Bio-Synthesis and Synergistic Effects of Cu-Ag Bimetallic Nanoparticles Using Argyreia Nervosa Leaf Extract for Antibacterial Applications

Parvathalu Kalakonda (parvathalu.k@gmail.com)
Government City College (A), Osmania University
Rajitha Kathi
Government City College (A), Osmania University
Merlinsheeba GL
University of Hyderabad
Naveen Kumar Dabbeta
Government City College (A), Osmania University
Naveen Kumar Madipoju
Government City College (A), Osmania University
Soujanya M L
Government City College (A), Osmania University
Vijay Morampudi
University of Hyderabad
Sreenivas Banne
Rice University-BRC
Pritam Mandal
Michigan Technological University
Ramu Naidu
University of Hyderabad
Sarvani Jowhar Khanam
University of Hyderabad
Murali Banavoth
University of Hyderabad
Nainavinodini Sudarsanam Eve
Government City College (A), Osmania University
Bala Bhaskar Podila
Government City College (A), Osmania University

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Abstract

A novel biosynthesis of metal nanoparticles and their potential applications have attracted many researchers, as metal nanoparticles are effectively used in biomedical applications. We study copper-silver bimetallic nanoparticles (Cu-Ag BMNPs) through this biosynthesis, which utilizes Argyreia Nervosa (AN) plant leaf green extract as a capping, reducing and stabilizing agents. The biosynthesis methodology, which is operationally simple and cost-effective, and silver nitrate ($AgNO_3$) and cupric oxide (CuO) are used as precursor materials. Comprehensive characterization of the Cu-Ag BMNPs was conducted using X-Ray Diffraction (XRD), UV-Vis Spectrometer, Scanning Electron Microscopy (SEM), Zeta Seizer, and Fourier Transformed Infrared spectrometer (FTIR). SEM analysis revealed the formation of Cu-Ag BMNP aggregates with sizes ranging from 25 to 100 nm. The FTIR results indicate successful removal of organic and inorganic impurities, which is corroborated by the XRD data. To assess antimicrobial properties of the Cu-Ag BMNPs, disk diffusion and Minimum Inhibition Concentration (MIC) assays are performed against Escherichia coli (E. coli) as the target pathogen, and results are compared to a standard reference of Gentamicin antibiotic. The antimicrobial tests demonstrate that the Cu-Ag BMNPs exhibit remarkable antimicrobial activity with high minimum inhibition concentration (MIC) values and a broad zone of inhibition in the disc diffusion assays against E. coli. The results showed the potential antimicrobial activity of the synthesized BMNPs against the tested bacteria. The green synthesis approach employed in this study provides a sustainable and eco-friendly method for synthesizing Cu-Ag-BMNPs with potential antimicrobial properties.

1. INTRODUCTION

Nanobiotechnology is a rapidly growing field that harnesses the unique properties of nanoparticles for various biomedical applications, including antimicrobial agents and drug delivery vehicles [1, 2]. Metal nanoparticles, particularly those synthesized through nanotechnology, have applications in biomedicine, drug delivery systems, energy, agriculture, and antimicrobial/anticancer activities [3–10]. With the rise of bacterial resistance to conventional antibiotics, there is an urgent need to explore alternative antimicrobial agents to combat pathogenic infections, which pose significant global health risks. In this context, mono/bi/tri/multi-metal nanoparticles (MNPs) have been identified to act as promising candidates for antimicrobial applications.

Efforts have been directed towards developing eco-friendly and cost-effective synthesis methods for MNPs. Current synthesis approaches involve chemical reduction processes, microwave-assisted methods, electron irradiation techniques, and plant-mediated biogenic methods [11–20]. However, challenges remain in controlling the stability, yield, and morphology of the as produced MNPs [21–23]. Chemical synthesis methods require substantial energy and cost investments, and they have negative environmental impacts, including pollution, eco-toxicity, and public health hazards.

Among the various synthesis methods, green synthesis/biogenic methods have gained popularity due to their environment friendly nature, low toxicity, cost-effectiveness, and simplicity of synthesis process.

These methods utilize plant extracts, such as leaves, fruits, or peels, as well as microorganisms, to reduce and stabilize MNPs [24–27]. While microorganism-based synthesis processes are time-consuming and require complex aseptic conditions, using plant extracts offers a more energy-efficient and may cost-effective practical approach. Plant extracts contain abundant natural biomolecules and complex molecular structures with rich medicinal values that can effectively cap MNPs and enhance their antibacterial properties [28–31].

MNPs are highly sought-after in nanotechnology due to their multifunctional properties, including optical, electrical, catalytic, magnetic, and antimicrobial characteristics, arising from their nano dimensions with large surface area to volume ratio [32–36]. Bimetallic nanoparticles, such as copper-silver (Cu-Ag), form core-shell nanostructures and exhibit multifunctional properties due to the synergistic effects of the constituent metal ions [37–41]. While the synthesis of mono-metallic nanoparticles using the extract of Argyreia Nervosa (AN) plant leaf has been reported, this plant has not yet been utilized for the synthesis of copper-silver bimetallic nanoparticles (Cu-Ag BMNPs)[42–46]. The AN plant is known for its medicinal properties, including anti-inflammatory and stress-reducing effects[47].

