and gradually intensified as the reaction proceeded, with the reaction mixture developing a progressively darker appearance and ultimately becoming dark blackish-grey. The mixture gradually turned color indicating the preliminary formation of nanoparticles. The collected nanoparticles were pelleted by centrifugation at 8000 rpm for 10 minutes. The resulting pellet was washed 3 times, firstly with ethanol followed by washes with deionized water to remove excess residues. The nanoparticles were then dried at 55 °C and placed in a sealed container until further physicochemical characterization and biological evaluation at room temperature (Figure 1).

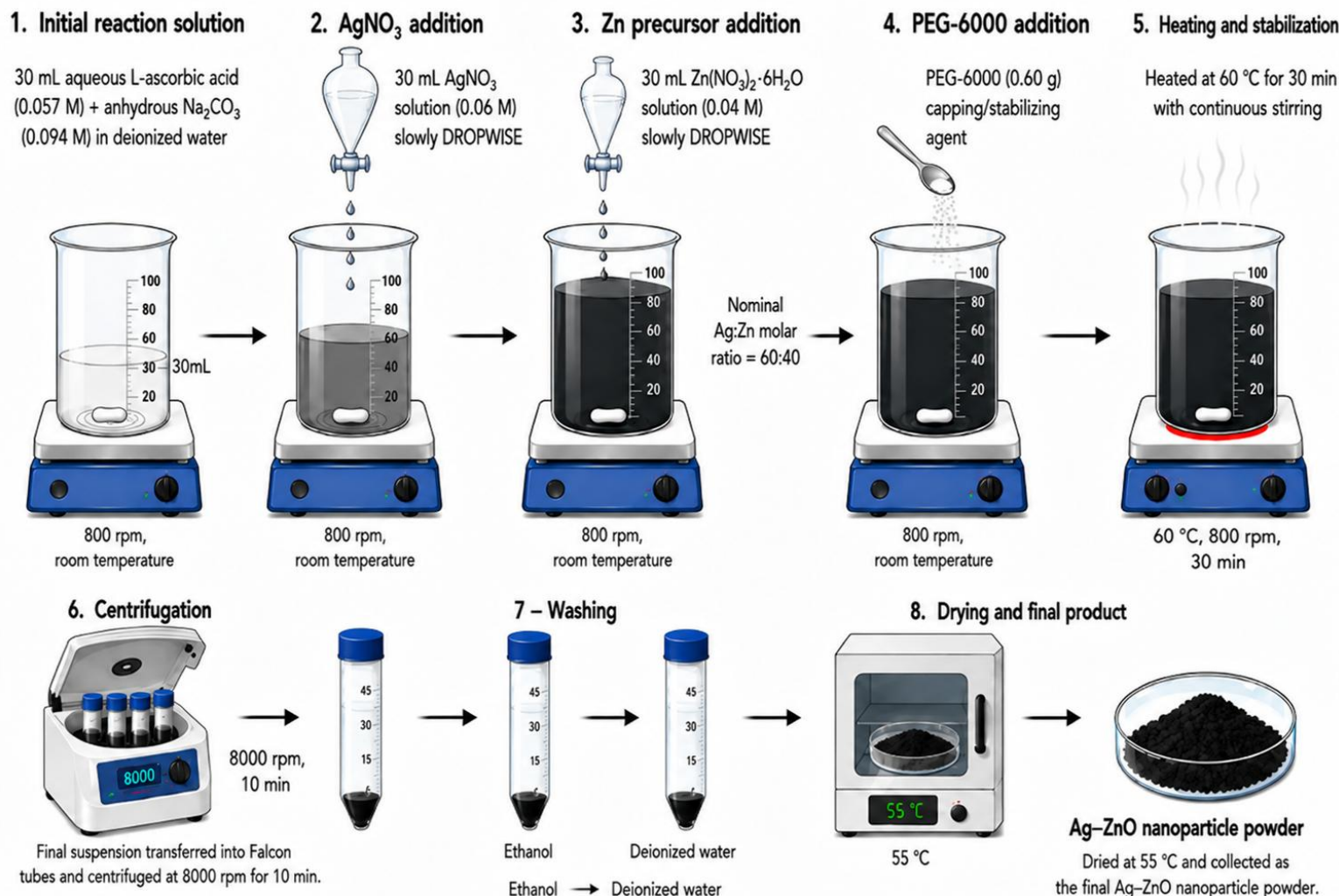


Figure-1. Synthesis of Ag-ZnO nanocomposite.

Characterization of Ag-ZnO nanocomposite

Characterization of the synthesized Ag-ZnO nanocomposites was carried out using X-ray diffraction (XRD; SmartLab SE, Rigaku Corporation, Japan) to determine their crystalline structure and phase composition. Surface morphology and elemental composition were investigated using scanning electron microscopy (SEM; Nova NanoSEM 450, FEI Company, USA) coupled with energy-dispersive X-ray spectroscopy (EDX). Surface functional groups were identified using Fourier transform infrared spectroscopy (FTIR; Spectrum II, PerkinElmer, USA). The average crystallite size was calculated from the XRD data using the Scherrer equation.

Antibacterial activity

The antibacterial activity of the synthesized Ag-ZnO nanocomposite was evaluated against *Pseudomonas aeruginosa*, *Pseudomonas chengduensis*, *Staphylococcus aureus* and *Staphylococcus haemolyticus* using agar well diffusion and broth microdilution methods. The bacterial strains were obtained from the laboratories of the Department of Zoology and the Department of Microbiology and Molecular Genetics, University of Okara, Pakistan. These included *P. aeruginosa* SSI.2 (GenBank accession no. PP587531.1), *P. chengduensis* AHH2 (OR342052.1), *S. aureus* SHB-1 (OR054191.1) and *S. haemolyticus* LYB-1 (OR054190.1). Fresh bacterial cultures were used to inoculate Mueller-Hinton agar

(MHA; Cat. No. M173, HiMedia) plates for the agar well diffusion assay. Wells of 6 mm diameter were aseptically created in the agar and filled with 25, 50, 75, and 100 μ L of Ag–ZnO nanocomposite suspension at a stock concentration of 1 mg/mL. Sterile distilled water served as the negative control. The plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured in millimeters (mm). The experiments were performed in triplicate ($n = 3$) using fresh bacterial cultures.

The minimum inhibitory concentration (MIC) of synthesized Ag–ZnO was determined by broth microdilution method using 96 well microtiter sterile plates. The mueller–Hinton broth was used for the dilution of synthesized Ag–ZnO nanoparticles to obtain two-fold serial concentrations ranging from 2.0 mg/mL to 0.015625 mg/mL. The two-fold serial concentrations used in this study were 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL. The bacterial suspensions were prepared to a concentration of approximately 1×10^6 CFU/mL for each test organism. The inoculum was added to wells containing above mentioned concentrations of the Ag–ZnO NPs. Negative control (sterile MH broth) and positive growth control (bacterial suspensions without Ag–ZnO NPs) were also maintained in wells of the microtiter plate. The inoculated wells were incubated at 37 °C for 24 h and subsequently examined further for visible bacterial growth. The MIC of the synthesized Ag–ZnO NPs was defined as lowest nanoparticle concentration that prevented visible bacterial growth.

