Development of Multi-concentration Cu:Ag Bimetallic Nanoparticles as a Promising Bactericidal for Antibiotic-Resistant Bacteria as Evaluated with Molecular Docking Study

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Abstract

The present study is concerned with evaluating the influence of various concentrations of Ag within Cu:Ag bimetallic nanoparticles developed for use as a promising anti-bacterial agent against antibiotic-resistant bacteria. Here, Cu:Ag bimetallic nanoparticles with various concentration ratios (2.5, 5.0, 7.5, and 10 wt%) of Ag in fixed amount of Cu labeled as 1:0.025, 1:0.050, 1:0.075, and 1:0.1 were synthesized using co-precipitation method with ammonium hydroxide and deionized water as solvent, polyvinyl pyrrolidone as a capping agent, and sodium borohydride and ascorbic acid as reducing agents. These formulated products were characterized through a variety of techniques. XRD confirmed phase purity and detected the presence of distinct fcc structures belonging to Cu and Ag phases. FTIR spectroscopy confirmed the presence of vibrational modes corresponding to various functional groups and recorded characteristic peak emanating from the bimetallic. UV–visible spectroscopy revealed reduction in band gap with increasing Ag content. SEM and HR-TEM micrographs revealed spherical morphology of Ag-doped Cu bimetallic with small and large scale agglomerations. The samples exhibited varying dimensions and interlayer spacing. Bactericidal action of synthesized Cu:Ag bimetallic NPs depicted statistically significant (\( P < 0.05 \)) inhibition zones recorded for various concentrations of Ag dopant against Staphylococcus aureus (\( S. aureus \)), Escherichia coli (\( E. coli \)), and Acinetobacter baumannii (\( A. baumannii \)) ranging from (0.85–2.8 mm), (0.55–1.95 mm) and (0.65–1.85 mm), respectively. Broadly, Cu:Ag bimetallic NPs were found to be more potent against gram-positive compared with gram-negative. Molecular docking study of Ag–Cu bimetallic NPs was performed against \( \beta \)-lactamase which is a key enzyme of cell wall biosynthetic pathway from both \( S. aureus \) (Binding score: −4.981 kcal/mol) and \( A. baumannii \) (Binding score: −4.013 kcal/mol). Similarly, binding interaction analysis against FabI belonging to fatty acid biosynthetic pathway from \( A. baumannii \) (Binding score: −3.385 kcal/mol) and \( S. aureus \) (Binding score: −3.012 kcal/mol) along with FabH from \( E. coli \) (Binding score: −4.372 kcal/mol) was undertaken. These theoretical computations indicate Cu-Ag bimetallic NPs as possible inhibitor of selected enzymes. It is suggested that exploring in vitro inhibition potential of these materials may open new avenues for antibiotic discovery.

Keywords: Bimetallic, Antimicrobial, Docking, HR-TEM, Cu:Ag

Introduction

A variety of micro-organisms composed of bacteria, fungi, viruses, and parasites are present within the earth and its environment. These species cause complications in the production and use of medical...
equipment, healthcare merchandise, processed foods, water purification systems, and domestic sanitation products [1, 2]. Antibiotics are routinely employed by physicians to kill bacteria that cause illness in humans and animals. The disadvantage of frequent use of antibiotics is that it makes bacteria drug-resistant with time. Antibiotics also serve to reduce the number of ‘good’ bacteria present in the body, which fight against infections. Illnesses resulting from infections caused by antibiotic-resistant bacteria have become a major cause for concern in the field of medicine today. In this respect, many germs have been identified to be drug-resistant [3–7]. Novel efforts are under way to address the issue of drug-resistant bacteria and substitute current antimicrobial agents with more efficient and complementary therapies. In this regard, nanotechnology has rendered a substantial contribution to the production of nanomaterials such as metallic and metal oxide NPs (i.e., Ag, Cu, CuO, TiO2, SiO2, MgO, and ZnO) to fight an ever-increasing number of antimicrobial-resistant microorganisms. Among these, Cu and Ag NPs have shown encouraging antimicrobial properties [8–12].

In recent years, bimetallic NPs have been developed and used for various applications in the fields of chemistry, material science, biotechnology, and environmental protection. Bimetallic NPs containing copper (Cu) and silver (Ag) with a high fraction of surface atoms and large specific surface area have been widely studied [13]. These bimetallic NPs are of great interest due to their enhanced chemical, optical, catalytic, biological, plasmonic, and especially antimicrobial properties [14–20]. Ag ions can be reduced by ethanol under atmospheric conditions at 800 to 1000 °C to obtain silver NPs [21, 22]. Silver NPs possess good antimicrobial efficacy; therefore, it is used in the production of sunscreen creams and water treatment [23]. Cu NPs are fabricated by the reduction of copper sulfate with hydrazine in ethylene glycol under microwave irradiation, and can also be used as an antibacterial agent [24–26].

