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RESEARCH PAPRE

Argyreia nervosa-driven biosynthesis of Cu–Ag bimetallic nanoparticles from plant leaves extract unveils enhanced antibacterial properties

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Abstract

Our study specifically explores the biosynthesis of copper–silver bimetallic nanoparticles (Cu–Ag BMNPs) using *Argyreia nervosa* (AN) plant leaf green extract as a versatile agent for capping, reducing, and stabilizing. This biosynthesis method is characterized by its simplicity and cost-effectiveness, utilizing silver nitrate ($AgNO_3$) and cupric oxide (CuO) as precursor materials. Our comprehensive characterization of the Cu–Ag BMNPs, employing techniques such as X-ray diffraction (XRD), UV–Vis spectrometry, scanning electron microscopy (SEM), Zetasizer, and Fourier transformed infrared spectrometry (FTIR). FTIR analysis reveals biofunctional groups and chemical bands, while SEM and XRD analyses provide morphological and structural details. To evaluate the antimicrobial properties of the Cu–Ag BMNPs, we conducted disc diffusion and minimum inhibitory concentration (MIC) assays against *Escherichia coli* (*E. coli*), with results compared to the standard gentamicin antibiotic. It is observed that the 2% and 5% CuO concentrations of AN Cu–Ag BMNPs exhibit substantial antibacterial activity in comparison to AN extract when tested on EPEC. Among these, the Cu–Ag BMNPs at a 2% concentration demonstrate higher antibacterial activity, potentially attributed to the enhanced dispersion of BMNPs facilitated by the lower CuO doping concentration. These two assays showcased the improved antimicrobial activity of Cu–Ag BMNPs, highlighting their synergistic effect, characterized by high MIC values and a broad zone of inhibition in the disc diffusion tests against *E. coli*. These results emphasize the significant antibacterial potential of the synthesized BMNPs, with a medicinal plant AN leaf extract playing a pivotal role in enhancing antibacterial activity.

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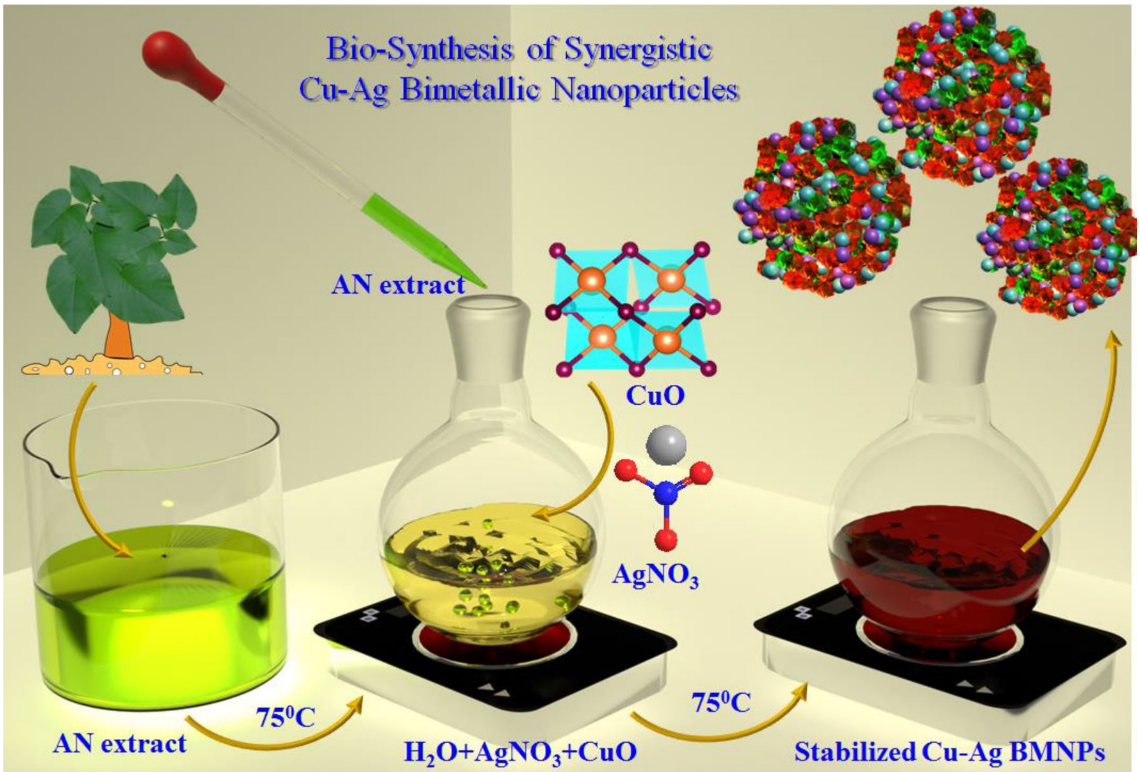
Graphical abstract

Novelty:

The most important aspect of this research project is the role of the *Argyreia nervosa* plant leaf extract in improving and enhancing the antibacterial activity of the Cu–Ag BMNPs. This underscores the novel synergy between plant extracts and nanomaterials for practical applications. Also, this study's novelty arises from its innovative approach to biosynthesis, the pivotal role of AN plant leaf extract, and the potential for addressing real-world challenges related to bacterial infections and resistance. The enhanced antibacterial properties brought about by this unique synergy have far-reaching implications in diverse applications and underscore the potential of bimetallic nanoparticles in nano-biotechnology.



Keywords Bimetallic nanoparticles · Green synthesis · Synergetic effect · Disc diffusion · Antimicrobial activity · *Escherichia coli*

Introduction

Nano-biotechnology stands as a swiftly expanding field that taps into the distinctive characteristics of nanoparticles for diverse biomedical applications, encompassing roles as antimicrobial agents and carriers for drug delivery [1, 2]. Metal nanoparticles, especially those synthesized through nanotechnology, find applications across biomedicine, drug delivery systems, energy, agriculture, and in the combat against microbial infections and cancers [3–10]. Given the growing resistance of bacteria to conventional antibiotics, there arises an urgent need to seek out alternative antimicrobial agents to address pathogenic infections, which present substantial global health risks. Within this context, mono/bi-nanoparticles have emerged as highly promising candidates for specific antimicrobial applications over the antibiotics.

Recent endeavors have been focused on the creation of environmentally friendly and economically viable techniques for manufacturing metal nanoparticles (MNPs). Presently, synthesis methodologies encompass chemical reduction processes, microwave-assisted procedures, electron irradiation techniques, and biogenic methods mediated by plants [11–20]. Nevertheless, challenges persist in terms of effectively regulating the stability, output, and structural features of the resulting MNPs [21–23]. Chemical synthesis routes require significant energy and financial resources, leading to adverse environmental consequences such as pollution, ecological toxicity, and public health risks.

Among the array of synthesis techniques available, green synthesis or biogenic methods have gained great prominence due to their eco-friendly attributes, low toxicity, cost-efficiency, and streamlined synthesis procedures. These approaches make use of plant extracts, derived from sources like leaves, fruits, or peels, as well as microorganisms, to both reduce and stabilize MNPs [24–27]. While microorganism-driven synthesis routes tend to be time-consuming and necessitate intricate aseptic conditions, the utilization of plant extracts presents a more energy-efficient and economically viable alternative. Plant extracts boast abundant natural biomolecules and intricate molecular structures with significant medicinal value, effectively serving as capping agents for MNPs and augmenting their antibacterial attributes [28–31].

MNPs are highly sought after in the field of nanotechnology due to their multifunctional properties. These properties encompass optical, electrical, catalytic, magnetic, and antimicrobial characteristics, which emanate from their nanoscale dimensions and the resulting large surface area-to-volume ratio [32–36]. Bimetallic nanoparticles, such as the copper–silver (Cu–Ag) combination, form core–shell nanostructures, demonstrating multifunctional attributes owing to the synergistic effects between the constituent metal ions [37–41]. Despite reports of monometallic nanoparticle synthesis using *Argyreia nervosa* (AN) plant leaf extract, this plant has not yet been harnessed for the creation of copper–silver bimetallic nanoparticles (Cu–Ag BMNPs) [42–46]. Notably, the AN plant is renowned for its medicinal properties, including anti-inflammatory and stress reduction effects [47].