This study presents the first-ever utilization of the AN plant leaf green extract as a reducing, capping, and stabilizing agents to synthesize stable Cu-Ag BMNPs. Previous research has demonstrated the efficient antimicrobial activity of AgNPs against E. coli bacteria, and we expect the Cu-Ag BMNPs to exhibit enhanced antibacterial properties[47]. AgNPs have been extensively explored for various technological applications, including antimicrobial agents, drug delivery vehicles, water purification, and the textile industry. They are known for their high stability and low toxicity towards human cells. Despite numerous studies reporting the synthesis and antimicrobial activities of various MNPs, the green synthesis of Cu-Ag BMNPs using the AN plant and their antibacterial properties, as far as knowledge is concerned, have not been investigated. In this work, we synthesized Cu-Ag BMNPs using the AN plant leaf extract and evaluated their antibacterial activities against one of the *Escherichia coli* serotype known as Enteropathogenic E.coli (EPEC) via disc diffusion and minimum inhibitory concentration (MIC) assays. Our findings provide valuable insights into the potential of these green-synthesized Cu-Ag BMNPs as antibacterial agents. Moreover, we assert that the green synthesis method employed in this study, utilizing the AN plant leaf extract, offers a promising, energy-efficient, and cost-effective approach for large-scale production of Cu-Ag BMNPs for commercial applications.

2. MATERIALS AND METHODS

2.1 MATERIALS

Copper (II) Oxide, silver nitrate, solvents were purchsed from Sigma-Aldrich, Bangalore, India. Argyreia Nervosa (AN) plant leafs were collected from our institute green garden Hyderabad, India.

2.2 SYNTHESIS OF SILVER-COPPER BIMETALLIC NANOPARTICLES (Cu-Ag BMNPs)

Argyreia Nervosa (AN) leaves were cleaned in a vial repeatedly with water to remove all dust particles. Then, leaves were dried at ambient conditions, and they were ground thoroughly into fine powders. Subsequently, 1.5g of AN leaf powder was added with 75ml of DI water and stirred with 500 rpm at a temperature of 75°C for 20 minutes. As a result, AN extract was filtered using a filter paper and then centrifuged for 20 minutes at 3000 rpm speed.

To fabricate copper-silver bimetallic nanoparticles (Cu-Ag BMNPs), 75ml of the AN leaf extract was transferred to burette and added drop-wise (one drop per second) to various mixtures consisting of 200 ml of $AgNO_3$ and 50ml of cupric oxide (with CuO concentrations of 2%, 5%, 10%, and 20% in $AgNO_3$) in a 200ml beaker. Both solutions were added at a 80°C temperature for 15 minutes at a stirring rate of 500 rpm. The addition of leaf extract from the burette was controlled as drop-wise, while the resulting solution was stirred at 500 rpm at temperature 80^0C. The mixed solution was heated on a hot plate at 80°C temperature until a observable change in the color. Then, the final solution was centrifuged at 3000 rpm for 20 min and dried at 60^0 C in a oven to collect the powder nanoparticles. The schematic synthesis procedure ois f Cu-Ag BMNPs is shown in Fig. 1.

2.3 CHARACTERIZATION OF SYNTHESIZED Cu-Ag BMNPs

The confirmation of formation of Cu-Ag bimetallic nanoparticles (Cu-Ag BMNPs) was detected using UV-visible spectroscopy over a range of 200-800nm wavelength. This analysis notify the characteristic absorption peaks of Cu-Ag BMNPs. FTIR was used to identify the specific functional phytochemical groups responsible for forming these Cu-Ag BMNPs. The FTIR analysis provide valuable insights into the functional groups and vibrational chemical bonds involved in stabilization, reducing, and capping Cu-Ag BMNPs.The surface morphology details and grain sizes of the Cu-Ag BMNPs were examined using Scanning Electron Microscopy (SEM). The SEM analysis help to reveal visualization of the Cu-AgBMNPs and the determination of their surface size distribution.. The details of crystal structure of the Cu-Ag BMNPs was examined using X-Ray Diffraction (XRD). This technique provide information about the Cu-Ag BMNPs crystalline structure.

Furthermore, the zeta potential of the Cu-Ag BMNPs was determined using a Zeta-Seizer Nano-ZS instrument. The zeta potential values were calculated based on electrophoretic mobility using the Smoluchowski equation. This analysis provided insights into the surface charge and stability of the nanoparticles. Overall, the combination of UV-visible spectroscopy, FTIR, SEM, XRD, and zeta potential measurements facilitated a comprehensive characterization of the synthesized Cu-Ag BMNPs, including their optical properties, chemical composition, morphology, crystal structure, and surface charge.

2.4 ANTIBACTERIAL ACTIVITY

Antimicrobial activity of the Cu-Ag bimetallic nanoparticles (Cu-Ag BMNPs) was examined antibacterial activity of the Cu-Ag BMNPs under sterile lab conditions with a biosafety cabinet. All culture media equipemnet, glassware used for monitoring microbial activity were sterilized via autoclaving unit. In order to determine the antibacterial activity of the synthesized Cu-Ag BMNPs, we studied their effects on

Eneropathogenic Escherichia (EPEC) along with a reference antibiotic, Gentamicin. In the following sections, we briefly describe the two methods; we employed to assess the antibacterial activity of the as-synthesized Cu-Ag BMNPs.