Metals such as Cu and Ag individually do not have promising optical, catalytic, and structural properties and cannot be converted into bimetals. On the other hand, combining both metals (Cu:Ag) offers new opportunities to tune the structure and morphology of the resulting product for desired applications. Based upon its final structure, e.g., core–shell, dumb-bell structure, two-interface structure, randomly-mixed structure, or flower-shape structure, bimetallic NPs can exhibit a range of antimicrobial activity [27–31]. Various methods are available for the synthesis of bimetallic NPs including co-precipitation, sol–gel, hydrothermal, reduction, micro-emulsion, and polyol method [32–37].

In this study, Cu:Ag bimetallic NPs were synthesized through co-precipitation method using ammonium hydroxide and deionized water as solvent, polyvinyl pyrrolidone (PVP) as a capping agent, and sodium borohydride and ascorbic acid as reducing agents. Four samples with various concentrations were prepared. With increasing concentrations of Ag in prepared bimetallic NPs, samples showed enhanced activity against bacteria acinetobacter baumannii that causes fever and nausea. The synthesized material assumed red appearance during rapid growth suggesting that antimicrobial activity was enhanced with increasing concentrations of Ag in bimetallic NPs. Furthermore, In silico predictions using molecular docking study were performed to identify the interaction pattern of Cu:Ag bimetallic NPs against β-lactamase enzyme of cell wall biosynthetic pathway alongside FabI and FabH enzymes of fatty acid biosynthetic pathway.

**Methods**

The current study was aimed to synthesize various concentrations of Ag within Cu:Ag bimetallic nanoparticles through hydrothermal route to investigate the efficacy of antibacterial agent against antibiotic-resistant bacteria.

**Materials**

Copper (II) chloride (CuCl2 0.2 H2O, 98.9%), and silver nitrate (AgNO3) as precursors, polyvinyl pyrrolidone (PVP, an average molecular weight of 40,000) as capping agent, sodium borohydride (NaBH4, 99.9%) and L-Ascorbic acid (C6H8O6, 99.0%) as reducing agents and ammonium hydroxide (NH4OH) were used in the present study after acquiring them from Sigma Aldrich, USA.

**Synthesis of bimetallic Cu:Ag NPs**

Bimetallic Cu:Ag NPs were prepared using co-precipitation method as portrayed in Fig. 1. In deionized water, 1.25 g of PVP and 0.5 g of ascorbic acid were added and stirred vigorously at 100 °C. Two solutions of 40 mL of ammonium hydroxide were prepared individually; in one solution, 1.7 g copper chloride and in the other solution 1.7 g silver nitrate were added. These two solutions are then poured one after the other into the initially prepared solution with the addition of 0.5 g of NaBH4. Afterwards, the final solution was stirred at 100 °C for 4 h to make it homogeneous and later centrifuged at 6000 rpm for complete extraction of NPs. The obtained bimetallic NPs were dried at 100 °C for complete elimination of moisture and impurities, to make sure that the prepared bimetallic products were in pure form [12]. Similarly, four samples with various Ag concentrations (mol 2.5%, 5%, 7.5%, and 10%) were prepared with fixed Cu ratios.
Antimicrobial activity
In vitro bactericidal potential of Cu:Ag bimetallic NPs was evaluated against pathogenic bacteria S. aureus, E. coli and A. baumannii isolates obtained from bovine mastitic milk using well diffusion method. Mannitol salt agar, MacConkey agar and Lauria Bertani agar were swabbed with isolated bacteria activated growth $1.5 \times 10^8$ CFU/ml. After media solidification, five wells were prepared using yellow pipette possessing dimensions of 15 mm diameter and ten microliter (5 µg/mL). Freshly prepared Cu:Ag bimetallic NPs were loaded into wells with different ratios in comparison with ten microliters of amoxicillin (5 µg/mL) as positive control and 50 µl of DIW as negative control. The bactericidal activity of synthesized Cu:Ag bimetallic NPs was determined by measuring inhibition zones (in mm) formed after incubation for 15 h at 37 °C.

Statistical analysis
The bactericidal activity of synthesized NPs with inhibition zone (mm) measurements was considered statistically significant using SPSS 20.0, one-way analysis of variance (ANOVA) [57].