Molecular docking

Molecular docking experiments of the Ag–ZnO nanocluster were performed using HDOCK server (Yan et al., 2017) against target proteins of bacterial pathogens. HDOCK, as a web-based server, employs a hybrid approach that integrates template-based modeling with ab initio free docking methods to predict accurate docked conformations. In this study, the optimized Ag–ZnO nanocluster was docked into the active sites of MurC (PDB ID: 8EWA), DNA gyrase B (PDB ID: 7PTF) and Sortase A (PDB ID: 2MLM). The generated protein–nanocluster models were evaluated using HDOCK scores and confidence scores. Amino acid residue interactions and the corresponding molecular interactions were further analyzed for the selected protein–nanocluster

complexes. The selected complexes were visualized and analyzed using PyMOL version 2.6.0 (Schrödinger (2010), LLC, New York, NY, USA) to examine the three-dimensional interaction interfaces and interacting residues.

Statistical analysis

Inhibition-zone measurements were determined in triplicate for each bacterial strain and expressed as mean $\pm$ SD. The statistical analysis was performed using one-way ANOVA. The differences were considered statistically significant at $p < 0.05$ and the effect size was expressed as eta squared (η^2).

Results

Characterization of Ag–ZnO

XRD and FTIR analysis

X-ray diffraction (XRD) was used to investigate the crystalline components present in Ag–ZnO nanocomposite. Figure 2A shows the XRD pattern obtained from the nanocomposite. Sharp peaks corresponding to 2θ values of about 31.7° (100), 36.2° (101) and 67.9° (112) are indicative of a wurtzite hexagonal structure of zinc oxide (JCPDS File No. 36-1451). In addition to these peaks, other peaks corresponding to 2θ values of approximately 38.2° (111), 44.3° (200), 64.4° (220), and 77.3° (311) are ascribed to silver in a face-centered cubic (fcc) structure (JCPDS File No. 04-0783). The XRD peaks corresponding to silver were more intense than those of corresponding zinc oxide peaks, particularly the most intense peak corresponding to Ag (111) reflection at about 38.2°. Using the Debye–Scherrer equation, the average crystallite size of the Ag–ZnO nanocomposite was found 15.6 nm.

FTIR spectrum of Ag–ZnO nanocomposite (Figure 2B) exhibited broad absorption band at 3308.7 cm^{-1} , assigned to O–H stretching vibrations associated with surface hydroxyl groups. The band at 1595.0 cm^{-1} was linked to O–H bending vibrations and/or surface oxygen-containing groups. The absorption band at 1408.6 cm^{-1} may be associated with carbonate species, while the band at 1011.4 cm^{-1} may correspond to C–O stretching vibrations originating from residual surface species from the synthesis process. Whereas, in low-wavenumber region, band observed at 540.5 cm^{-1} was attributed to metal–oxygen vibrations, particularly Zn–O lattice vibrations.

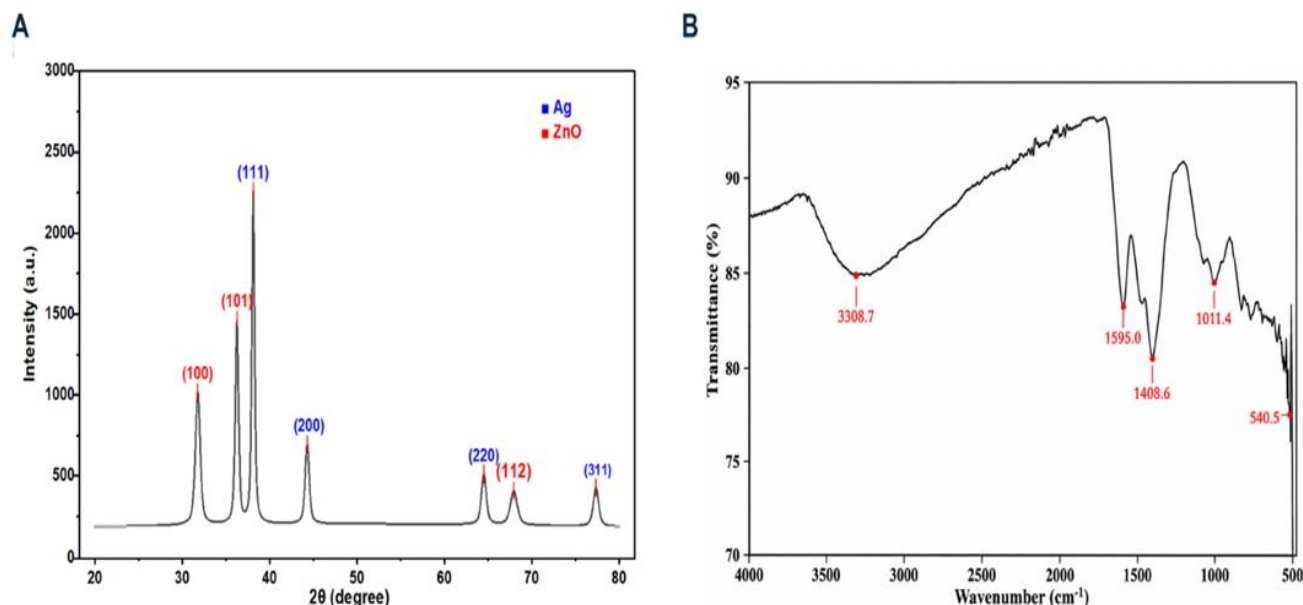


Figure-2. (A) XRD pattern (B) FTIR spectrum of the Ag–ZnO nanocomposite.

SEM and EDX analysis

The surface morphology of Ag–ZnO nanocomposite was examined by using scanning electron microscopy (SEM). The SEM images of the nanocomposite at different magnifications are shown in Figure 3 (a–b). At 10,000× magnification Figure 3 (A), the nanostructured surface under study revealed heterogeneous morphology consisting of aggregation of particles and presence of numerous voids which were distributed irregularly over the surface. Aggregated particles were interconnected and formed clusters. In addition, surface showed rough and porous nature. At higher magnification of 100,000× Figure

3(B), fine granular nanostructures were observed. Most of these nanostructures were irregular to quasi-spherical in shape and were in close contact with one another.

The energy-dispersive X-ray spectroscopy (EDX) was used to assess elemental composition of the obtained Ag–ZnO nanocomposite as shown in Figure 3 (C). It can be observed from the spectrum that intense signal at 3 keV is due to Ag, signals in lower energy region along with a peak at 8.6 keV are due to Zn and similarly signal at 0.5 keV corresponded to O. The low-intensity C signal was also observed and few Au peaks in the spectrum due to the conductive gold coating applied on the samples for SEM studies.