2.4.1 BACTERIAL CULTURE

Enteropathogenic *Escherichia coli* (EPEC), a rod-shaped Gram-negative pathogen was cultured in Luria Bertani (LB) broth revived from glycerol stocks in -80°C storage. The bacteria were grown at 37°C for 12 to 14 hours in the shaking incubator at 180rpm. LB agar plates were streaked with EPEC bacterial culture for growth and maintenance to perform assays. Later, overnight culture of EPEC at OD_{600nm} = 1 was used in performing disc diffusion and minimum inhibitory concentration

2.4.2 DISC DIFFUSION ASSAY PROCEDURE

Antibacterial bio activity of as-produced Cu-Ag BMNPs was evaluated against Gram-negative Enteropathogenic *E. coli* by disc diffusion method. 100µl of EPEC at OD_{600} = 1 was plated on LB agar media plates. Whatman filter paper designed to cut sterile 6mm discs impregnated with 50µl of AN extract and AN Cu-Ag BMNPs of 2%, 5%, 10% and 20% concentration were placed on the EPEC coated LB agar media plates and incubated entire night at 37°C temperature post drying. LB broth media was added as negative control and Gentamicin (30ug), an effective antibiotic against Gram-negative bacteria including E. *coli* served as a positive control. The inhibition zones due to bacterial clearance by the Cu-Ag BMNPs were recorded by measuring the diameter in millimeter.

2.4.3 MINIMUM INHIBITION CONCENTRATION (MIC) ASSAY PROCEDURE

To determine this assay of the synthesized Cu-Ag BMNPs, 50µl of EPEC (~ 1.22×10^8 CFU/ml) at OD_{600} = 1 was incubated with 50µl of AN leaf extract, 2%, 5%, 10% and 20% of AN Cu-Ag BMNPs in a 96-well plate. LB broth and Gentamicin (30µg) were used as negative and positive control respectively. The plate was incubated for 6 hours at 37°C and samples were diluted UP TO 10-fold up to 10^6, from which 10µl was taken from each dilution and added on LB agar media plates. Then, plated are incubated overnight at 37°C, MIC was calculated by comparing bacterial CFU/ml given by the 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 results obtained from the disc diffusion and MIC assays provide valuable insights into the antimicrobial potential of the synthesized Cu-Ag BMNPs. The disc diffusion assay allows for the qualitative evaluation of the Cu-Ag-BMNPs' efficacy in inhibiting bacterial growth, as indicated by the formation of inhibition zones. On the other hand, the MIC assay provides quantitative data regarding the lowest concentration of Cu-Ag BMNPs.

3. RESULTS

3.1 UV-VIS SPECTROSCOPY

The optical properties of metal nanoparticles are influenced by surface morphology, size, and shape. Our study observed the absorption peak intensity resulting from the Surface Plasmon Resonance (SPR) in the UV-Vis spectroscopy analysis of the Cu-Ag bimetallic nanoparticles (Cu-Ag BMNPs). Broad absorption peaks were observed in the wavelength range of 350 to 550 nm, and the broadness of the peak increased with higher copper concentrations in the Cu-Ag BMNPs. The maximum absorption was recorded at a wavelength of 450 nm shown in Fig. 2. This may be due to increased binding of the nanoparticles with the BMNPs caused copper complexation.

3.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR analysis provides insights into the functional groups and chemical bands present in the 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 spectrum confirmed the absence of surfactants on the nanoparticle surface.

3.3 MORPHOLOGY -SCANNING ELECTRON MICROSCOPY (SEM)

Figure 4 shows the grain size and surface morphology details of the Cu-Ag BMNPs. The micrograph images of the Cu-Ag-BMNPs reveal different irregular shapes with sizes ranging from 100 to 500 nm, while the AgNPs exhibited clear spherical shapes with an average size of approximately 20–50 nm[36]. The SEM images of Cu-Ag BMNPs observed irregular clusters with rough surfaces and different sizes. The varying crystallization growth of CuNPs and AgNPs might attribute the observed agglomerated cluster formation.

3.4 X-RAY DIFFRACTION (XRD)

XRD measurements and analysis were employed to determine the details of the crystalline structure of the Cu-Ag BMNPs. The XRD diffraction patterns disclose that the Cu-Ag Cu-Ag BMNPs are of composite crystalline structures and free of impurities. The XRD diffraction pattern of individual CuNPs and AgNPs reveal that are crystalline structure (Fig. 5a) and hybrid composite XRD spectrum observed polycrystalline structure (Fig. 5b). The diffraction pattern of Cu-Ag BMNPs show broader peaks, which confirms that the produced synthesized Cu-Ag BMNPs possess polycrystalline nanomaterial. Scherer's formula was used to estimate the crystalline size details of Cu-Ag BMNPs. The strong peak intensities were observed at 37.5^0, 45.6^0, 64^0, 77^0, which suggest the crystalline phases of CuO and Cu-Ag BMNPs. The reflection planes 111 (37.5^0), 111(43.6^0), 200 (45.6^0), 200 (53^0), 220 (64^0), 220 (74^0), 311 (77^0) verified the crystalline phase of Cu-Ag BMNPs. The high intense peak is noticed at (111) and the lattice details were calculated using Bragg's equation.