Molecular docking study
Antibiotics decrease bacterial growth and cause death of bacteria through cell wall damage, disrupting biochemical processes, cell membrane damage, and penetration through biofilm [38]. In recent decades, plenty of nanoparticles with potential bactericidal activity have been reported, which kill bacteria either through cell wall disruption or by blocking the food source by a mechanism similar to known antibiotics [39–41]. Hence, enzymes belonging to these biochemical pathways are thought to be an important and attractive target for antibiotic discovery [12]. Here, key enzymes from cell wall biosynthetic pathway (i.e. β-lactamase) and fatty acid biosynthetic pathway (i.e. FabH and FabI) were selected as possible targets to evaluate the mechanism of interaction of Cu:Ag bimetallic NPs with their active pocket as inhibitors (see Fig. 2).

3D structural parameters of selected enzymes were fetched from protein data bank with PDB code: 4U0X (2.03 Å Resolution) for β-lactamase [42] and 6AH9; Resolution 1.74 Å [43] for Enoyl-[acyl-carrier-protein] reductase (FabI) from A. baumanni. The β-lactamase (3D structure) with PDB ID: 1MWU; Resolution 2.6 Å [44] and FabI with PDB code: 4CV1; Resolution 1.95
Molecular docking study of Cu:Ag bimetallic NPs was performed using ICM Molsoft v3.8–4a or above (Molsoft L.L.C., La Jolla, CA) software to identify binding interactions with key residues of active site [47]. The protein/receptor preparation tool of ICM was used for optimization and structure preparation of selected enzyme targets. Steps involved were addition of polar H-atoms, deletion of water molecules, and energy minimization using default parameters. The co-crystallized ligand molecule was removed to provide room for docking of NPs. The binding pocket was defined using grid box specifying position of crystallized ligand. The conformation with lowest binding energy out of top 10 docked conformations was selected in each case to analyze interaction pattern and binding tendency of Ag–Cu bimetallic NPs inside active pocket. Pymol and discovery studio visualizer software were employed for analysis and 3D-view depiction of binding interactions [48]. The structure of Cu:Ag bimetallic NPs was retrieved from PubChem in sdf format.

Characterization
Structural analysis and phase purity were observed by engaging XRD (PAN analytical X’pert pro XRD) with Cu-Kα radiation ($\lambda = 0.154$ nm, 20° to 80°). The presence of vibrational modes corresponding to various functional groups was evaluated using Fourier transform infrared spectroscopy-FTIR with Perkin Elmer spectrometer. Absorption spectra were acquired using a UV–visible-Genesys 10S spectrophotometer. FESEM coupled with EDS spectrometer (JSM-6610LV) and HR-TEM (JEOL JEM 2100F) were employed to visualize surface morphologies. The ICM v3.8-4a or above (Molsoft L.L.C., La Jolla, CA) software was used for molecular docking analysis.

Results and Discussion
XRD analysis was undertaken to assess the constitution of phases and crystal structure of formulated products. Figure 3a reveals the XRD pattern plotted between 20° and 80°. In bimetallic Cu:Ag, observed reflections around ~38.2°, 46°, 64.4°, and 77.1° are attributed to (111), (200), (220), and (311) facets of fcc Ag phase according to JCPDS No. 04-0783 [32, 49–52]. Whereas, in the case of
Cu, diffractions appearing at 32.6°, 44.2°, and 51° denoted (110), (111), and (200) lattice planes that confirmed the presence of fcc structured CuO and metallic Cu, respectively, and well-matched with JCPDS No. 04-0836 [32, 53–56]. In extracted pattern, both Ag and Cu peaks were observed which signifies the presence of NPs constituting both Ag and Cu phases. Moreover, the existence of CuO in samples with lower Ag content (e.g., 1:0.025, 1:0.050, and 1:0.075) reveals that Cu NPs were oxidized and exhibited non-protective behavior at high-temperature due to lower Ag concentrations [52]. Conversely, in the sample with the highest Ag content (1:0.1), CuO peak exhibits low intensity which indicates the formation of partially oxide-free product [57]. This suggests that improved oxidation resistance of bimetallic NPs will occur due to the addition of Ag [52]. No additional peak of impurity was detected within the instrument detection limits while each crystallographic plane comprises energetically distinct sites based on atom density. Both Cu and Ag NPs have high atom density facets at (111) that served to expose the maximum orientation of planes [51, 58]. Using Bragg’s law, d-spacing of Ag and Cu were found to be 0.24 and 0.21 nm, respectively which corresponds to distinct plane (111) of both elements and was in line with HR-TEM findings (Fig. 6) [51, 59–62]. Corresponding SAED rings (Fig. 3b–d) obtained from prepared
bimetallic products display distinct ring patterns that demonstrate well-crystallized products and accord well with XRD patterns.