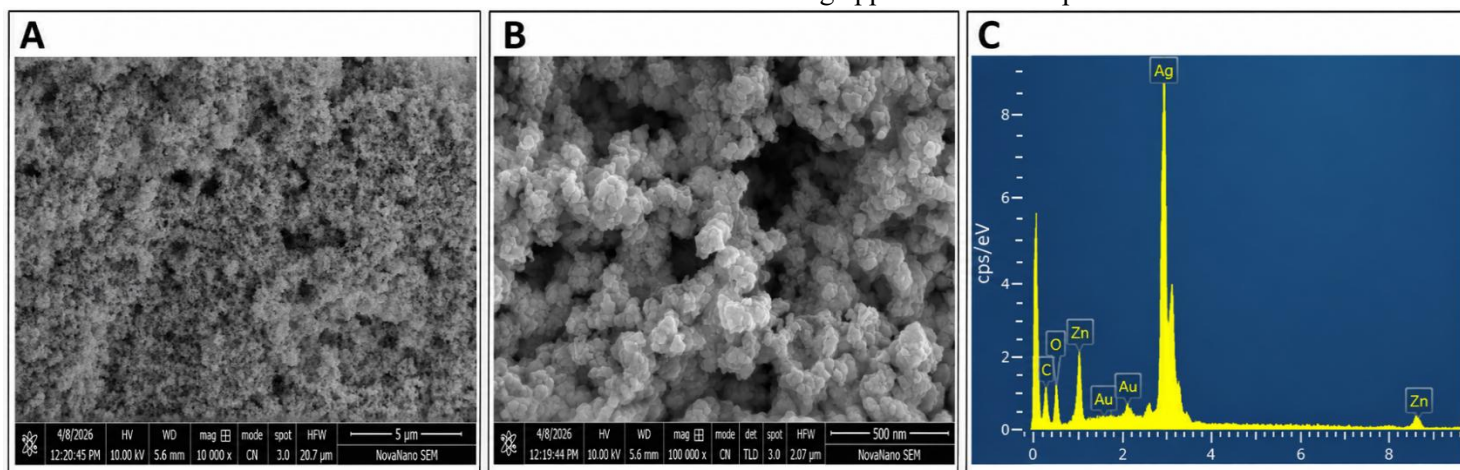


Figure-3. (A) SEM image at 10,000× magnification, (B) SEM image at 100,000× magnification (C) EDX spectrum of the Ag–ZnO nanocomposite.

Antibacterial activity

The antibacterial activity of synthesized Ag–ZnO nanocomposite was determined against four kinds of bacteria, including two Gram-negative bacteria *Pseudomonas aeruginosa*, *Pseudomonas chengduensis* and two Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus haemolyticus*. The antibacterial assay was performed by using the agar well diffusion method and MIC determination. As shown in Figure 4 and Table 1, the synthesized Ag–ZnO nanocomposite showed marked antibacterial activities against the tested bacteria, and the inhibition zones were increased with the increase of used volume of test samples from 25 μ L to 100 μ L. There was no inhibition zone for negative control (NC). For *P. aeruginosa*, the mean inhibition zone increased from 13.33 ± 0.58 mm at 25 μ L to 18.33 ± 0.58 mm at 50 μ L, 21.33 ± 0.58 mm at 75 μ L, and 23.00 ± 1.00 mm at 100 μ L. A similar pattern was observed for *P. chengduensis*, with inhibition zones of 13.33 ± 0.58 , 20.00 ± 1.00 , 22.00 ± 1.00 , and 23.67 ± 1.15 mm at 25, 50, 75, and 100 μ L, respectively. Among the four

tested strains, *P. chengduensis* showed the largest mean inhibition zone at 100 μ L (23.67 ± 1.15 mm). The Gram-positive strains also showed an increase in inhibition zone diameter with increasing volume. For *S. aureus*, the inhibition zones were 13.89 ± 0.58 , 16.67 ± 0.58 , 19.33 ± 0.58 , and 23.00 ± 1.00 mm at 25, 50, 75, and 100 μ L, respectively. At the corresponding volumes, *S. haemolyticus* showed inhibition zones of 12.00 ± 1.00 , 16.67 ± 0.58 , 19.67 ± 0.58 , and 22.17 ± 0.76 mm. Thus, although *S. haemolyticus* showed the smallest inhibition zone at 25 μ L and a slightly smaller zone than *S. aureus* at 100 μ L.

One-way ANOVA showed that the applied volume had a significant effect on inhibition zone diameter for all four bacterial strains ($p < 0.001$). The corresponding F values were 108.000 for *P. aeruginosa*, 67.242 for *P. chengduensis*, 94.444 for *S. aureus*, and 83.296 for *S. haemolyticus* [F (3,8)]. Large effect sizes were observed for all strains, with η^2 values ranging from 0.962 to 0.976, indicating that the applied volume accounted for a substantial proportion of the variation in inhibition zone diameter.

Table-1. Zones of inhibition (mm) of Ag–ZnO nanocomposite against tested bacterial species at different applied volumes. Values are expressed as mean $\pm$ SD (n = 3).

Bacterial strain	Negative control (Mean $\pm$ SD, mm)	25 μ L	50 μ L	75 μ L	100 μ L	F(3,8)	p	η^2
<i>P. aeruginosa</i>	0.00 $\pm$ 0.00	13.33 $\pm$ 0.58	18.33 $\pm$ 0.58	21.33 $\pm$ 0.58	23.00 $\pm$ 1.00	108.000	<0.001	0.976
<i>P. chengduensis</i>	0.00 $\pm$ 0.00	13.33 $\pm$ 0.58	20.00 $\pm$ 1.00	22.00 $\pm$ 1.00	23.67 $\pm$ 1.15	67.242	<0.001	0.962
<i>S. aureus</i>	0.00 $\pm$ 0.00	13.89 $\pm$ 0.58	16.67 $\pm$ 0.58	19.33 $\pm$ 0.58	23.00 $\pm$ 1.00	94.444	<0.001	0.973
<i>S. haemolyticus</i>	0.00 $\pm$ 0.00	12.00 $\pm$ 1.00	16.67 $\pm$ 0.58	19.67 $\pm$ 0.58	22.17 $\pm$ 0.76	83.296	<0.001	0.969