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

The Cu-Ag BMNPs composite crystalline 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), θ is the angle of diffraction. The composite polycrystalline size of nanoparticles was determined about 25 to100 nm.

3.5 ZETA POTENTIAL AND ZETA SEIZER

The zeta potential analysis of the dispersed Cu-Ag BMNPs provides insights into the nanoparticles dispersion's surface charge interactions and long-term stability. The variation of zeta potential as a function of copper doping concentration in the Cu-Ag BMNPs is shown in Fig. 6. The recorded zeta potential values ranged from – 20 to -10 mV in deionized water. Zeta potential values from + 25 mV to -25 mV indicate a high degree of dispersion stability, while ultra-lower zeta potential values suggest the formation of aggregates due to inter-particle attractions.

3.6 ANTIBACTERIAL ACTIVITY OF Cu-Ag BMNPs

3.6.1 DISC DIFFUSION ASSAY

EPEC pathogen bacteria have been known to cause persistent diarrhea in human beings and it remains challenging infection to tackle all over the globe. Negative impacts such as bacteria gaining antibiotic resistance, antibiotic-associated diarrhea and the level of toxicity call for a new active antimicrobials with low toxicity. The antibacterial effect of *Argyreia nervosa* mediated bimetallic Cu-Ag BMNPs on EPEC was investigated by the disc diffusion assay with Gentamicin is being used as a reference antibiotic. The antibacterial activity of AN Cu-Ag BMNPs was high at 12 hours of incubation compared to 16 hours. AN extract did not show any clear zone of inhibition after 16 hours unlike at 12 hours, stating that bimetallic nanoparticles offer an additive advantage in bacterial inhibition and killing up to 16 hours when compared with only AN extract. Figure 7 shows the variation of antibacterial activity of Cu-Ag BMNPs as a function of concentration of Cu doping in the Cu-Ag BMNPs. Synthesized Cu-Ag BMNPs showed significant activity on EPEC and its activity slightly increased as the concentration of Cu doping in the Cu-Ag BMNPs increased. Figure 8 shows a histogram comparing the antibacterial activity of Cu-Ag BMNPs on EPEC pathogen as a function of the concentration of Cu doping in the composite Cu-Ag BMNPs.

3.6.2 MINIMUM INHIBITION CONCENTRATION (MIC) ASSAY

Figure 9 shows antimicrobial activity of Cu-Ag BMNPs against EPEC pathogen and it was validated using minimum inhibitory concentration (MIC) assay procedure. Figure 10 shows a histogram comparing the antibacterial activity of Cu-Ag BMNPs on EPEC bacteria as a function of Cu doping concentrations. It is observed that 2% and 5% CuO concentrations of AN Cu-Ag BMNPs display significant antibacterial activity when compared with AN extract on EPEC. Cu-Ag BMNPs at 2% concentration exhibit higher antibacterial activity, possibly due to higher degree of dispersion of BMNPs at lower CuO doping. Hence, the Minimum inhibitory concentration at which EPEC growth is inhibited at 2% AN Cu-Ag BMNPs. The high efficiency of Cu-Ag BMNPs can act as a better antibacterial agent. This ability might be due to the synergistic effect of both metal ions (Cu and Ag) present in the hybrid metal nanocomposites.

4. DISCUSSION

Metallic nanoparticles (MNPs), including mono-metallic, bimetallic, tri-metallic, and multi-metallic nanoparticles, have emerged as promising candidates for diagnosing and treating of chronic infections. Incorporating multiple metal ions in the hybrid nanostructure of MNPs has led to the exploration of their synergistic effects, resulting in the generation of multifunctional physiochemical properties and enhanced antimicrobial activity. In this study, we focused synthesizing copper-silver bimetallic nanoparticles (Cu-Ag BMNPs) using a biogenic synthesis method, in which Argyreia Nervosa (elephant creeper) medicinal plant leaves extract was used as a reducing, capping, and stabilizing agents.

The physiochemical properties of Cu-Ag BMNPs were extensively characterized using various analytical techniques. UV-Vis spectroscopy confirmed the successful formation of Cu-Ag BMNPs, as evidenced by the characteristic absorption peak. XRD) and FTIR analyses further elucidated the crystalline nature of the Cu-Ag BMNPs and provided insights into their structural details. The presence of two distinct crystalline phases in the synthesized Cu-Ag BMNPs was confirmed by XRD, signifying the successful incorporation of copper and silver elements in the composite nanoparticle structure.