FTIR spectra were recorded between 500 and 4000 cm$^{-1}$ as presented in Fig. 3e; the transmitted band positioned between 600 and 900 cm$^{-1}$ is caused by the formation of Cu:Ag bonding [63]. The observed band around ~1200 and 1400 cm$^{-1}$ is attributed to C–O and C–H, respectively; peaks appearing at ~1800 and 2100 cm$^{-1}$ corresponds to C=O and N–H bonding due to PVP and NH$_4$OH [64]. Transmittances observed around ~2800 cm$^{-1}$ and 3400 cm$^{-1}$ are ascribed to the presence of C≡N and hydroxyl group (O–H) [64].

Figure 4a shows the absorption spectra of Ag–Cu bimetallic NPs with clear absorption bands sited at 340, 410, and 500 nm, which are ascribed to surface plasmonic resonance absorption of metallic Ag and Cu [52]. The band appearing at 410 nm typically arises due to the presence of Ag NPs, and latter peak positioned at 510 nm is attributed to the existence of Cu NPs [52, 57–59, 65–67]. It may be suggested that bimetallic NPs are developed with distinct Ag and Cu phases, instead of bimetallic development that was also affirmed by XRD results as discussed earlier [52]. Slight redshift in absorption band at 410 nm and an increase in maximum absorption with increasing Ag content was observed [58]. Using the Tauc equation,

\[ \alpha h \nu = K (h \nu - E_g)^n \]

where \( \alpha \) is considered as coefficient of absorption [2.303 log \((T/d)\)], \( h \) symbolizes Planck’s constant \( (6.62607015 \times 10^{-34} \text{ Js}) \), \( \nu \) is frequency of light, \( K \) shows absorption index, and \( E_g \) is equal to band gap energy in eV. The value of “\( n \)” is related to electronic transition type of band gap [13, 26, 68, 69]. The band gap of prepared bimetallic products was calculated and found to be 3.2, 2.9, 2.7, and 2.6 eV, as demonstrated in Fig. 4b–e.

From SEM images (Fig. 5a–d) of as-synthesized Cu:Ag bimetallic nanocomposites, it was observed that small-sized particles were deposited on the surface of large particles. An increase in Ag content from 2.5 to 7.5% led to the formation of various particles with varying morphology which finally culminated into chunky Cu:Ag NPs. Further, accumulation of uneven and tiny Ag particles was augmented with increasing dopant concentration suggesting appearance of more scattered blocks on its surface. This signifies the major influence that Ag doping into Cu has on morphology, which was further confirmed with HR-TEM micrographs (see Fig. 5e–h).

To further elaborate on the morphology and d-spacing of prepared bimetallic, HR-TEM with 10 nm resolution was engaged. In Fig. 6a, d-spacing (0.21 nm) of Cu NPs corresponds to (111) facet of Cu, as also evident in XRD results (Fig. 3a). Figure 6b portrays a slight increase in layer spacing (0.21 to 0.22 nm) and shows Ag NPs with 0.24 nm interplanar distance that matched with (111) plane. Similarly, Fig. 6c, d shows calculated layer spacings and separate phases of bimetallic while Fig. 6e demonstrates particle shape of Ag and Cu NPs. The particles in HR-TEM images are seen to possess a core–shell structure. In Fig. 6d within a single particle, lattice fringes emanating from Cu and Ag were recorded. This strongly suggests the formation of core–shell bimetallic NPs with different Cu:Ag ratios yielding irregular quasi-spherical NPs. Furthermore, TEM image showed particles seem like as dark and bright region. This variation in contrast
within a single particle may indicate the presence of two distinct constituent materials suggesting the creation of bimetallic Cu:Ag particles [70, 71].

The presence of distinct peak in EDS spectrum and elemental composition originating from bimetallic affirmed successful formation of Cu:Ag NPs. Figure 7a represents EDS spectrum obtained from 1:0.050 sample which
Fig. 6 a–d HR-TEM (10 nm) images for d-spacing measurement for all prepared samples, e low magnification image showing bimetallic particles.

Fig. 7 EDS profiles obtained from bimetallic NPs.
shows clear peaks of Cu and Ag. Figure 7b is taken from 1:010 sample where peaks for C and O were detected in doped samples. These show up since carbon tabs are utilized to hold samples during SEM examination and/or due to background counts in SEM–EDS sensor.