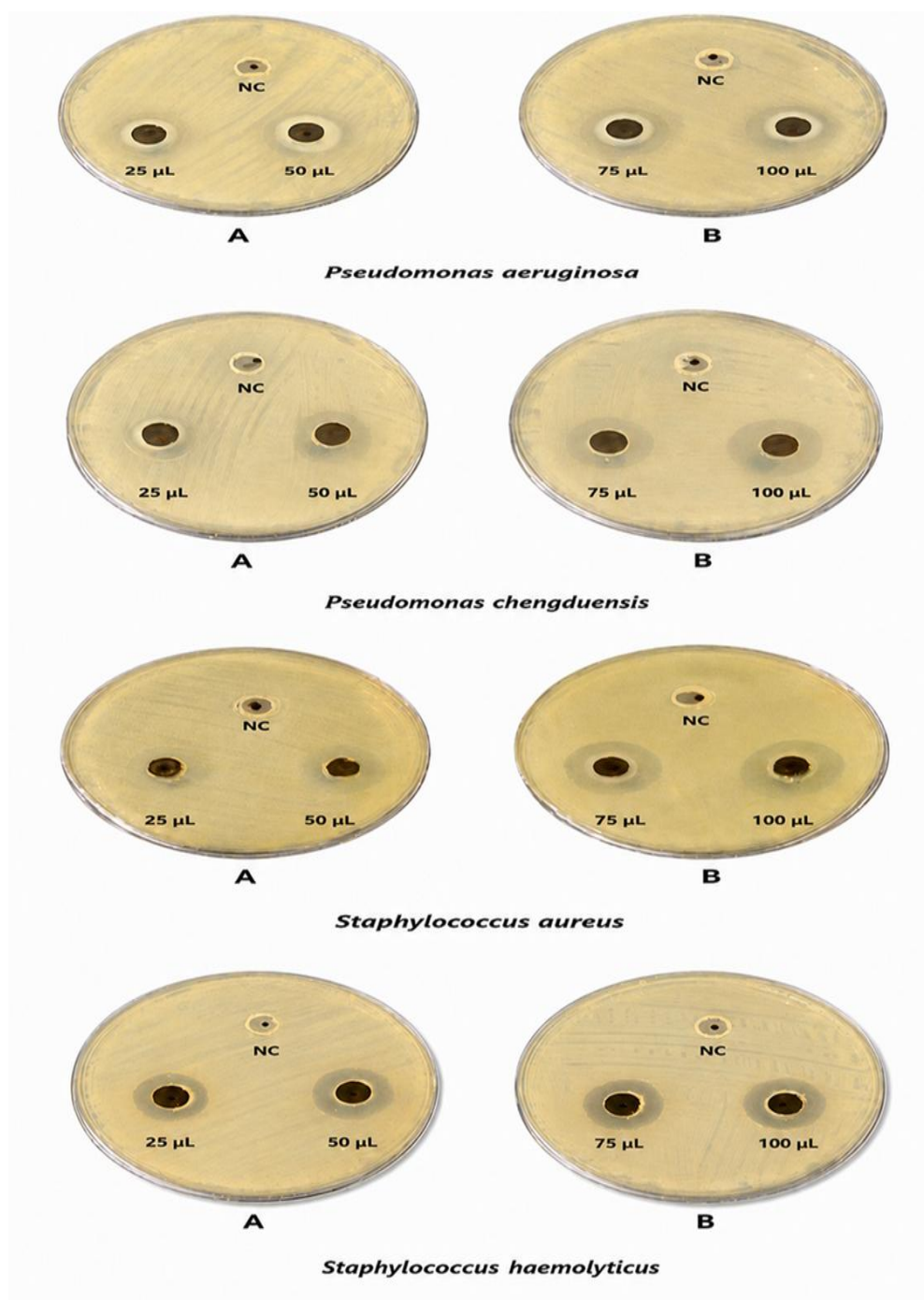


Figure-4. Agar well diffusion assay showing the antibacterial activity of Ag-ZnO nanocomposite against *P. aeruginosa*, *P. chengduensis*, *S. aureus*, and *S. haemolyticus*. (A) 25 and 50 μL ; (B) 75 and 100 μL of nanocomposite suspension. NC represents the negative control (sterile distilled water).

For the MIC test, MIC values of Ag-ZnO NPs were determined at concentrations ranging from 0.015625 to 2.0 mg/mL. As shown in Table 2, the lowest MIC value was observed for *P. chengduensis* (0.125

mg/mL), followed by *P. aeruginosa* and *S. aureus*, with MIC values of 0.25 mg/mL each. In contrast, *S. haemolyticus* exhibited the highest MIC value of 0.50 mg/mL.

Table-2. MIC values of the Ag–ZnO nanocomposite against the tested bacterial strains.

Bacterial strain	Gram status	Tested concentration range (mg/mL)	MIC (mg/mL)
<i>Pseudomonas aeruginosa</i>	Gram-negative	0.015625–2.0	0.25
<i>Pseudomonas chengduensis</i>	Gram-negative	0.015625–2.0	0.125
<i>Staphylococcus aureus</i>	Gram-positive	0.015625–2.0	0.25
<i>Staphylococcus haemolyticus</i>	Gram-positive	0.015625–2.0	0.50

Molecular docking study

Nanocluster design and optimization

The Ag–ZnO nanocluster was built in Materials Studio (BIOVIA, 2024) by taking a piece out of the bulk Ag and wurtzite-phase ZnO lattices ($a = 3.2495 \text{ \AA}$, $c = 5.2069 \text{ \AA}$) using the Nanocluster tool. The resulting

structure had Ag atoms located mainly on the outside and at the corners, while Zn and O atoms formed a tetrahedral network inside, with oxygen atoms linking nearby Ag and Zn centers (Figure 5A).

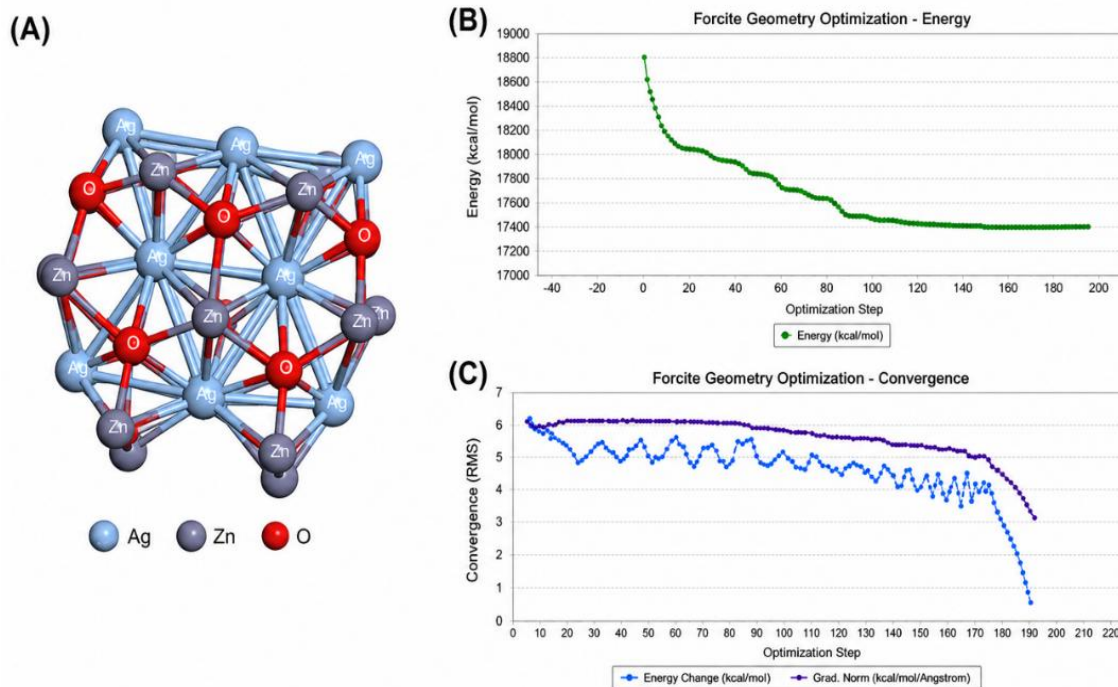


Figure-5. Ag–ZnO nanocluster optimization: (A) constructed model, (B) energy minimization, and (C) convergence profile using the Forcite module.