This study marks the pioneering use of AN plant leaf green extract as a trifunctional agent, encompassing reduction, capping, and stabilization, for the synthesis of stable Cu–Ag BMNPs. While prior research has established the efficient antimicrobial properties of AgNPs against *Escherichia coli* bacteria, we anticipate that Cu–Ag BMNPs will exhibit even more potent antibacterial characteristics [47]. AgNPs have been widely explored for a spectrum of technological applications, including their use as antimicrobial agents, carriers for drug delivery, components in water purification, and additives in the textile industry. They are notably acclaimed for their remarkable stability and low toxicity toward human cells. Despite numerous investigations reporting the synthesis and antimicrobial features of diverse MNPs, the green synthesis of Cu–Ag BMNPs utilizing AN plant extract, along with their antibacterial attributes, remains an uncharted territory.

In this study, we successfully synthesized Cu–Ag BMNPs with the aid of AN plant leaf extract and assessed their antibacterial efficacy against enteropathogenic *E. coli* (EPEC), a specific *Escherichia coli* serotype, employing disc diffusion and minimum inhibitory concentration (MIC) assays. Our findings yield valuable insights into the potential of these environmentally synthesized Cu–Ag BMNPs as potent antibacterial agents. Furthermore, we assert that the green synthesis approach employed in this study, utilizing AN plant leaf extract, represents a promising, energy-efficient, and cost-effective avenue for the large-scale production of Cu–Ag BMNPs, catering to various commercial antibacterial applications.

Methods and characterizations

Materials

We procured copper (II) oxide (CuO), silver nitrate ($AgNO_3$), and all standard solvents from Sigma-Aldrich, located in Bangalore, India. The AN plant leaves were meticulously collected from the herbal garden at EPTRI, Hyderabad.

Synthesis of silver–copper bimetallic nanoparticles (Cu–Ag BMNPs)

The green synthesis process is simple and economic for evaluating the practical feasibility of the Cu–Ag bimetallic nanoparticles' production for industrial applications. The AN leaves were carefully cleaned in a vial, undergoing multiple rinses with water to eliminate all dust particles. Following this, the leaves were air dried under ambient conditions and finely ground into a powder. Subsequently, 1.5 g of the AN leaf powder was mixed with 75 mL of deionized (DI) water and stirred at 500 rpm, maintaining a temperature of 75 °C for a duration of 20 min. This process yielded the AN extract, which was then subjected to filtration using filter paper and subsequently centrifuged for 20 min at 3000 rpm.

To synthesize Cu–Ag BMNPs, 75 mL of the AN leaf extract was carefully dispensed drop by drop (at a rate of one drop per second) from a burette into various mixtures. These mixtures comprised 200 mL of 0.05 M $AgNO_3$ and 50 mL of (0.005–0.01 M) CuO (with CuO concentrations at 2%, 5%, 10%, and 20% in $AgNO_3$) within a 300 mL beaker. The solutions were combined and maintained at 80 °C for 15 min while stirring at a rate of 500 rpm. The addition of leaf extract from the burette was meticulously controlled as it dropped into the solution being stirred at 500 rpm, all at a consistent temperature of 80 °C. The combined solution was heated on a hot plate until a visible change in color was observed. Subsequently, the resulting solution underwent centrifugation at 3000 rpm for 20 min and was then dried in an oven at 60 °C to obtain the powdered nanoparticles. A schematic representation of the Cu–Ag BMNPs synthesis procedure is depicted in Fig. 1. All experiments are repeated to see consistency in the results.

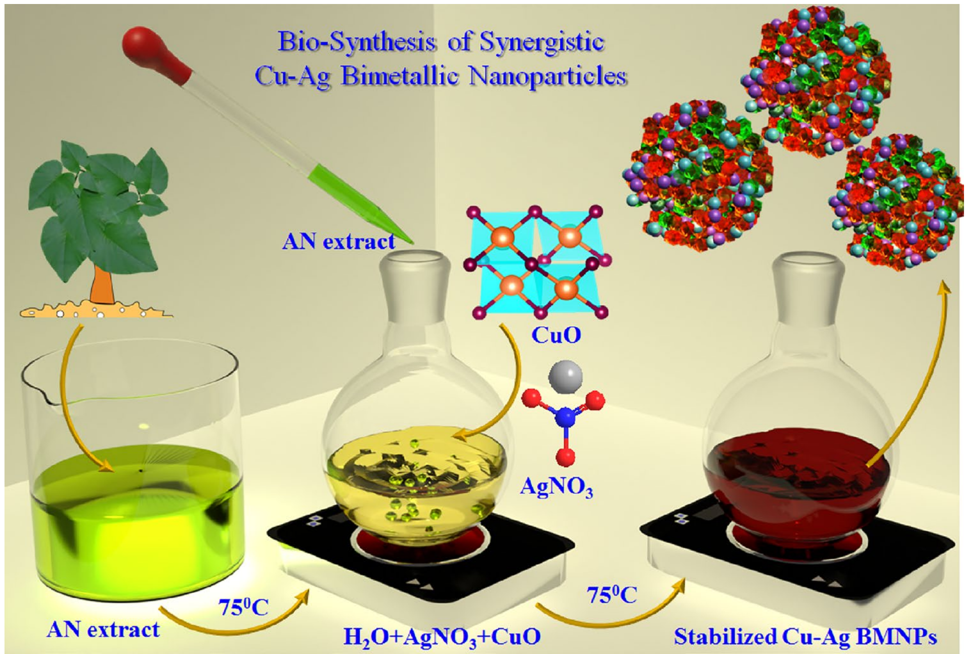


Fig. 1 Nucleation process of AN leaf extract-mediated Cu–Ag BMNPs

Characterization of synthesized Cu–Ag BMNPs

The formation of Cu–Ag BMNPs was confirmed through UV–visible spectroscopy, which analyzed wavelengths ranging from 200 to 800 nm. This analysis revealed the characteristic absorption peaks of Cu–Ag BMNPs. Furthermore, FTIR was employed to identify the specific functional phytochemical groups responsible for the formation of Cu–Ag BMNPs. The FTIR analysis yielded valuable insights into the functional groups and vibrational chemical bonds involved in stabilizing, reducing, and capping Cu–Ag BMNPs. To examine the surface morphology, grain sizes, and size distribution of Cu–Ag BMNPs, SEM was utilized. SEM allowed for the visualization of Cu–Ag BMNPs and facilitated the determination of their surface size distribution. Additionally, the crystal structure details of Cu–Ag BMNPs were investigated using XRD, which provided information about the crystalline structure of these nanoparticles.

The zeta potential of the Cu–Ag BMNPs was ascertained using a Zetasizer Nano ZS instrument. Zeta potential values were calculated by employing the Smoluchowski equation based on electrophoretic mobility. This analysis offered valuable insights into the surface charge and stability of the nanoparticles. In summary, the combination of UV–visible spectroscopy, FTIR, SEM, XRD, and zeta potential measurements provided a comprehensive characterization of the synthesized Cu–Ag BMNPs. This characterization encompassed their optical properties, chemical composition, morphology, crystal structure, and surface charge.