In vitro bactericidal efficacy of Cu:Ag bimetallic NPs using agar well diffusion assay is presented in Table 1. The results demonstrate direct proportional relationship between synthesized NPs concentration and inhibition zones (mm). Statistically significant (P<0.05) inhibition zones recorded for (2.5, 5, 7.5 and 10 wt%) Ag dopant against S. aureus, E. coli and A. baumannii ranged from 0.85–2.8 mm, 0.55–1.95 mm and 0.65–1.85 mm, respectively, see Table 1. All results were compared with DIW (0 mm) and amoxicillin (4 mm) as negative and positive control, respectively. Similarly, %age efficacy of doped NPs increased (21.2–70%), (13.7–48.7%) and (16.2–46.2%) against S. aureus, E. coli and A. baumannii, respectively. Overall Cu:Ag bimetallic NPs were found to be more potent against S. aureus (i.e. gram +ive) compared with E. coli and A. baumannii (i.e. gram −ive).

Size, concentration, and shape of NPs directly affect oxidative stress produced by nanostructures. Bactericidal efficacy in the form of inhibition zones (mm) improved due to greater wt% doping of Ag-doped Cu bimetallic NPs due to increased cations (+++) availability. Bactericidal action in regard to size and concentration depicts an inverse relationship to size [58, 59]. Nano-sized structures produce reactive oxygen species (ROS) efficiently which reside in bacterial cell membranes leading to extrusion of cell organelles and ultimately death of bacteria [60]. Besides ROS production, cationic interaction of Ag⁺ and Cu²⁺ with negatively charged parts of bacterial cell membrane results in improved bactericidal efficacy at increasing concentrations through cell lysis and bacteria collapse [58, 61].

Biological applications of various classes of nanoparticles have been extensively studied since the last few decades. Owing to unique characteristics of NPs, they have been widely utilized for their potential as bactericidal agent with the ability to substitute traditional antibiotics. NPs interact with bacterial cells, disrupt cell membrane permeation and destroy key metabolic pathways [72]. The specific mechanism of nanoparticle toxicity towards bacteria needs to be explored. It is believed that NPs interact with bacterial cell involving electrostatic forces, van der Waals forces or hydrophobic interactions that ultimately result in death of bacteria. Enzymes have been reported as main virulence factor involved in bacterial infection and targeting them to inhibit their activity aid in tackling the caused infection [73]. Here, molecular docking study of Cu:Ag NPs against enzyme targets of cell wall alongside fatty acid biosynthetic pathway identified binding interaction pattern of these NPs inside active pocket. Keeping in view in vitro antibacterial potential of these NPs against A. baumannii, S. aureus and E. coli, the enzyme targets were selected from these microorganisms to get an insight into possible mechanism behind their bactericidal activity.

Best docked conformation observed in case of Cu:Ag Bimetallic NPs with β-lactamase from A. baumannii revealed hydrogen bonding interaction with Glu272 (2.8 Å) and Ser286 (3.2 Å) along with metal contact interaction with Val292 while the docking score was −4.013 kcal/mol (Fig. 8a). Similarly, binding score of Ag–Cu bimetallic NPs observed against β-lactamase from S. aureus was −4.981 kcal/mol possessing H-bonding interaction with Ser403 (3.2 Å), Tyr519 (3.6 Å), Gln521 (3.0 Å) and Asn464 (3.1 Å) as shown in Fig. 8b.

Second enzyme target selected in the current study FabI belong to fatty acid biosynthetic pathway and molecular docking predictions suggested Cu:Ag bimetallic NPs as potential inhibitor against this target. The Cu:Ag Bimetallic NPs showed good binding score (-3.385 kcal/mol) against FabI from A. baumannii having H-bonding with Ser201 (2.7 Å), Ala199 (3.5 Å), and Leu198 (3.3 Å) as depicted in Fig. 8c. Similarly, best docked conformation of Ag–Cu NPs with active site of FabI from S. aureus showed H-bonding with Gly202 (2.5 Å) and Gln155 (2.5 Å) having binding score –3.012 kcal/mol (Fig. 8d).

| Sample | Inhibition zone (mm)² | Inhibition zone (mm)² | Inhibition zone (mm)² | Ampicillin | DIW |
|--------|----------------------|----------------------|----------------------|------------|-----|
|        | 0.005 mg/ml          | 0.005 mg/ml          | 0.005 mg/ml          | 0.005 mg/ml |     |
| 1.25%  | 0.85                 | 0.55                 | 0.65                 | 4          | 0   |
| 1.5%   | 1.45                 | 0.95                 | 1.05                 | 4          | 0   |
| 1.75%  | 2.05                 | 1.45                 | 1.5                  | 4          | 0   |
| 1.10%  | 2.8                  | 1.95                 | 1.85                 | 4          | 0   |