The Forcite module, Universal force field and Smart minimization algorithm were used for geometry relaxation and the total potential energy decreased from about 18,840 kcal/mol to 17,290 kcal/mol (Figure 5B), corresponding to a reduction of approximately 1,550 kcal/mol. This reduction indicated successful energy minimization within the selected computational mode. The convergence profile (Figure 5C) showed that both the energy

change and gradient norm decreased during optimization, with a pronounced decline after approximately 180 optimization step.

Molecular docking

The docking results showed that the top-ranked docking scores for the three protein targets ranged from -160.69 to -166.28 . MurC (PDB ID: 8EWA) showed the most negative docking score of -166.28

and the highest confidence score of 0.5807, with a root mean square deviation (RMSD) of 31.54 Å. DNA gyrase B (PDB ID: 7PTF) showed a docking score of -160.72, a confidence score of 0.5534, and an RMSD of 22.99 Å, while Sortase A (PDB ID: 2MLM) showed a docking score of -160.69, a confidence score of 0.5532, and an RMSD of 25.86 Å. Among the three targets, MurC showed the most negative docking score and the highest confidence score. The reported ligand

RMSD values were calculated by comparing the ligand structures in the predicted docking models with their corresponding input or modeled structures, rather than experimentally determined binding poses. The confidence scores (0.5532–0.5807) suggested possible interactions between the Ag–ZnO nanocluster and the selected bacterial proteins, according to the HDock confidence scoring framework.

Table-3. HDock docking results for the Ag–ZnO nanocluster against bacterial target proteins.

Target protein	PDB ID	HDock score	Confidence score	Ligand RMSD (Å)
MurC	8EWA	-166.28	0.5807	31.54
DNA gyrase B	7PTF	-160.72	0.5534	22.99
Sortase A	2MLM	-160.69	0.5532	25.86

Interaction analysis

All interaction analyses of the Ag–ZnO nanocluster with the three tested target proteins were performed using PyMOL. Of the three, MurC (8EWA) interacted mostly with polar residues of the protein, notably through hydrogen-bond donor interactions with Arg113 (A), Arg115 (B) and Arg113 (B) at distances between 2.2 and 4.0 Å. Furthermore, there were also hydrogen-bond acceptor contacts with His116, as well as a π -interaction with His13 at 4.2 Å. Additional contacts of the nanoparticle were via van der Waals interactions with Tyr114, Tyr220 and Met184. In contrast, Sortase A (2MLM) featured a binding environment of predominantly hydrophobic. The nanoparticle was found to interact with Ala34, Ala46, Ala60, Pro36, Leu39, Val108 and Ile124 of the protein of which many of these contacts were predominantly

hydrophobic and van der Waals in nature, at distances of 3.8 to 4.2 Å. The interactions also included a number of π -interactions, notably with Trp136 and with Arg139 at 3.5 and 3.6 Å, respectively, as well as a contact with the sulfur atom of Cys126 at approximately 2.9 Å. Lastly, for DNA Gyrase B (7PTF), the interaction pattern was clearly dominated by His and Lys metal-contact interactions. These were with His122, His102, His140, His141 and Lys76 with distances between 3.1 and 4.0 Å. There were also Glu carboxylate contacts from Glu205 and Glu168 at 3.8 to 4.0 Å. The MurC interface was characterized predominantly by polar and aromatic contacts, the Sortase A interface by hydrophobic and π -related contacts, including contacts with the catalytic residues Cys126 and Arg139 and the DNA gyrase B interface by metal-associated, polar and some charged interactions.