Antibacterial activity

The assessment of the antimicrobial activity of the Cu–Ag BMNPs took place within a sterile laboratory environment, utilizing a biosafety cabinet. All equipment and glassware used for monitoring antimicrobial activity were sterilized through autoclaving. To evaluate the antibacterial properties

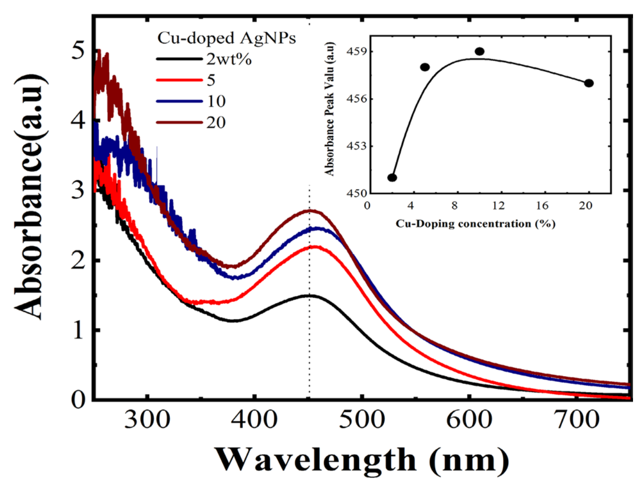


Fig. 2 UV–Vis absorption spectra of Cu–Ag BMNPs showing peak absorbance

of the synthesized Cu–Ag BMNPs, we conducted and repeated experiments involving EPEC, in comparison with a reference antibiotic, gentamicin. In the subsequent sections, we provide a brief overview of the two methods employed to evaluate the antibacterial activity of the Cu–Ag BMNPs.

Bacterial culture

EPEC, a rod-shaped Gram-negative pathogen, was cultured by reviving glycerol stocks stored at −80 °C in Luria–Bertani (LB) broth. The bacteria were cultivated at 37 °C for 12–14 h in a shaker incubator at 180 rpm. To facilitate growth and maintenance for subsequent assays, EPEC bacterial culture was streaked on LB agar plates. Subsequently, the overnight culture of EPEC with an optical density at 600 nm (OD600 nm) of 1 was employed in conducting the disc diffusion and minimum inhibitory concentration assays.

Disc diffusion assay

To assess the antibacterial activity of the synthesized Cu–Ag BMNPs, a disc diffusion method was employed against Gram-negative enteropathogenic *E. coli* (EPEC). Initially, 100 µL of EPEC culture at OD 600 = 1 was evenly spread on LB agar media plates. Sterile 6 mm filter paper discs, impregnated with 50 µL of AN leaf extract, as well as AN Cu–Ag BMNPs at concentrations of 2%, 5%, 10%, and 20% (solution-based), were carefully placed on the LB agar media plates already coated with EPEC. These plates were then incubated overnight at a temperature of 37 °C following the drying of the impregnated discs. As a control, LB broth media was added as a negative control, while gentamicin (30 µg), a known effective antibiotic against Gram-negative bacteria, including *E. coli*, served as a positive control. The zones of inhibition resulting from bacterial clearance due to the action of the Cu–Ag BMNPs were quantified by measuring their diameter in millimeters.

Minimum inhibition concentration (MIC) assay

To determine the MIC of the synthesized Cu–Ag BMNPs, a series of experiments were conducted. In a 96-well plate, 50 µL of enteropathogenic *E. coli* at an approximate concentration of 1.22×10^8 CFU/mL (OD600 = 1) was incubated with 50 µL of AN leaf extract, along with varying concentrations of AN Cu–Ag BMNPs, specifically 2%, 5%, 10%, and 20%. As controls, LB broth and gentamicin (30 µg) were used as negative and positive controls, respectively. The plate was then incubated for 6 h at 37 °C. Following incubation, samples were subjected to serial 10-fold dilutions, resulting in dilutions up to 106-fold. From each dilution, 10 µL was extracted and plated onto LB agar media plates. Subsequently, these plates were incubated overnight at 37 °C. The MIC was calculated by comparing the bacterial colony-forming units per milliliter (CFU/mL) using a designated formula.

$$\text{Colony forming units per mL} = \left(\frac{\text{number of colonies counted} * \text{dilution factor}}{\text{volume of culture plated in mL}}\right).$$

The outcomes derived from both the disc diffusion and MIC assays offer significant insights into the antimicrobial capabilities of the synthesized Cu–Ag BMNPs. The disc diffusion assay enables a qualitative assessment of the Cu–Ag BMNPs' effectiveness in curtailing bacterial proliferation, manifested by the presence of inhibition zones. Conversely, the MIC assay furnishes quantitative information regarding the minimum concentration of Cu–Ag BMNPs required for bacterial inhibition. We explore further studies with other methods such as time-kill kinetics, biofilm inhibition assays, and cytotoxicity assessments would offer a more comprehensive understanding of the efficacy, mechanism of action, and safety profile of the Cu–Ag bimetallic nanoparticles against bacterial pathogens.

Results

UV–VIS spectroscopy

In our investigation, we delved into the optical characteristics of metal nanoparticles, acknowledging their susceptibility to variations in surface morphology, size, and shape. During our analysis using UV–Vis spectroscopy, we honed in on the intensity of absorption peaks associated with surface plasmon resonance (SPR). What we found intriguing were the broad absorption peaks spanning from 350 to 550 nm, their width expanding in tandem with the rise in copper concentration within the Cu–Ag BMNPs. Of particular interest was the peak at 450 nm, exhibiting the highest absorption intensity. We hypothesize that this phenomenon may signify an augmented interaction among nanoparticles within the Cu–Ag BMNPs, potentially spurred by copper complexation [48]. This observation is further elucidated in Fig. 2.

Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis provides insights into the functional groups and chemical bands present in the Cu–Ag BMNPs.

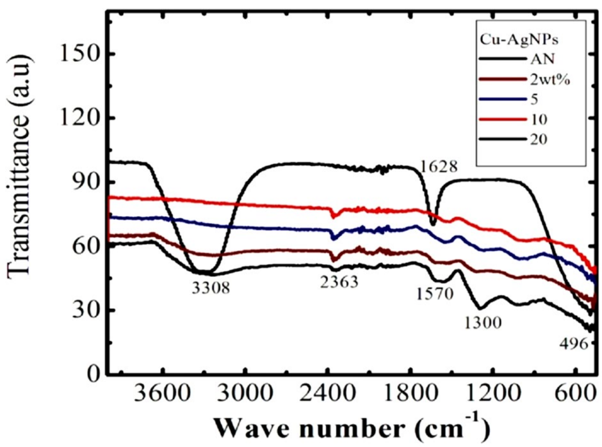


Fig. 3 FTIR spectra of AN leaf extract-mediated Cu–Ag BMNPs

The infrared spectra, as shown in Fig. 3, revealed the presence of specific functional biomolecules responsible for reducing the bimetallic ions and stabilizing the nanoparticles. The observed peaks were compared with standard values to identify the surface functional groups. The peaks at 496 cm^{-1} were attributed to the stretching vibration of Cu–O bands, while the bands at 1450 cm^{-1} and 1630 cm^{-1} were assigned to N–H stretch vibration and C–N/C–C stretching vibrations, respectively, indicating the presence of proteins. Additionally, the peak at 3308 cm^{-1} suggested the presence of phenolic compounds in the plant extract, which played a crucial role in the synthesis and formation of Cu–Ag BMNPs. Notably, the FTIR spectra confirmed the absence of surfactants on the nanoparticle surface.