Antibacterial properties of the Cu-Ag BMNPs were examined against Enteropathogenic *E. coli*, against a standard reference antibiotic, Gentamicin to which Enteropathogenic E.*coli* are susceptible. The results demonstrated that the as-synthesized Cu-Ag BMNPs exhibited higher antimicrobial activity against Enteropathogenic E. coli, suggesting their potential as effective antimicrobial agent. These findings align with previous studies highlighting the benefits of bimetallic nanoparticles in terms of improved physiochemical properties and increased antimicrobial activity. The superior antibacterial activity of Cu-

Ag BMNPs can be assigned to the synergistic effects between the incorporated metal ions, which enhance the overall antimicrobial efficacy. The possible mechanism and interactions of Cu-Ag-BMNPs with bacteria cell is shown in Fig. 11. There are four possible mechanism of the antibacterial agent Cu-Ag-BMNPs with the E. coli bacteria pathogen cell. The probable mechanisms are: 1.Cu-Ag-BMNPs adhere to bacteria cell surface wall and damage cell membrane; 2. Cu-Ag-BMNPs diffuse into the bacteria cell membrane and interact with cellular organelles; 3. Cu-Ag-BMNPs cause an enhance reactive oxygen species (ROS) inside the membrane cells, which causes cell damage, and 4. Cu-Ag-BMNPs modulate the cellular signal system which results deactivation of cells, eventually killing the cells.

In the context of green synthesis methods, Moreover, our study highlights the utilization of Argyreia Nervosa plant extracts as a viable plant extract source for the green synthesis of Cu-Ag BMNPs. Argyreia Nervosa is a rich medicinal plant known for its diverse array of minerals and vitamins [36–39, 48, 49]. The plant leaf green extract served as an effective capping, reducing, and stabilizing agents during the sample fabrication process, producing high quality Cu-Ag BMNPs successfully. These findings are consistent with recent research emphasizing the potential of biogenic BMNPs combined with phytochemicals or phenolic compounds from medicinal plants as alternative treatments for chronic infections.

The rise in multi-drug resistant microbes and superbugs demands a quest for new safer alternatives to potential control and reduce the deadly impact of microbial infections. In particular, antibacterial agents which are non-toxic to patients and do not pose a threat of microbes gaining resistance against mode of its antibacterial action, are immediate need. To address this issue, candidature of MNPs (mono/bi/tri/multi-metallic NPs) are explored since they possess a variety of bioactive compounds with effective antibacterial properties. Recently, biogenic syntheses of mono-metallic nanoparticles (MNPs) and bimetallic nanoparticles (BMNPs) have been accepted as a sustainable synthesis route due to its environment-friendly, non-toxic processing, and cost-effectiveness. It presents a sustainable alternative to conventional synthesis methods that often involve the use of hazardous chemicals and high energy process. Additionally, we highlight the use of these synsthesized Cu-Ag BMNPs can be employed for potential therapeutic applications in chronic infection and microbial diseases with further cell-based vivo studies model.

The use of plant leaf extract has become a specific synthesis technique for producing MNPs, as these plant leaves extract act as both reducing, capping, and stabilizing agents to the metal nanoparticles during the synthesis process. Overall, these findings from our present work contribute to the growing body of knowledge on the applications of bimetallic nanoparticles in combating chronic infections and pave the way for further research and development in nano-biotechnology research field.

5. CONCLUSION

In the current study, we employed single step novel biosynthesis method to synthesize copper-silver bimetallic nanoparticles (Cu-Ag BMNPs). The Argyreia Nervosa plant leaf green extract acted as an perfect capping, reducing and stabilizing agent during the bio-synthesis process. The UV-Vis spectroscopy and XRD analysis confirmed the high quality and structural details of the Cu-Ag BMNPs, which exhibited diameters ranging from approximately 25 to 100 nm. The antibacterial activity of the Cu-Ag BMNPs against Enteropathogenic *E.coli* pathogenic bacteria was evaluated using disc diffusion and minimum inhibitory concentration assays, demonstrating significant antibacterial activity and highlighting the potential for large-scale applications. Future research directions include assessing biocompatibility of Cu-Ag BMNPs, conducting in-vivo studies for cancer diagnosis and antimicrobial activity against various pathogens, and exploring targeted drug delivery systems. Additionally, we suggest that the practical applications of Cu-Ag BMNPs, such as antimicrobial agents, drug delivery vehicles, water purification agents, in textile industries, and dental treatments should be further explored. This study establishes a foundation for investigating the performance of Cu-Ag BMNPs in diverse practical applications and emphasizes the potential advantages of bimetallic nanoparticles over mono-metallic counterparts. The successful green synthesis of Cu-Ag BMNPs and their antimicrobial activity against Enteropathogenic *E. coli* opens avenue for development of advanced nanomaterial's with enhanced properties for biomedical and industrial applications. Further studies should focus on optimizing the synthesis process, understanding the antibacterial mechanisms, measuring potential toxicity, and assessing the stability. These results will facilitate large production of Cu-Ag BMNPs from the laboratory scale to industrial scale applications, benefiting human health and the environmental applications. The promising results obtained in this study highlight the significant potential of bimetallic nanoparticles and their green synthesis methodologies in advancing nano-biotechnology and its diverse applications.

Declarations

AUTHOR'S CONTRIBUTION

Parvathalu Kalakonda: Supervision, writing-original draft and editing, visualization

Rajitha Kathi: Designing experiment, and Data collection,

Merlin Sheeba: Designing experiment procedure and investigation antibacterial activity

S. Rami Nayudu & Pritam Mandal: Review of articles and data collection

All other authors were involved in various parts of discussion of project.

All authors read and approved the final submission.

DATA AVAILABILITY

Data will be made available on request.

ETHICAL APPROVAL

Internal review board committee of our lab state that we do not have any consent to publish this antimicrobial and other data.