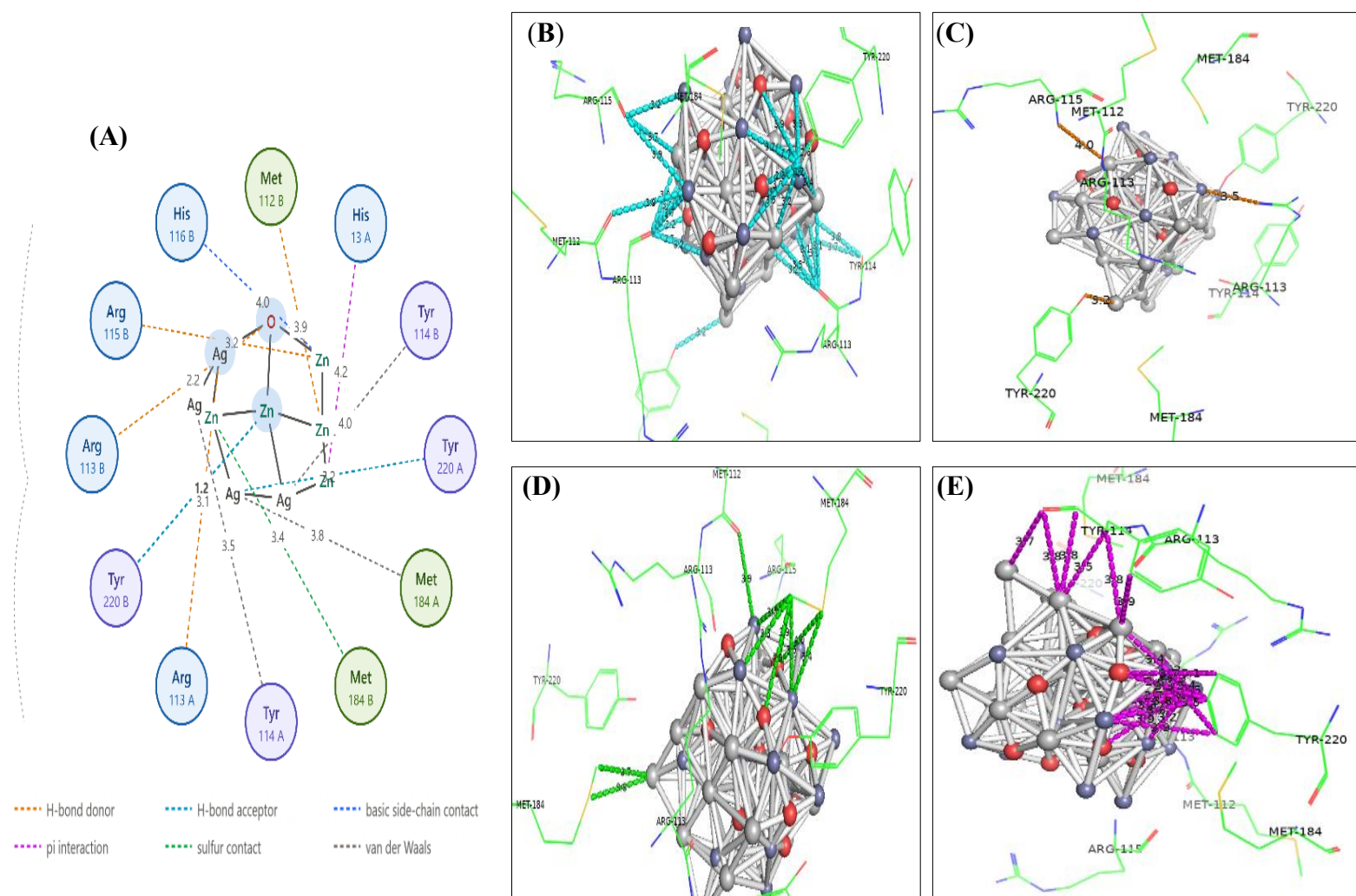


Figure-6. MurC (8EWA) and Ag-ZnO docked complex interaction analysis (A) 2D interaction analysis diagram, (B) H-acceptor bonds, (C) H-donor bonds, (D) Metal Sulphur Bonds and (E) pi-pi metal interaction.

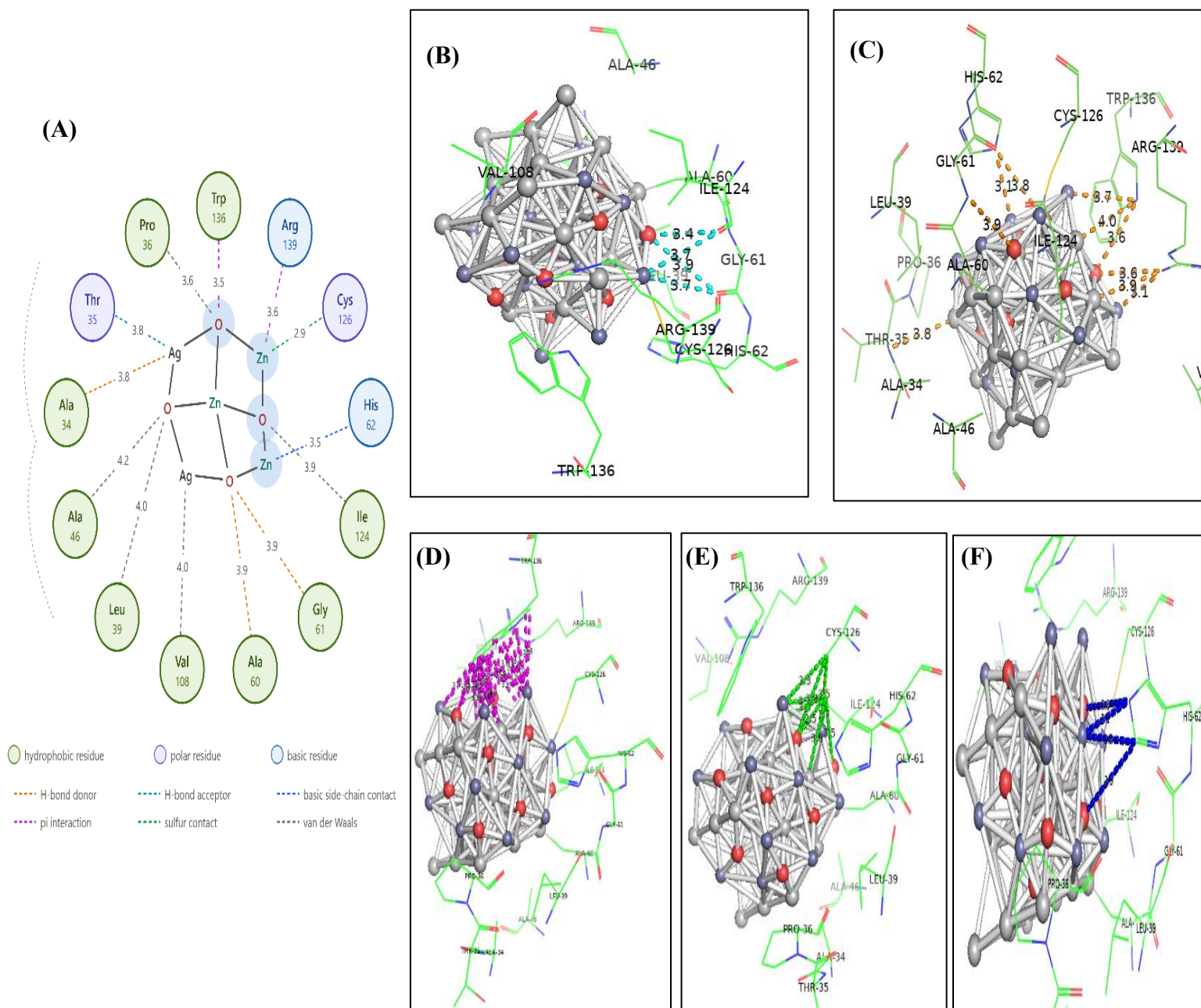


Figure-7. Sortase A (2MLM) and Ag-ZnO docked complex interaction analysis (A) 2D interaction analysis diagram, (B) H-acceptor bonds, (C) H-donor bonds, (D) Metal Sulphur Bonds, (E) pi-pi metal interaction and (F) Metal oxyniro bonds.