Morphology: scanning electron microscopy (SEM)

Figure 4 displays the size and surface morphology features of the Cu–Ag BMNPs [48–51]. The micrograph images of the Cu–Ag BMNPs depict diverse irregular shapes with sizes ranging from 200 to 500 nm, whereas the AgNPs display distinct spherical shapes with an average size of approximately 100–300 nm [36, 50, 51]. The SEM images of Cu–Ag BMNPs reveal irregular clusters with rough

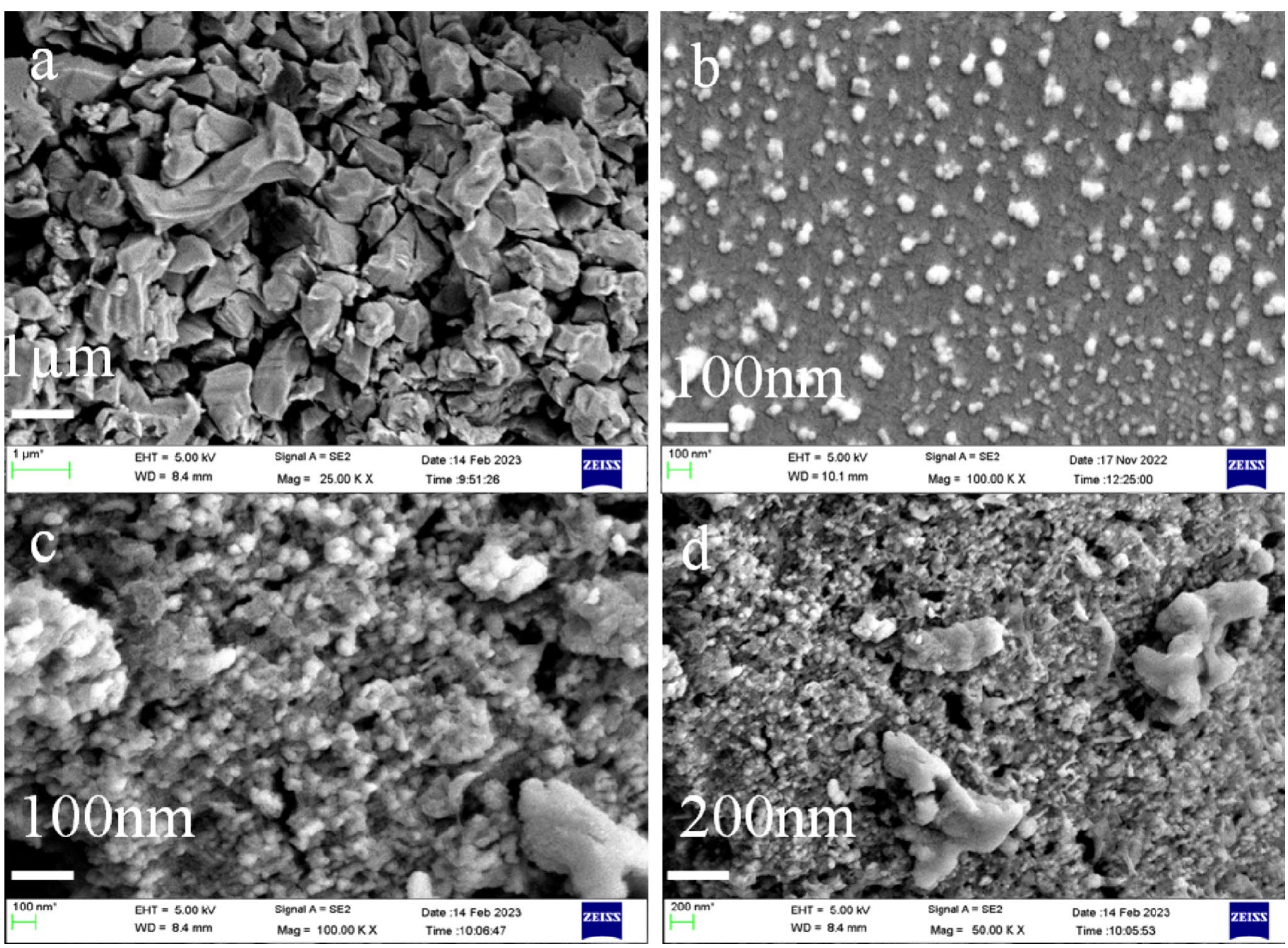


Fig. 4 SEM image of the **a** CuNPs, **b** AgNPs, **c** and **d** Cu–Ag BMNPs

surfaces and varying sizes. The formation of these agglomerated clusters could be attributed to the differing crystallization growth patterns of CuNPs and AgNPs [48, 52–54]. It is required to modify surface strategies inorder to prevent or minimize nanoparticles aggregation. Our future research directions aimed at further elucidating and mitigating nanoparticle aggregation issues. This could involve exploring alternative formulation strategies, optimizing surface functionalization approaches, or investigating novel nanoparticle stabilization techniques.

X-ray diffraction (XRD)

XRD measurements and analysis were employed to determine the details of the crystallite structure of the Cu–Ag BMNPs. The XRD diffraction patterns disclose that the Cu–Ag BMNPs are of composite crystallite structures and free of impurities. The XRD diffraction pattern of individual CuNPs and AgNPs reveal that are crystallite structure (Fig. 5a) and hybrid composite XRD spectrum observed polycrystallite structure (Fig. 5b). The diffraction pattern of Cu–Ag BMNPs show broader peaks, which confirms that the produced synthesized Cu–Ag BMNPs possess polycrystallite nanomaterial. Scherer's formula was used to estimate the crystallite size details of Cu–Ag BMNPs. The strong peak intensities were observed at 37.5°, 45.6°, 64°, and 77°, which suggest the crystallite phases of CuO and Cu–Ag BMNPs. The reflection planes 111 (37.5°), 111(43.6°), 200 (45.6°), 200 (53°), 220 (64°), 220 (74°), 311 (77°) verified the crystallite phase of Cu–Ag BMNPs. The high intense peak is noticed at (111) and the lattice details were calculated using Bragg's equation [51].

$$a = \lambda\left(\frac{\sqrt{h^2 + k^2 + l^2}}{2\sin\theta}\right)A^0.$$

The Cu–Ag BMNPs composite crystallite size was calculated using Debye-Scherr's equation ($D = 0.9\lambda/\beta\cos\theta$) where λ is the wavelength of X-rays (used in the experiment), β is the full width of half maximum (FWHM), and θ is the angle of diffraction. The composite polycrystallite size of nanoparticles was determined about 25–100 nm and it also depends on doping levels od CuO [50, 51, 55, 56].

Zeta potential

The analysis of zeta potential for the dispersed Cu–Ag BMNPs offers valuable insights into the surface charge interactions and long-term stability of these nanoparticles. Figure 6 illustrates the variation in zeta potential as a function of copper doping concentration in the Cu–Ag BMNPs. The recorded zeta potential values fell within the range of −20 to −10 mV when dispersed in deionized water. Zeta potential values spanning from +25 mV to −25 mV are indicative of a high degree of dispersion stability, while extremely low zeta potential values suggest the formation of aggregates due to inter-particle attractions.

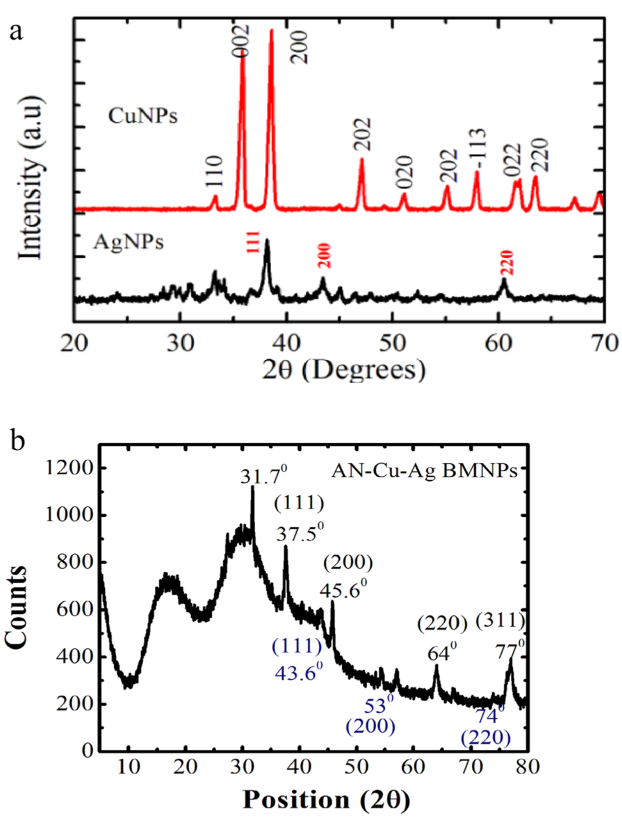


Fig. 5 **a** Stacked XRD pattern of CuNPs and AgNPs. **b** XRD pattern of AN Cu–Ag BMNPs