² Inhibition zone (mm) of Cu:Ag bimetallic NPs for S. aureus

² Cu:Ag bimetallic NPs inhibition zones measurements for E. coli

² Bimetallic NPs zones of inhibition (mm) for A. baumannii

The results demonstrate direct proportional relationship between synthesized NPs concentration and inhibition zones (mm). Statistically significant (P<0.05) inhibition zones recorded for (2.5, 5, 7.5 and 10 wt%) Ag dopant against S. aureus, E. coli and A. baumannii ranged from 0.85–2.8 mm, 0.55–1.95 mm and 0.65–1.85 mm, respectively, see Table 1. All results were compared with DIW (0 mm) and amoxicillin (4 mm) as negative and positive control, respectively. Similarly, %age efficacy of doped NPs increased (21.2–70%), (13.7–48.7%) and (16.2–46.2%) against S. aureus, E. coli and A. baumannii, respectively. Overall Cu:Ag bimetallic NPs were found to be more potent against S. aureus (i.e. gram +ive) compared with E. coli and A. baumannii (i.e. gram −ive).

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In addition, the binding capacity of Cu:Ag bimetallic NPs against FabH from *E. coli* was also evaluated and the binding score observed was $-4.372$ kcal/mol having H-bonding interaction with Thr254 (3.5 Å), HIE244 (2.6 Å) and Glu302 (3.0 Å) shown in Fig. 8e.

**Conclusion**

Cu:Ag bimetallic nanoparticles were prepared through co-precipitation method for use in applications to combat bacteria-related ailments. XRD profiles confirmed the presence of fcc structured CuO and metallic Cu and Ag particles. Both Ag and Cu peaks were observed which signifies bimetallic NPs entailing Ag and Cu phases. Planes observed in XRD analysis correspond well to SAED rings. Attached chemical groups with formulated products and characteristic transmittance band between 600 and 900 cm$^{-1}$ was caused by the formation of Cu:Ag bonding. The plotted spectra of UV–vis showed absorption at 410 nm which typically arises due to the presence of Ag NPs, and latter peak positioned at 510 nm was attributed to the existence of Cu NPs. The particles in HR-TEM images were seen to have a core–shell structure. Cu:Ag NPs clearly showed the formation of bimetallic NPs with different Cu:Ag ratios yielding irregular quasi-spherical NPs. Further, d-spacing of Cu NPs i.e., 0.21 nm corresponds to (111) facet of Cu detected in XRD results. A slight increase in layer spacing (from 0.21 to 0.22 nm) also shows that Ag NPs with 0.24 nm layer distance matched with (111) plane. Molecular docking study showed good agreement with in vitro bactericidal activity. The binding tendency of Cu:Ag bimetallic NPs against β-lactamase enzyme of cell wall biosynthetic pathway alongside FabI and FabH enzymes of fatty acid biosynthetic pathway demonstrated their inhibition potential that needs to be explored further through enzyme inhibition studies.

**Abbreviations**

EDS: Energy dispersive X-ray spectroscopy; FTIR: Fourier transform infrared spectroscopy; G+ve: Gram-positive; G−ve: Gram negative; HR-TEM: High resolution transmission electron microscopy; JCPDS: Joint committee on powder diffraction standards; Ag: Silver; UV–Vis: Ultra-violet visible spectroscopy; XRD: X-ray diffraction.

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**Authors’ contributions**

SM and MI performed the whole experiments and AR wrote the manuscript. AG and RG provided the novel idea to carry out the experiment. AH performed antimicrobial and SA and JH participated in molecular docking study. participated in the data analysis of the results and discussion portion. M. Inshad and AS reviewed the manuscript, corrected the English. AUH carried out the FESEM and HRTEM analysis. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data are fully available on demand.
References

1. Ma Z, Kim D, Adesogan AT, Ko S, Galvao K, Jeong KC (2016) Chitosan microparticles exert broad-spectrum antimicrobial activity against antibiotic-resistant microorganisms without increasing resistance. ACS Appl Mater Interfaces 8(17):10700–10709

2. Niu J, Sun Y, Wang F, Zhao C, Ren J, Qu X (2018) Photomodulated nanomaterials used for a gram-selective antimicrobial. Chem Mater 30(20):7027–7033

3. Gupta A, Mumtaz S, Li C-H, Hussain I, Rotello VM (2019) Combatting antibiotic-resistant bacteria using nanomaterials. Chem Soc Rev 48(2):415–427

4. Zhao X et al. (2020) Smart Ti3C2Tx MXene fabric with fast humidity response and joule heating for healthcare and medical therapy applications. ACS Nano 17:8793–8805

5. Gu Z et al. (2018) Exploring the nanotoxicology of MoS2: a study on the interaction of MoS2 nanoparticles and K+ channels. ACS Nano 12(1):705–717

6. Yin W et al. (2016) Functionalized nano-MoS2 with peroxidase catalytic and near-infrared photothermal activities for safe and synergistic wound antibacterial applications. ACS Nano 10(12):11000–11011