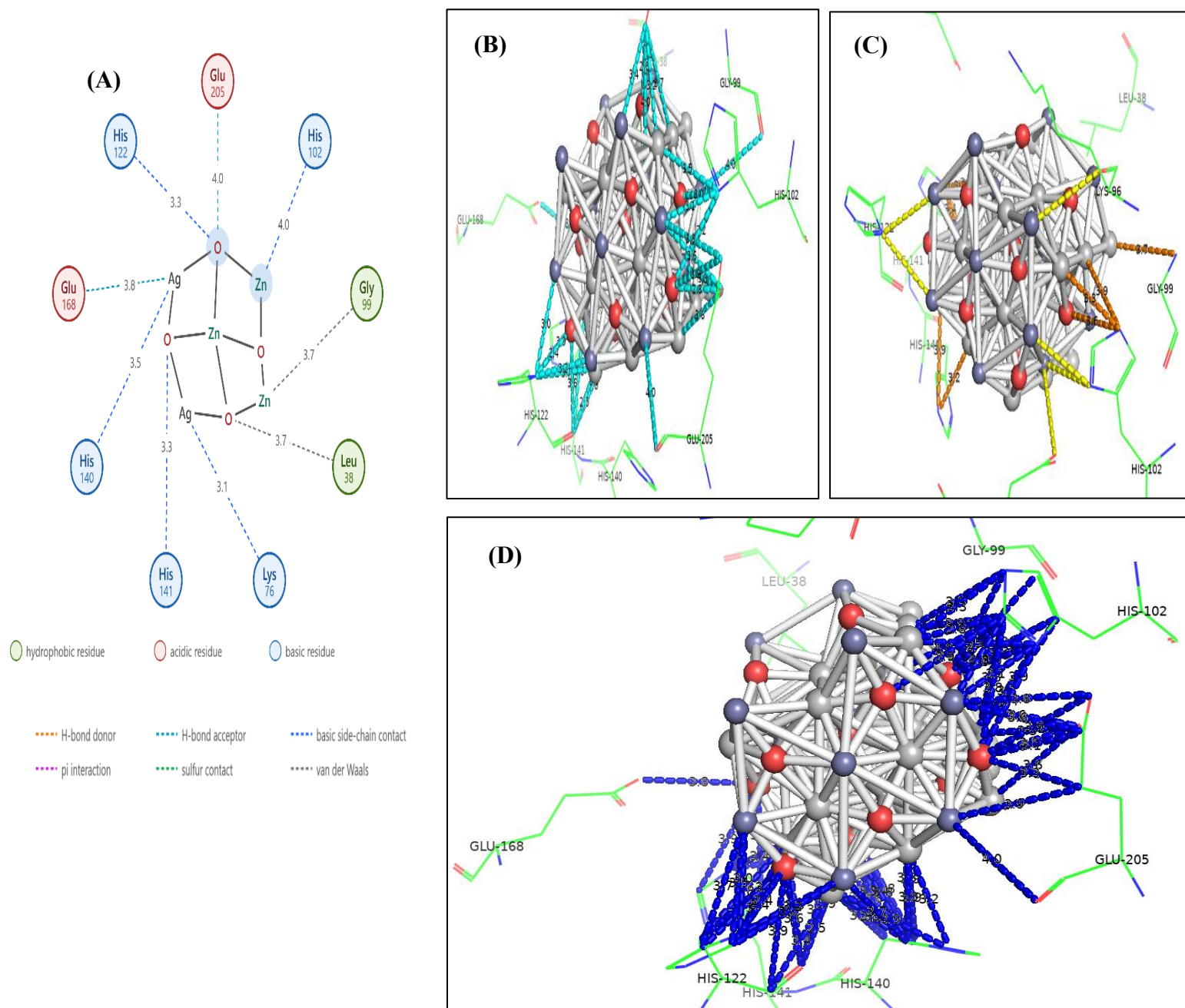


Figure-8. DNA Gyrase B (7PTF) and Ag-ZnO docked complex interaction analysis (A) 2D interaction analysis diagram, (B) H-acceptor bonds, (C) H-donor bonds and (D) Metal oxyniro bond.

Discussion

Metallic nanoparticles, ranging from mono- to multimetallic systems, have gained considerable attention for their multifunctional properties, biomedical applications and promising antibacterial activity against pathogenic bacteria (Shahlaei et al.,

2024; Skłodowski et al., 2023). In the present study, the Ag-ZnO nanocomposite was successfully synthesized through a chemical reduction approach, with its formation and physicochemical characteristics supported by XRD, FTIR, SEM and EDX analyses. The XRD pattern showed distinct crystalline reflections attributable to both ZnO and metallic Ag

phases. The peaks observed approximately at 31.7° , 36.2° and 67.9° were assigned to the (100), (101) and (112) planes of hexagonal wurtzite ZnO, respectively, whereas the reflections at 38.2° , 44.3° , 64.4° and 77.3° corresponded to the (111), (200), (220) and (311) planes of face-centered cubic Ag. Similar structural characteristics have been reported for Ag-containing ZnO nanomaterials. Akbarizadeh et al. (2022) similarly reported wurtzite ZnO with Ag peaks at 37.84° , 44.16° and 64.32° , while Khatir and Sabbagh (2022) reported Ag reflections at 38.28° , 44.44° and 64.57° . The close agreement between the observed diffraction peaks and previously reported Ag/ZnO reflections provides clear structural evidence for the coexistence of crystalline ZnO and metallic Ag phases in the synthesized nanocomposite. The average crystallite size of approximately 15.6 nm further supports the nanoscale crystalline nature of the synthesized material.

The FTIR spectrum of the synthesized Ag–ZnO nanocomposite showed bands corresponding to surface functional groups as well as metal–oxygen vibrations. The bands were found at 3308.7 cm^{-1} for O–H stretching and 1595.0 cm^{-1} for O–H bending and/or surface oxygen containing groups. The spectrum also displayed two distinctive bands centered at 1408.6 cm^{-1} for carbonate species and 1011.4 cm^{-1} for C–O stretching whereas, 540 cm^{-1} was characteristic of Zn–O/metal–oxygen vibrations. These findings were consistent with the reported values for O–H absorption in the range of $3500\text{--}3200\text{ cm}^{-1}$ and C–O stretching at 1012 cm^{-1} reported by Suresh et al. (2024). Other vibrations were found at 3433 and 1625 cm^{-1} for O–H groups, 1421 and 1490 cm^{-1} for carbonate species and at 459 cm^{-1} for Zn–O was consistent with the finding of Mousavi-Kouhi et al. (2021). These surface functional groups along with Zn–O bonds were established in the herein synthesized Ag–ZnO nanocomposite.

SEM images revealed quasi-spherical to granular Ag–ZnO nanoparticles with pronounced surface roughness. It is expected that highly surface-energized small nanoparticles would tend to agglomerate, thereby forming granular to porous structures. The observed aggregation may be associated with strong interparticle forces and the high surface energy of nanoscale materials. During synthesis and subsequent drying, neighboring particles may come into close contact and form larger aggregates. This nanoparticle morphology is in good agreement with the reported SEM images for Ag–ZnO nanocomposites by Raza et

al. (2024). In addition, the EDX spectrum of the synthesized Ag–ZnO nanocomposite established the presence of Ag, Zn and O and provided elemental composition of the nanocomposite in good agreement with the results obtained from XRD. Together, the SEM and EDX findings support the formation of an Ag–ZnO nanocomposite with an agglomerated nanoscale morphology and the expected elemental constituents.

The synthesized Ag–ZnO nanocomposite showed pronounced antibacterial activities against all tested Gram-negative and Gram-positive bacterial pathogens. Ag–ZnO nanocomposite was assessed for its antibacterial activities using well diffusion method. The zone of inhibition was found to increase with increase in volume of tested nanocomposite from 25 μL to 100 μL . Similar results were obtained with other silver containing nanomaterials (Dutta et al., 2023; Madipoju et al., 2025; Malik et al., 2023). Among the tested bacteria two Gram-negative species *Pseudomonas aeruginosa* and *Pseudomonas chengduensis* exhibited higher levels of inhibition compared to Gram-positive species. The effect of applied volume on inhibition zone diameter was statistically significant for all tested strains (one-way ANOVA, $p < 0.001$), with large effect sizes ($\eta^2 = 0.962\text{--}0.976$). These results clearly indicated volume-dependent antibacterial activities of synthesized Ag–ZnO nanocomposite.