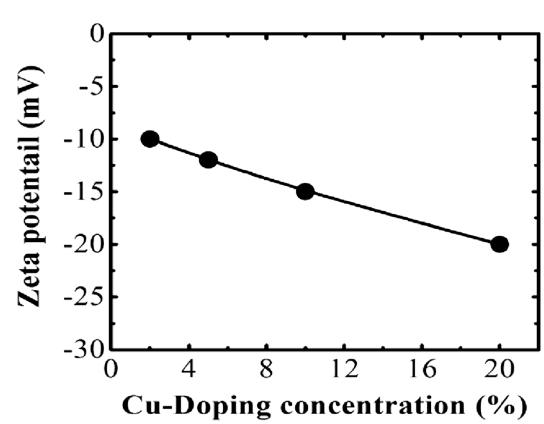


Fig. 6 Zeta potential of Cu–Ag BMNPs

Antibacterial activity of Cu–Ag BMNPs

Disc diffusion assay

EPEC, a known pathogen responsible for persistent diarrhea in humans, presents a formidable global health challenge. The emergence of antibiotic resistance, antibiotic-associated diarrhea, and rising toxicity levels underscore the urgent need for new, effective antimicrobials with low toxicity. This study investigated the antibacterial efficacy of AN-mediated bimetallic Cu–Ag BMNPs against EPEC using the disc diffusion assay, with gentamicin serving as a reference antibiotic. Notably, the antibacterial activity of AN Cu–Ag BMNPs was most pronounced at the 12-h incubation mark, as opposed to 16 h. After 16 h, AN extract failed to produce a clear zone of inhibition, unlike at 12 h. This observation suggests that bimetallic nanoparticles offer an added advantage in inhibiting and eliminating bacteria for an extended period, as compared to using only AN extract. Figure 7 illustrates the variation in antibacterial activity of Cu–Ag BMNPs relative to the concentration of Cu doping in these nanoparticles. The synthesized Cu–Ag BMNPs exhibited significant activity against EPEC, and this activity slightly intensified with higher concentrations of Cu doping, as demonstrated in Fig. 8, which provides a graphical representation of the antibacterial activity of Cu–Ag BMNPs against the EPEC pathogen, correlated with the concentration of Cu doping in the composite Cu–Ag BMNPs.

Minimum inhibition concentration (MIC) assay

Figure 9 illustrates the antimicrobial activity of Cu–Ag BMNPs against the EPEC pathogen, corroborated through the MIC assay. In Fig. 10, a histogram provides a comparative analysis of the antibacterial activity of Cu–Ag BMNPs against EPEC bacteria across various Cu doping concentrations. Significantly, it is evident that the 2% and 5% CuO concentrations of AN Cu–Ag BMNPs exhibit substantial antibacterial activity in comparison to AN extract when tested on EPEC. Among these, the Cu–Ag BMNPs at a 2% concentration demonstrate higher antibacterial activity, potentially attributed to the enhanced dispersion of BMNPs facilitated by the lower CuO doping concentration. This underscores the importance of the MIC at which EPEC growth is effectively inhibited by 2% Cu–Ag BMNPs at this optimum concentration. The heightened antibacterial efficacy of Cu–Ag BMNPs may be attributed to the synergistic effects of both metal ions, Cu and Ag, present in this

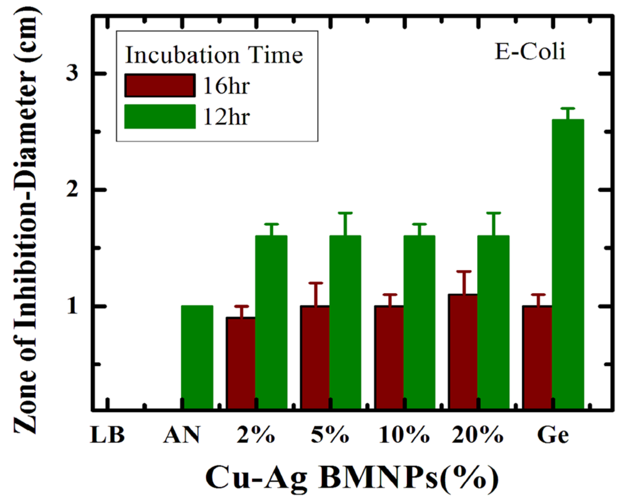


Fig. 8 Histogram showing zone of inhibition against EPEC as the function of Cu doping in Cu–Ag BMNPs

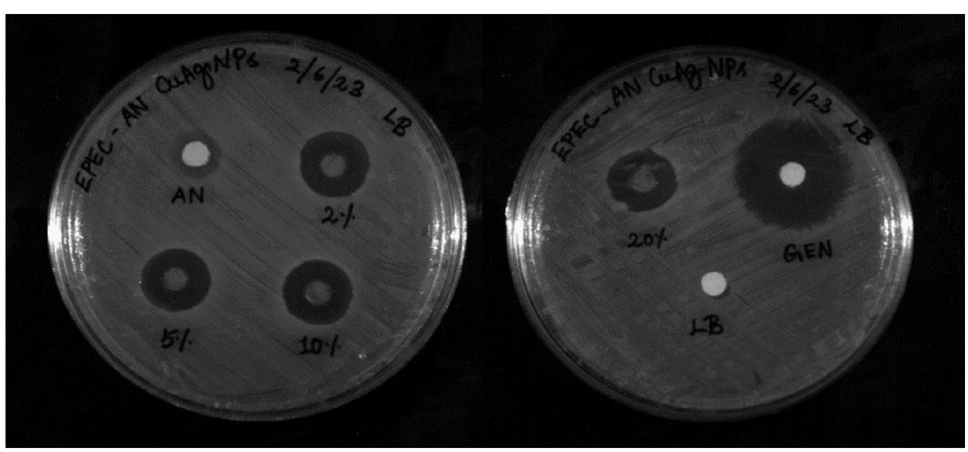


Fig. 7 Antibacterial activity of AN Cu–Ag BMNPs on EPEC via disc diffusion assay

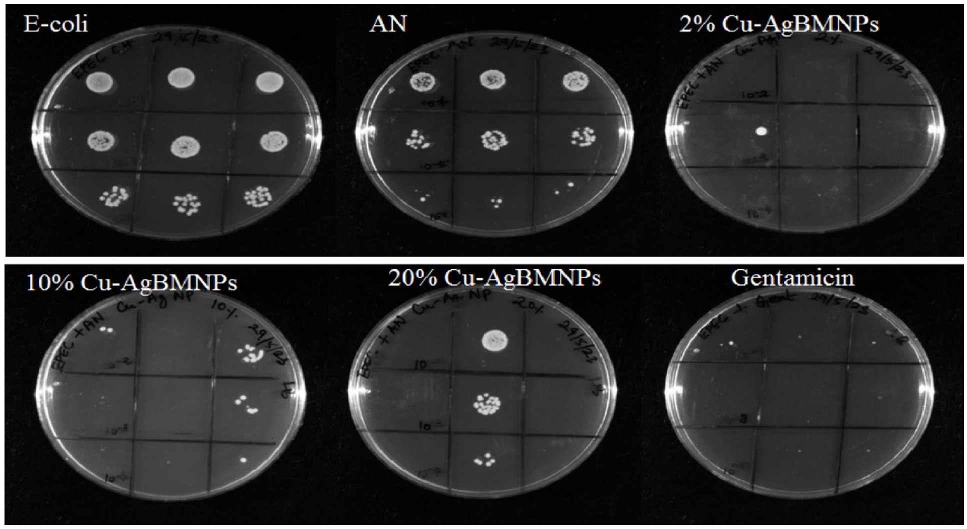


Fig. 9 Effect of AN leaf extract-mediated Cu–Ag BMNPs against EPEC via the MIC assay

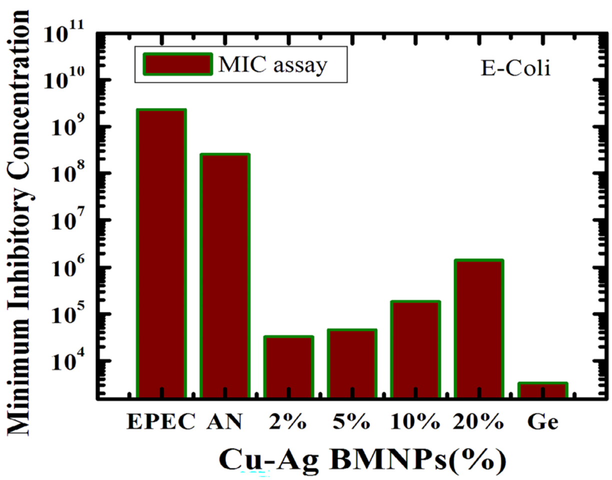


Fig. 10 Effect of AN leaf extract-mediated Cu–Ag BMNPs against EPEC via the MIC assay

hybrid metal nanocomposite, rendering it a potent antibacterial agent.