7. Cao F et al. (2017) An efficient and benign antimicrobial depot based on silver-infused MoS2. ACS Nano 11(S):4651–4659

8. Ruparelia JP, Chatterjee AK, Duttagupta SP, Pal A, Mukherji SJ (2008) Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 4(3):707–716

9. Zain NM, Stapley A, Shama GJ (2014) Green synthesis of silver nanoparticles and their characterization. Int J Mol Sci 5:1–6

10. Das N, Nath S, Chakdar D, Gope G, Bhattacharjee RJ (2009) Preparation of silver nanoparticles and their characterization. Int J Mol Sci 10(2):292

11. Zhu H, Zhang C, Yin YJ (2005) Novel synthesis of copper nanoparticles: influence of the synthesis conditions on the particle size. Nanotechnology 16(12):3079

12. Raza A et al. (2019) Enhanced industrial dye degradation using Co doped in chemically exfoliated MoS2 nanosheets. Appl Nanosci 10(5):1535–1544

13. Raza A et al. (2020) A comparative study of dirac 2D materials, TMDCs and 2D insulators with regard to their structures and photocatalytic/sono photocatalytic behavior. Appl Nanosci 10:3875–3899

14. Sánchez-López E et al. (2020) Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials (Basel) 10(2):292

15. Huang J, Lin L, Sun D, Chen H, Yang D, Li Q (2015) Bio-inspired synthesis of silver-infused MoS2. ACS Nano 11(5):4651–4659

16. Toshima N (2008) Capped bimetallic and trimetallic nanoparticles for near-infrared photothermal activities for safe and synergetic wound antibacterial applications. Langmuir 26(14):12225–12229

17. Perdikaki A et al. (2016) Ag and Cu monometallic and Ag/Cu bimetallic nano-enzyme used for a gram-selective antimicrobial. Chem Mater 28(25):14018–14025

18. Chen X et al. (2017) Enhanced antimicrobial efficacy of bimetallic porous CuO microspheres decorated with Ag nanoparticles. ACS Appl Mater Interfaces 7(25):14018–14025

19. Das R, Nath S, Chakdar D, Gope G, Bhattacharjee RJ (2009) Preparation of silver nanoparticles and their characterization. Int J Mol Sci 10(2):292

20. Tahir K et al. (2016) Visible light photon catalytic inactivation of bacteria and photo degradation of methylene blue with Ag/TiO2 nanocomposite prepared by a novel method. J Photochem Photobiol B Biol 162:189–198

21. Das N, Nath S, Chakdar D, Gope G, Bhattacharjee RJ (2009) Preparation of silver nanoparticles and their characterization. Int J Mol Sci 10(2):292

22. Sánchez-López E et al. (2020) Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials (Basel) 10(2):292

23. Zhu H, Zhang C, Yin YJ (2005) Novel synthesis of copper nanoparticles: influence of the synthesis conditions on the particle size. Nanotechnology 16(12):3079

24. Raza A et al. (2019) Enhanced industrial dye degradation using Co doped in chemically exfoliated MoS2 nanosheets. Appl Nanosci 10(5):1535–1544

25. Raza A et al. (2020) A comparative study of dirac 2D materials, TMDCs and 2D insulators with regard to their structures and photocatalytic/sono photocatalytic behavior. Appl Nanosci 10:3875–3899

26. Tahir K et al. (2016) Visible light photon catalytic inactivation of bacteria and photo degradation of methylene blue with Ag/TiO2 nanocomposite prepared by a novel method. J Photochem Photobiol B Biol 162:189–198

27. Sánchez-López E et al. (2020) Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials (Basel) 10(2):292

28. Thota S, Wang Y, Zhao J (2018) Collodial Au–Cu alloy nanoparticles: synthesis, optical properties and applications. Mater Chem Front 2(6):1074–1089. https://doi.org/10.1039/C7TM0538E

29. Duan S, Wang R (2013) Bimetallic nanostructures with magnetic and noble metals and their physicochemical applications. Prog Nat Sci Mater Int 23(2):113–126

30. Ikram M et al. (2020) 2D chemically exfoliated hexagonal boron nitride (hBN) nanosheets doped with Au nanoparticles and catalytic activity for the treatment of industrial wastewater. Appl Nanosci 10(9):3525–3528

31. Liu X, Wu Y, Xie G, Wang Z, Li Y, Li Q (2017) New green soft chemistry route to Ag-Cu bimetallic nanomaterials. Int J Electrochem Sci 12:3275–3282

32. McNamara K, Toifal SA (2015) Nanosystems: the use of nanomaterials and applications. Phys Chem Chem Phys 17(42):27981–27995