The minimum inhibitory concentration (MIC) values for bacterial growth by Ag–ZnO nanoparticles were found to be 0.125 mg/mL for *P. chengduensis*, 0.25 mg/mL for both *P. aeruginosa* and *S. aureus*, and 0.50 mg/mL for *S. haemolyticus*. These MIC values support the previous findings of antibacterial activity of Ag–ZnO, and the observed differences in MIC values for different bacterial species could be related to their susceptibility or resistance towards Ag–ZnO nanoparticles. The findings of the current study are consistent with those reported by Fattah et al. (2026) for the antibacterial activity of Ag–ZnO nanocomposites. According to their study, the MIC values for biosynthesized Ag–ZnO nanoparticles were found to be 0.125 mg/mL for *Staphylococcus aureus* ATCC 6538 and 0.25 mg/mL for a clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolate. Moreover, Hashem and El-Sayyad (2024) reported the MIC values for biosynthesized bimetallic Ag–ZnO nanoparticles (NPs) to be 0.125 mg/mL for *P. aeruginosa* and 0.0625 mg/mL for *S. aureus*. The observed differences in the obtained MIC values by

different investigators could be due to several factors including the type of nanocomposite used, characteristics of prepared nanoparticles, method of synthesis, tested bacterial species, used inoculum concentration and experimental conditions that are employed to determine the antibacterial activity. Overall, the MIC values support the previous obtained results from agar diffusion assay and thus confirm the antibacterial activity of Ag–ZnO against the tested pathogens under the used experimental conditions.

To explore protein-ligand interactions of Ag–ZnO nanoclusters, we carried out molecular docking of the optimized nanocluster to three bacterial target proteins: MurC (PDB ID: 8EWA), Sortase A (PDB ID: 2MLM) and DNA gyrase B (PDB ID: 7PTF). Prior to the molecular docking studies, we carried out a geometry optimization of the Ag–ZnO nanocluster, which decreased the total potential energy from 18,840 to 17,290 kcal/mol. HDOCK scores of the docking results for three proteins were –166.28 (MurC with confidence of 0.5807), –160.69 (Sortase A with confidence of 0.5532), and –160.72 (DNA gyrase B with confidence of 0.5534). The lowest score and highest confidence were obtained for interaction with MurC, making it the main target of the three tested proteins. However, the scores and confidence values of Sortase A and DNA gyrase B were also close to those of MurC. Thus, while these predictions support MurC as the leading predicted target, but they do not exclude Sortase A and DNA gyrase B as possible targets of the Ag–ZnO nanocluster. Such docking studies of protein–nanoparticle interactions were previously performed by Sazak et al. (2023) using HDOCK for a blind docking study of CuO nanoparticles and by Guzmán-Flores et al. (2025) who used HDOCK to model interactions of a functionalized gold nanoparticle with proteins from humans and bacteria.

The predicted interaction interfaces of the three proteins showed distinct physicochemical characteristics. MurC was dominated by polar, aromatic and van der Waals contacts involving Arg113, Arg115, His116, His13, Tyr114, Tyr220 and Met184, placing the Ag–ZnO nanocluster within a polar and solvent-accessible region. These contacts indicate that the predicted MurC association involved several chemically distinct surface interactions rather than a single contact type. Sortase A exhibited a predominantly hydrophobic interface, with additional contacts involving Trp136, Arg139 and Cys126. Notably, the interaction with the functionally

important residues Cys126 and Arg139 suggests possible interference with the catalytic environment. In contrast, DNA gyrase B showed mainly metal-associated, polar and charged contacts involving His, Lys and Glu residues within its ATPase subdomain, indicating a possible effect on the local environment involved in nucleotide-dependent function. Thus, the three proteins displayed chemically different predicted interaction interfaces, supporting the possibility that the Ag–ZnO nanocluster can associate with different bacterial protein surfaces through multiple types of contacts.

MurC, Sortase A and DNA gyrase B are involved in peptidoglycan biosynthesis, surface-protein anchoring and DNA replication, respectively. Mur enzymes are recognized as important antibacterial targets because of their essential role in cell-wall biosynthesis (Kumar et al., 2023), while MurC–MurF ligases are particularly attractive due to their conserved structural and active-site features (Hervin et al., 2023). Accordingly, the combination of the most negative HDOCK score, the highest confidence score and multiple predicted residue contacts make MurC the strongest computationally supported target among the three proteins investigated in this study. Nevertheless, these docking results represent predicted associations rather than direct evidence of enzyme inhibition or antibacterial mechanism.

Overall, the volume-dependent inhibition, MIC findings and molecular docking results provide complementary experimental and computational support for the antibacterial potential of the Ag–ZnO nanocomposite. MurC emerged as the leading predicted target, while the comparable scores and chemically distinct interfaces identified for Sortase A and DNA gyrase B suggest a broader potential for interaction with multiple bacterial proteins. In particular, the predicted contacts with Cys126 and Arg139 of Sortase A and with charged residues within the DNA gyrase B ATPase domain provide a structural basis for possible interference with functionally important bacterial processes. These protein-level interactions may complement the membrane disruption, Ag⁺ and Zn²⁺ release and ROS-mediated oxidative stress previously reported for Ag–ZnO systems, supporting a multi-mechanistic interpretation of the observed antibacterial activity. Taken together, the statistically strong volume-dependent inhibition, MIC findings and distinct predicted protein-interaction profiles provide

complementary evidence for antibacterial potential of Ag–ZnO nanocomposite under the tested conditions.

Conclusion

In conclusion, the synthesized Ag–ZnO nanocomposite showed promising antibacterial activity against Gram-negative and positive bacterial strains. The inhibition zones increased with increasing applied concentration of the tested samples, while minimum inhibitory concentration (MIC) values ranged from 0.125 to 0.50 mg/mL for all tested bacteria. The predicted protein–nanocluster interactions of the synthesized Ag–ZnO nanocluster with MurC, Sortase A and DNA gyrase B were also evaluated using the HDock molecular docking program. Among the three proteins, MurC emerged as the leading predicted target. Therefore, synthesized Ag–ZnO nanocomposite may have potential for the development of alternative antimicrobial materials; however, it is necessary to investigate its cytotoxicity and biological safety as well as the functional consequences of the predicted protein interactions. In addition, further studies are needed to determine antibacterial activity of synthesized Ag–ZnO nanocomposite against clinically isolated resistant pathogenic bacteria and to investigate its potential effects in combination with conventional antibiotics.

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Contribution of Authors

Saeed S: Performed the synthesis, experimental work, data collection and prepared the draft of the manuscript.

Khan MS: Supervised the research and validated the results.

Khan MS & Zahid S: Provided critical revisions to the manuscript.

Wajid M, Rehman SU & Ashfaq I: Assisted in biological assays and interpretation of results.

All authors read and approved the final draft of the manuscript.

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