Discussion

Metallic nanoparticles (MNPs), encompassing monometallic, bimetallic, trimetallic, and multi-metallic varieties, have emerged as promising candidates for the diagnosis and treatment of chronic infections. The incorporation of multiple metal ions into the hybrid nanostructure of MNPs has paved the way for exploring their synergistic effects, resulting in the generation of multifunctional physiochemical properties and heightened antimicrobial activity. In this study, our focus was on the synthesis of Cu–Ag BMNPs through a biogenic approach. We utilized AN(elephant creeper) medicinal plant leaf extract as a reducing, capping, and stabilizing agent. The physiochemical properties of Cu–Ag BMNPs underwent extensive characterization using various analytical techniques. UV–Vis spectroscopy served to confirm the successful formation of Cu–Ag BMNPs, substantiated by the presence of characteristic absorption peaks. Furthermore, XRD and FTIR analyses shed light on the crystalline nature of the Cu–Ag BMNPs and offered insights into their structural intricacies. Importantly, the vital role of AN plant leaf extract in facilitating the formation of complex nanostructures was evident. It significantly contributed to the enhancement of antibacterial activity in these composite nanoparticles, as substantiated.

The antibacterial properties of Cu–Ag BMNPs were assessed against Enteropathogenic *E. coli*, in comparison to the standard reference antibiotic gentamicin, known for its effectiveness against this strain. The results unequivocally demonstrated that the synthesized Cu–Ag BMNPs exhibited significantly higher antimicrobial activity against enteropathogenic *E. coli*, underscoring their potential as formidable antimicrobial agents. These findings are in concordance with prior studies emphasizing the advantages of bimetallic nanoparticles, which boast enhanced physiochemical properties and heightened antimicrobial efficacy. The superior antibacterial activity of Cu–Ag BMNPs can be attributed to the synergistic effects arising from the incorporation of different metal ions. This synergy serves to augment the overall antimicrobial efficacy. To shed light on the potential mechanisms underlying this enhanced antibacterial activity, Fig. 11 illustrates four possible interactions between Cu–Ag BMNPs and *E. coli* pathogenic cells. These mechanisms encompass the following: 1. Cu–Ag BMNPs adhering to the bacterial cell surface and disrupting the cell membrane; 2. Cu–Ag BMNPs infiltrating the bacterial cell membrane and

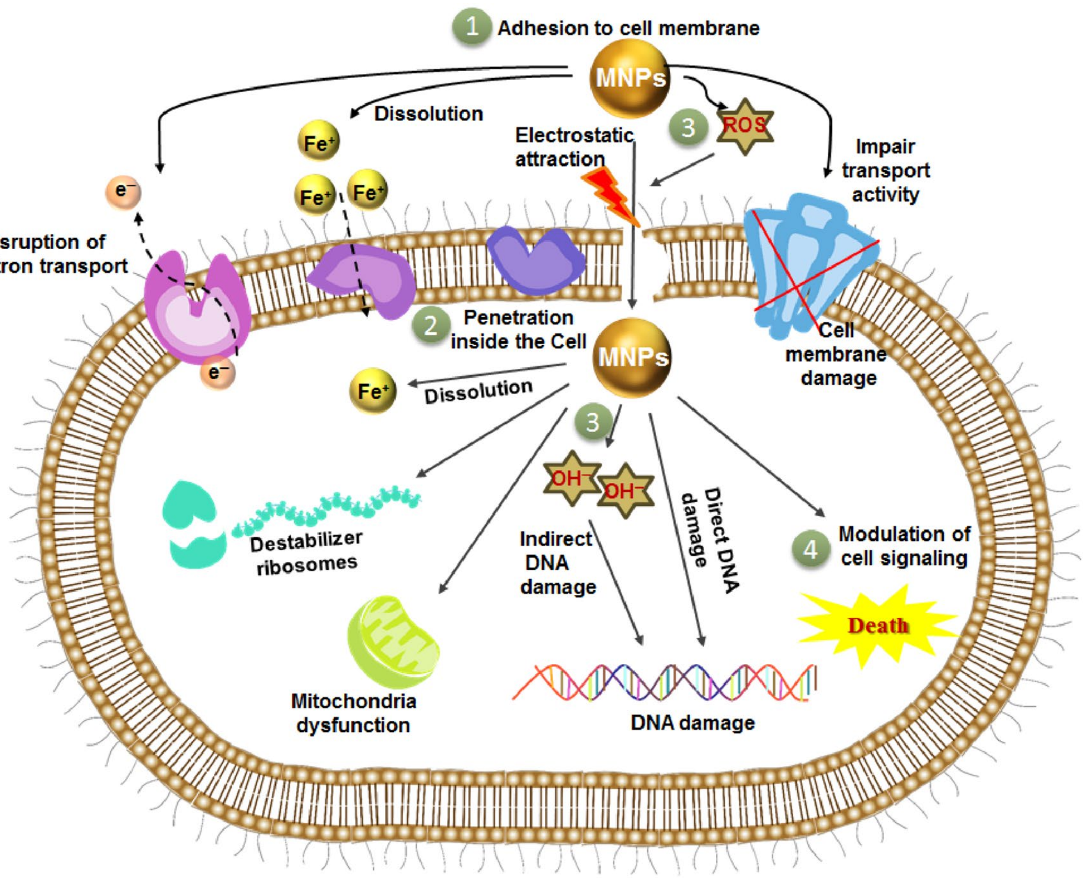


Fig. 11 The possible mechanism routes of the antibacterial action of Cu–Ag BMNPs nanoparticles

interacting with cellular organelles; 3. Cu–Ag BMNPs triggering an increase in reactive oxygen species (ROS) within the cell membrane, leading to cell damage; and 4. Cu–Ag BMNPs modulating the cellular signaling system, ultimately deactivating the cells and resulting in cell death. Further elucidation of the molecular interactions and pathways involved could provide valuable insights into their mode of action and enhance their therapeutic potential with bacteria cell. The Cu–Ag bimetallic nanoparticles showed potentialantibacterial effect which could be helpful for understanding their long-term effectiveness and safety, warranting further research into the mechanisms underlying bacterial adaptation and resistance aquasition.

Within the realm of green synthesis methods, our study brings to the forefront the utilization of AN plant extracts as a robust resource for the eco-friendly production of Cu–Ag BMNPs. AN is a medicinal plant renowned for its rich array of minerals and vitamins [36–39, 52, 57]. In our research, the plant's leaf extract proved to be a highly efficient capping, reducing, and stabilizing agent, facilitating the successful production of high-quality Cu–Ag BMNPs. These results align with recent studies underscoring the potential of biogenic BMNPs when combined with phytochemicals or phenolic compounds derived from medicinal plants as promising alternatives for the treatment of chronic infections.