CONFLICT OF INTEREST

The authors declare no competing interest.

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Figures

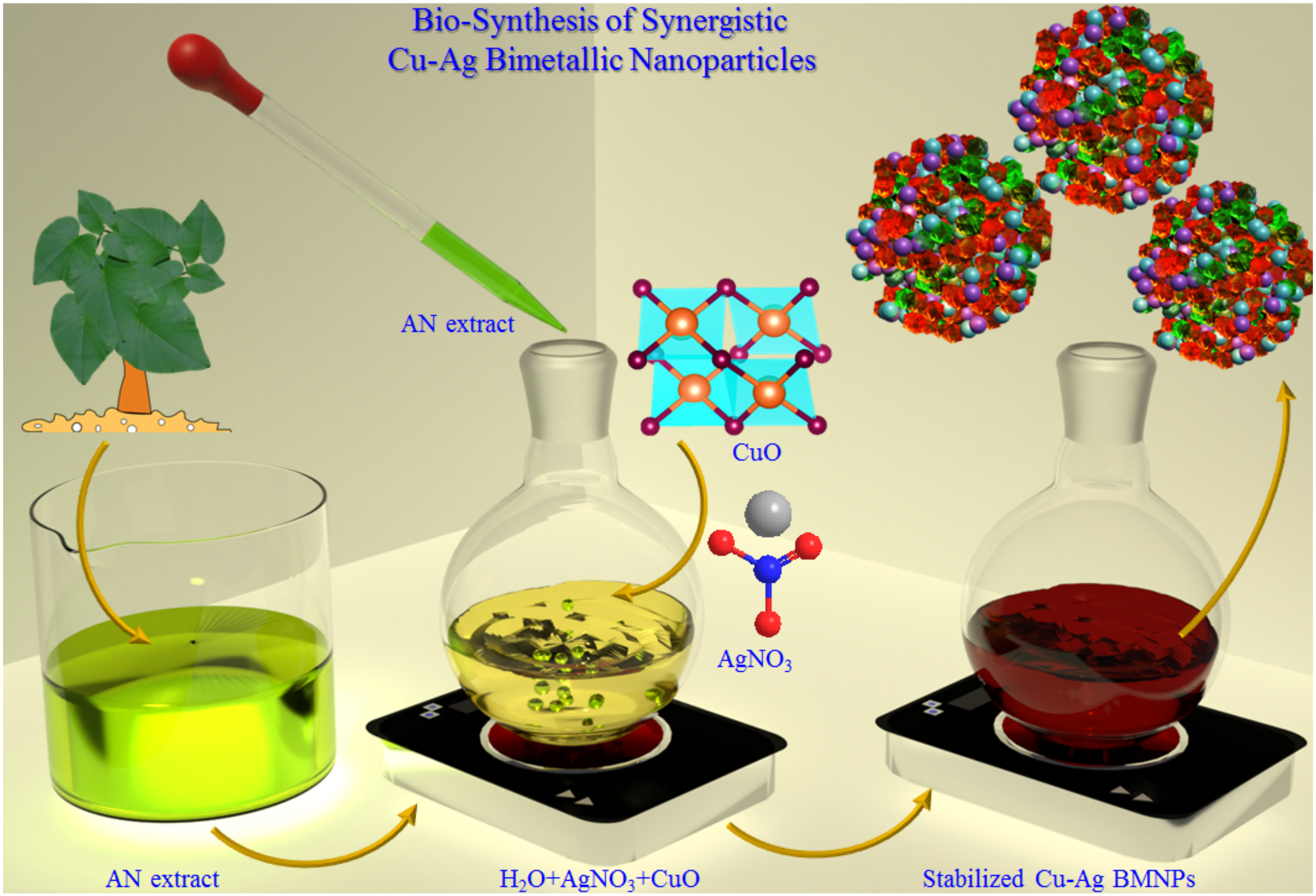

Figure 1