33. Huang J, Lin L, Sun D, Chen H, Yang D, Li Q (2015) Bio-inspired synthesis of metal nanomaterials and applications. Chem Soc Rev 44(17):6330–6374. https://doi.org/10.1039/C5CS00133A

34. Rao CNR, Ramakrishna Matte HSS, Vagga R, Govindaraj A (2012) Recent progress in the synthesis of inorganic nanoparticles. Dalton Trans 41(17):5089–5120. https://doi.org/10.1039/C2DT12266A

35. Ikram M et al. (2020) Photocatalytic and bacticidal properties and molecular docking analysis of TiO2 nanoparticles conjugated with Zr for environmental remediation. RSC Adv 10(50):30007–30024. https://doi.org/10.1039/D0RA05862A

36. Ikram M et al. (2020) Dye degradation performance, bacticidal behavior and molecular docking analysis of Cu-doped TiO2 nanoparticles. RSC Adv 10(41):24215–24233

37. Dakal TC, Kumar A, Majumdar RS, Yadav V (2016) Mechanistic basis of antimicrobial actions of silver nanoparticles. Front Microbiol 7:1831
39. Rai M, Deshmukh S, Ingle A, Gade A (2012) Silver nanoparticles: the pow¬
erful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 112(S):841–852

40. White RJ, Cutting K, Kingsley A (2006) Topical antimicrobials in the control of wound bioburden. Ostomy Wound Manag 52(8):26–58

41. Lee N-Y, Hseuh P-R, Ko W-C (2019) Nanoparticles in the treatment of infections caused by multidrug-resistant organisms. Front Pharmacol 10:1153

42. Powers RA et al (2014) Biochemical and structural analysis of inhibitors targeting the ADC-7 cephalosporinase of Acinetobacter baumannii. Biochemistry 53(48):7670–7679

43. Crystal structure of enoyl-ACP reductase from Acinetobacter baumannii in complex with NAD and Triclosan. http://www.rcsb.org/structure/6AH9.

44. Lim D, Strynadka NC (2002) Structural basis for the β lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. Nat Struct Biol 9(11):870–876

45. Schiebel J et al (2014) Rational design of broad spectrum antibacterial activity based on a clinically relevant enoyl-acyl carrier protein (ACP) reductase inhibitor. J Biol Chem 289(23):15987–16005

46. McKinney DC et al (2016) Antibacterial FabH inhibitors with mode of action validated in Haemophilus influenzae by in vitro resistance muta¬tion mapping. ACS Infect Dis 2(7):456–464

47. Abagyan R, Totrov M (1994) Based probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins. J Mol Biol 235(3):983–1002

48. Systems D (2015) BIOVIA, discovery studio modeling environment. Release 4.5, Dassault Systemes: San Diego, CA

49. Li Y, Guo S, Yang H, Chao Y, Jiang S, Wang CJ (2018) One-step synthesis of ultra-long silver nanowires of over 100 μm and their application in flexible transparent conductive films. RSC Adv 8(15):8057–8063

50. Lange AS, Sharma SJ, Pode RB (2010) Synthesis of silver nanoparticles: a safer alternative to conventional antimicrobial and antibacterial agents. Pharm Res 27(3):478–483

51. Khan Z, Obaid AY (2016) Seedless, copper-induced synthesis of stable Ag/Cu bimetallic nanoparticles and their optical properties. RSC Adv 6(35):29116–29126

52. Chen Z, Mochizuki D, Maiani MM, Wada YIN (2013) Facile synthesis of bimetallic Cu–Ag nanoparticles under microwave irradiation and their oxidation resistance. Nanotechnology 24(26):265602

53. Ahamed M, Alhadlaq HA, Khan M, Karuppiah P, Al-Dhabi NA (2014) Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. J Nanomater 2014:657854

54. Saxmal AK, Dutta S, Pal TJDT (2016) A ternary Cu2O–Cu–CuO nanocomposite: a catalyst with intriguing activity. Dalton Trans 45(1):3139–3150

55. Betancourt-Galindo R et al (2014) Synthesis of copper nanoparticles by thermal decomposition and their antimicrobial properties. J Nanomater 2014:980545

56. Mardiansyah D et al (2018) Effect of temperature on the oxidation of Cu nanowires and an easy way to produce it: oxidation-resistant transparent conducting electrode using a PEDOT: PSS coating. Sci Rep 8:1–9

57. Rout L, Kumar A, Dhaka RS, Dash PJR (2016) Bimetallic Ag–Cu alloy nanoparticles as a highly active catalyst for the enamination of 1, 3-dicarboonyl compounds. RSC Adv 6(55):49923–49940

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