The escalating challenge of multi-drug-resistant microbes and superbugs necessitates a relentless quest for safer alternatives to mitigate the deadly impact of microbial infections compared other mono metal nanoparticles [52–54]. Specifically, the need for antibacterial agents that are non-toxic to patients and do not encourage microbial resistance is pressing. In response, mono/bi/tri/multi-metallic nanoparticles (MNPs) are under investigation for their diverse array of bioactive compounds with potent antibacterial properties. Recent advances in the biogenic synthesis of both monometallic nanoparticles (MNPs) and bimetallic nanoparticles (BMNPs) have gained recognition as sustainable pathways [58–61]. This approach is characterized by its environmentally friendly, non-toxic processes, and cost-effectiveness, providing a sustainable alternative to traditional synthesis methods that often entail the use of hazardous chemicals and energy-intensive procedures [62–64]. Furthermore, we underscore the potential therapeutic applications of the synthesized Cu–Ag BMNPs in addressing chronic infections and microbial diseases, highlighting the need for further in vitro and in vivo cell-based and animal model studies.

The utilization of plant leaf extracts has emerged as a specialized synthesis technique for generating MNPs. These plant leaf extracts serve the triple purpose of reducing, capping, and stabilizing the metal nanoparticles during the synthesis process. Collectively, the results of our current study enrich the expanding pool of insights into the utilization of bimetallic nanoparticles for combatting chronic infections. This research sets the stage for future exploration and advancement in the field of nano-biotechnology. The study should help and incorporate a comprehensive life cycle assessment to evaluate the environmental impact (water purification through catalysis) of Cu–Ag bimetallic nanoparticles across their production, use, and disposal stages [46–48, 57]. The study would also benefit from comparative analysis with other types of nanoparticles to assess the performance of Cu–Ag bimetallic nanoparticles in terms of their physicochemical properties, stability, and efficacy in relevant applications [52–54]. The study acknowledges limitations to scaling up large-scale production of these nanoparticles, but suggests that overcoming these hurdles may be feasible with further investigation, particularly focusing on optimizing extract consumption, both salts concentration, and synthesis time.

Conclusion

In summary, this study presents a pioneering single-step biosynthesis method for the production of Cu–Ag BMNPs using the medicinal rich plant AN leaf extract. The study employed XRD and SEM techniques to examine the morphological and structural characteristics of the bimetallic nanoparticles, while FTIR and UV spectroscopy were utilized to identify functional groups and confirm plasmonic absorption, validating their bimetallic nature. Furthermore, the optimization of copper concentration within the Cu–Ag BMNPs hybrid composite demonstrated potential antibacterial activity, highlighting the multifaceted approach taken to characterize and assess the bimetallic nanoparticles' properties and their potential applications. The Cu–Ag BMNPs displayed substantial antibacterial activity against Enteropathogenic *E. coli*, as validated through disc diffusion and MIC assays. The importance of this research is underscored by the substantial promise of bimetallic nanoparticles with a synergistic effect and their environmentally friendly synthesis methods, contributing to the advancement of nano-biotechnology. Our findings pave the way for further exploration into the potential anticancer and antitoxic effects of the Cu–Ag BMNPs, along with their efficacy against both gram-positive and gram-negative pathogens. By elucidating the optical characteristics and potential mechanisms of action of these nanoparticles, our study sets the stage for broader investigations aimed at unlocking their therapeutic potential across various biomedical applications.

Author contributions PK: supervision, writing—original draft and editing, visualization; RK, NKD, NKM, and SLM: designing experiment and data collection; MSGL and VM: designing experiment procedure and investigation antibacterial activity; RNS, MB, NSE, BSP, SJK, and PM: review of articles and data analysis. Note: all authors read and approved the final submission.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interest.

Ethical approval The internal review board committee of our laboratory states that we do not have any consent to publish this antimicrobial and other data.

References

1. Ahmad J et al (2016) Differential cytotoxicity of copper ferrite nanoparticles in different human cells. J Appl Toxicol 36(10):1284–1293
2. He HM et al (2022) Metal-organic framework supported Au nanoparticles with organosilicone coating for high-efficiency electrocatalytic N_2 reduction to NH_3. Appl Catal B-Environ 302
3. Sahooli M, Sabbaghi S, Saboori R (2012) Synthesis and characterization of mono sized CuO nanoparticles. Mater Lett 81:169–172
4. Das SS et al (2020) Stimuli-responsive polymeric nanocarriers for drug delivery, imaging, and theragnosis. Polymers 12(6)
5. Chu YM et al (2021) Enhancement in thermal energy and solute particles using hybrid nanoparticles by engaging activation energy and chemical reaction over a parabolic surface via finite element approach. Fract Fraction 5(3)
6. Chu YM et al (2022) Combined impact of Cattaneo-Christov double diffusion and radiative heat flux on bio-convective flow of Maxwell liquid configured by a stretched nano-material surface (Retracted article. See vol. 450, 2023). Appl Math Comput 419
7. Wang YG et al (2016) Experimental evaluation of the lubrication properties of the wheel/workpiece interface in minimum quantity lubrication (MQL) grinding using different types of vegetable oils. J Clean Prod 127:487–499
8. Guo SM et al (2017) Experimental evaluation of the lubrication performance of mixtures of castor oil with other vegetable oils in MQL grinding of nickel-based alloy. J Clean Prod 140:1060–1076
9. Wang YG et al (2016) Experimental evaluation of the lubrication properties of the wheel/workpiece interface in MQL grinding with different nanofluids. Tribol Int 99:198–210
10. Jia DZ et al (2014) Experimental verification of nanoparticle jet minimum quantity lubrication effectiveness in grinding. J Nanoparticle Res 16(12)