Nucleation process of AN leaf extract mediated Cu-Ag BMNPs

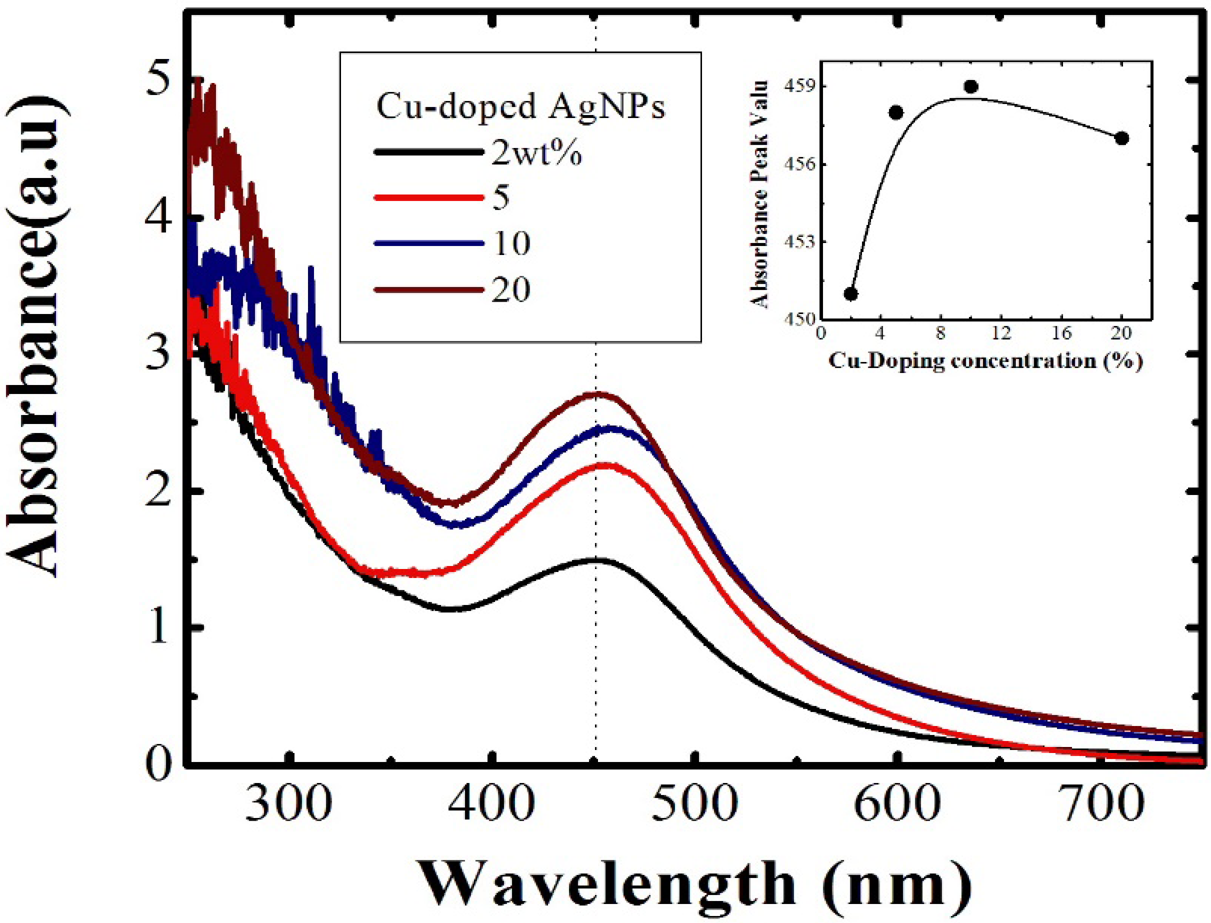

Figure 2

UV-Vis Absorption Spectroscopy of Cu-Ag BMNPs showing peak absorbance

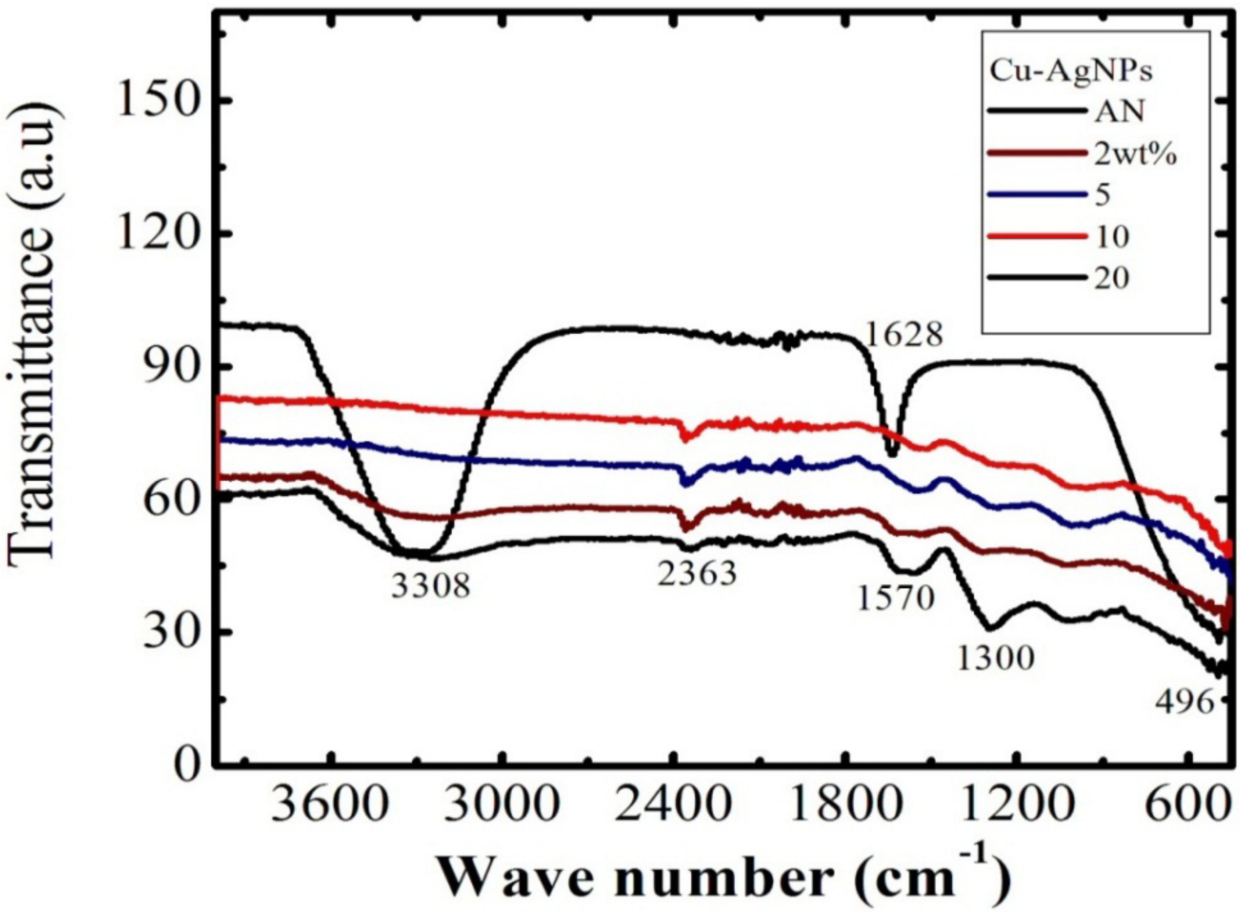