11. Sharma VK, Yngard RA, Lin Y (2009) Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 145(1–2):83–96
12. Arkaban H et al (2022) Polyacrylic acid nanoplatforms: antimicrobial, tissue engineering, and cancer theranostic applications. Polymers 14(6)
13. Salarpour S et al (2022) The application of exosomes and exosome-nanoparticle in treating brain disorders. J Mol Liquids 350
14. Wang FZ et al (2022) Numerical solution of traveling waves in chemical kinetics: time-fractional fishers equations. Fractals 30(2):2240051
15. Ghazal SEA (2021) Sol-gel Synthesis of selenium-doped nickel oxide nanoparticles and evaluation of their cytotoxic and photocatalytic properties. Inorg Chem Res 5:37–49
16. Sharma R, Gyergyek S, Andersen SM (2022) Microwave-assisted scalable synthesis of Pt/C: impact of the microwave irradiation and carrier solution polarity on nanoparticle formation and aging of the support carbon. ACS Applied Energy Materials 5(1):705–716
17. Haghighat M et al (2022) Cytotoxicity properties of plant-mediated synthesized K-doped ZnO nanostructures. Bioprocess Biosyst Eng 45(1):97–105
18. Cao Y et al (2022) Ceramic magnetic ferrite nanoribbons: eco-friendly synthesis and their antifungal and parasiticidal activity. Ceram Int 48(3):3448–3454
19. Hamidian K et al (2022) Cytotoxic performance of green synthesized Ag and Mg dual doped ZnO NPs using *Salvadora persica* extract against MDA-MB-231 and MCF-10 cells. Arab J Chem 15(5)
20. Hashemi N et al (2021) Leishmanicidal activities of biosynthesized $BaCO_3$ (witherite) nanoparticles and their biocompatibility with macrophages. Bioprocess Biosyst Eng 44(9):1957–1964
21. Ren SC et al (2022) Well-defined coordination environment breaks the bottleneck of organic synthesis: single-atom palladium catalyzed hydrosilylation of internal alkynes. Nano Res 15(2):1500–1508
22. Gao T et al (2020) Surface morphology assessment of CFRP transverse grinding using CNT nanofluid minimum quantity lubrication. J Cleaner Prod 277
23. Yang M et al (2019) Predictive model for minimum chip thickness and size effect in single diamond grain grinding of zirconia ceramics under different lubricating conditions. Ceram Int 45(12):14908–14920
24. Song JY, Kim BS (2009) Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 32(1):79–84
25. Vigneshwaran N et al (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett 61(6):1413–1418
26. Kalishwaralal K et al (2010) Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. Colloids Surfaces B-Biointerfaces 77(2):257–262
27. Kharissova OV et al (2013) The greener synthesis of nanoparticles. Trends Biotechnol 31(4):240–248
28. Bosetti M et al (2002) Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials 23(3):887–892
29. Sharma G et al (2019) Novel development of nanoparticles to bimetallic nanoparticles and their composites: a review. J King Saud Univ Science 31(2):257–269
30. Kulkarni N, Muddapur U (2014) Biosynthesis of metal nanoparticles: a review. J Nanotechnol 2014:1–8
31. Pandey P et al (2014) Antimicrobial properties of CuO nanorods and multi-armed nanoparticles against *B. anthracis* vegetative cells and endospores. Beilstein J Nanotechnol 5:789–800
32. Kalakonda P (2016) Synthesis of silver nanowires for highly conductive and transparent films. Nanomater Nanotechnol 6
33. Kalakonda P, Sreenivas B (2017) Synthesis and optical properties of highly stabilized peptide-coated gold nanoparticles. Plasmonics 12(4):1221–1225
34. Kalakonda P, Banne S (2018) Synthesis and optical properties of highly stabilized peptide-coated silver nanoparticles. Plasmonics 13(4):1265–1269
35. Kalakonda P et al (2017) Microfibrous silver-coated polymeric scaffolds with tunable mechanical properties. RSC Adv 7(55):34331–34338
36. Parvathalu K et al (2023) Facile synthesis of silver nanoparticles using green tea leaf extract and evolution of antibacterial activity. Plasmonics
37. Asamoah RB et al (2020) Synthesis and characterization of zinc and copper oxide nanoparticles and their antibacteria activity. Results Mater 7
38. Cao Y et al (2021) Green synthesis of bimetallic ZnO-CuO nanoparticles and their cytotoxicity properties. Scientific Rep 11(1)
39. Darvish M et al (2022) Biosynthesis of Zn-doped $CuFe_2O_4$ nanoparticles and their cytotoxic activity. Scientific Rep 12(1)
40. Mohamed EA (2020) Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. Heliyon 6(1):e03123
41. Jyoti K, Baunthiyal M, Singh A (2016) Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. J Radiat Res Appl Sci 9(3):217–227
42. Dubey SP, Lahtinen M, Sillanpaa M (2010) Tansy fruit mediated greener synthesis of silver and gold nanoparticles. Process Biochem 45(7):1065–1071
43. Kasthuri J, Kathiravan K, Rajendiran N (2009) Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. J Nanoparticle Res 11(5):1075–1085
44. Boddu SR et al (2011) Gold, silver, and palladium nanoparticle/nano-agglomerate generation, collection, and characterization. J Nanoparticle Res 13(12):6591–6601
45. Sheny DS, Philip D, Mathew J (2012) Rapid green synthesis of palladium nanoparticles using the dried leaf of *Anacardium occidentale*. Spectrochim Acta Part A Mol Biomol Spectrosc 91:35–38
46. Torres SK et al (2012) Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity. J Nanoparticle Res 14(11)
47. Subramanyam GK et al (2021) *Argyreia nervosa* (Samudra pala) leaf extract mediated silver nanoparticles and evaluation of their antioxidant, antibacterial activity, in vitro anticancer and apoptotic studies in KB oral cancer cell lines. Artif Cells Nanomed Biotechnol 49(1):635–650
48. Parvathalu K et al (2023) Green synthesis of silver nanoparticles using argyreia nervosa leaf extract and their antimicrobial activity. Plasmonics 18(3):1075–1081
49. Faisal S, Al-Radadi NS, Jan H, Abdullah Shah SA, Shah S, Rizwan M, Afsheen Z, Hussain Z, Uddin MN et al. (2021) *Curcuma longa* mediated synthesis of copper oxide, nickel oxide and Cu-Ni bimetallic hybrid nanoparticles: characterization and evaluation for antimicrobial, anti-parasitic and cytotoxic potentials. Coatings 11:849
50. Kumar A, Shah SR, Jayeoye TJ, Kumar A, Parihar A, Prajapati B, Singh S, Kapoor DU (2023) Biogenic metallic nanoparticles: biomedical, analytical, food preservation, and applications in other consumable products. Front Nanotechnol 5:1175149
51. Ismail M, Khan MI, Khan SB, Khan MA, Akhtar K, Asiri AM (2018) Green synthesis of plant supported CuAg and CuNi

bimetallic nanoparticles in the reduction of nitrophenols and organic dyes for water treatment. J Mol Liquids 260:78–91
52. Idris DS, Roy A (2023) Synthesis of bimetallic nanoparticles and applications—an updated review. Crystals 13(4)
53. Kalakonda P, Mandal P, Laxmi Mynepally S et al. (2024) Comparison of multi-metallic nanoparticles-alternative antibacterial agent: understanding the role of their antibacterial properties. J Inorg Organomet Polym
54. Kalakonda P, Bashitangu A, Mandal P, Khanam SJ, Banovath M, Hasan I, Podila BB (2024) Sustainable solutions for clean water: Green synthesized Cu–Ag-bimetallic nanoparticles based nano catalys. Mater Sci Eng: B 301:117147
55. Saurabh S, Khan R, Daverey A (2021) Synthesis and characterization of magnetic nanoparticles, and their applications in wastewater treatment: a review. Environ Technol Innov 24:101924
56. Hussain M, Ansari MA, Mir FA (2024) Preparation, characterization and cooling performance of ZnO based nanofluids. Discov Appl Sci 6:92
57. Smith J, Johnson A (2023) Enhanced absorption of Cu–Ag bimetallic nanoparticles at 450 nm due to copper complexation. J Nanoparticle Res 15(3):210–225
58. Parvathalu K, Rajitha K, Chandrashekar B et al (2023) Biomimetic synthesis of copper nanoparticles using tinospora cordifolia plant leaf extract for photocatalytic activity applications. Plasmonics
59. Khan MI, Shah S, Faisal S, Gul S, Khan S, Abdullah Shah SA, Shah WA (2022) Monotheca buxifolia driven synthesis of zinc oxide nano material its characterization and biomedical applications. Micromachines 13:668
60. Zafar S, Faisal S, Jan H, Ullah R, Rizwan M, Abdullah Alotaibi A, Bibi N, Rashid AU, Khattak A (2022) Development of iron nanoparticles (FeNPs) using biomass of enterobacter: its characterization, antimicrobial, anti-Alzheimer's, and enzyme inhibition potential. Micromachines 13:1259
61. Hussain T, Faisal S, Rizwan M, Zaman N, Iqbal M, Iqbal A, Ali Z (2022) Green synthesis and characterization of copper and nickel hybrid nanomaterials: investigation of their biological and photocatalytic potential for the removal of organic crystal violet dye. J Saudi Chem Soc 26(4):101486
62. Ullah R, Shah S, Muhammad Z, Shah S, Faisal S, Khattak U, Haq T, Taj Akbar M (2021) In vitro and in vivo applications of *Euphorbia wallichii* shoot extract-mediated gold nanospheres. Green Proces Synth 10(1):101–111
63. Al-Radadi NS, Faisal S, Alotaibi A, Ullah R, Hussain T, Rizwan M, Zaman N, Iqbal M, Iqbal A, Ali Z (2022) Zingiber officinale driven bioproduction of ZnO nanoparticles and their anti-inflammatory, anti-diabetic, anti-Alzheimer, anti-oxidant, and anti-microbial applications. Inorg Chem Commun 140:109274
64. Shah S, Shah SA, Faisal S et al (2022) Engineering novel gold nanoparticles using *Sageretia thea* leaf extract and evaluation of their biological activities. J Nanostruct Chem 12:129–140