Figure 3

FTIR spectrum of AN leaf extract mediated Cu-Ag BMNPs

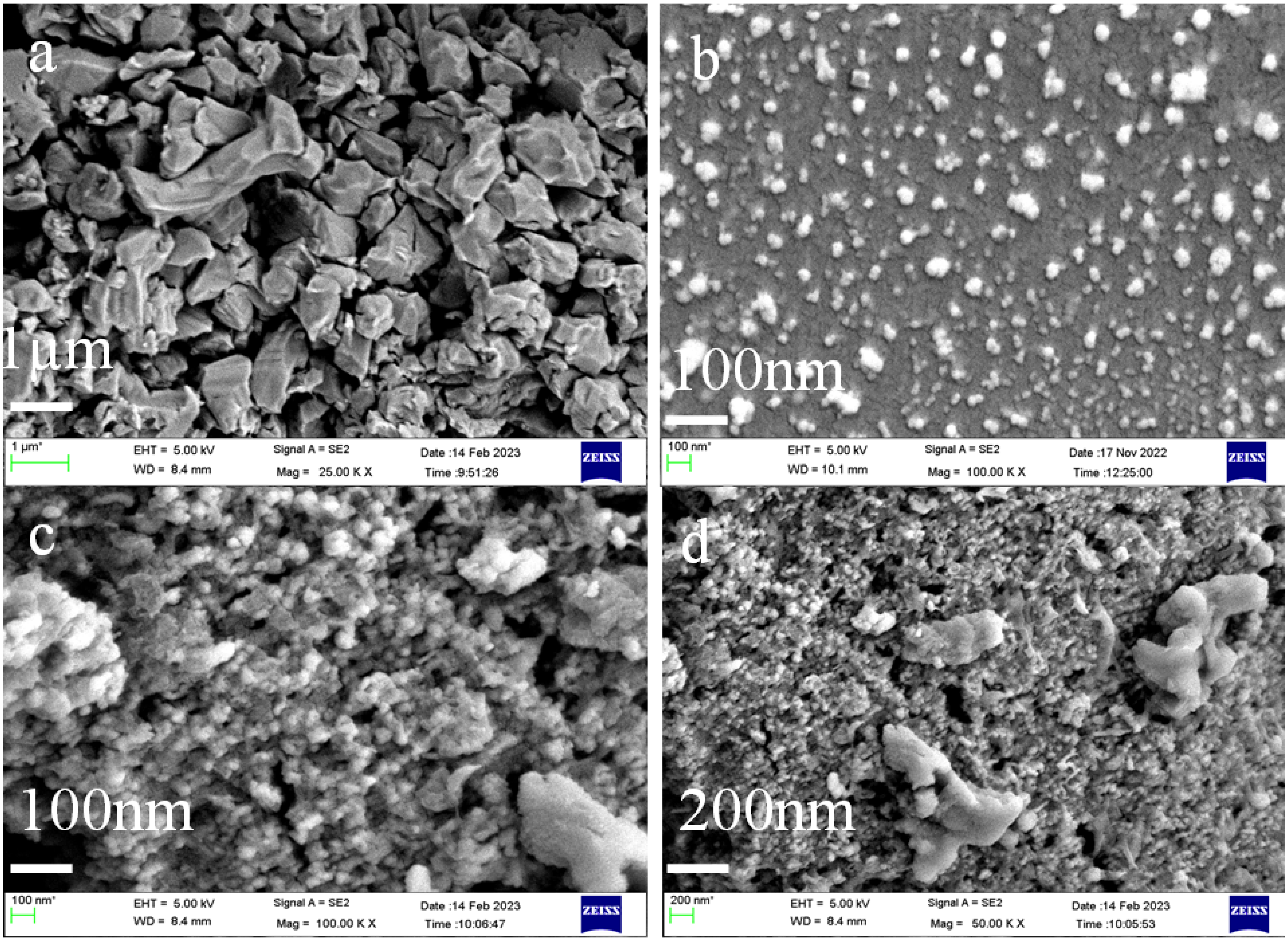

Figure 4

SEM image of the (a) CuNPs (b) AgNPs (c) & (d) Cu-Ag BMNPs

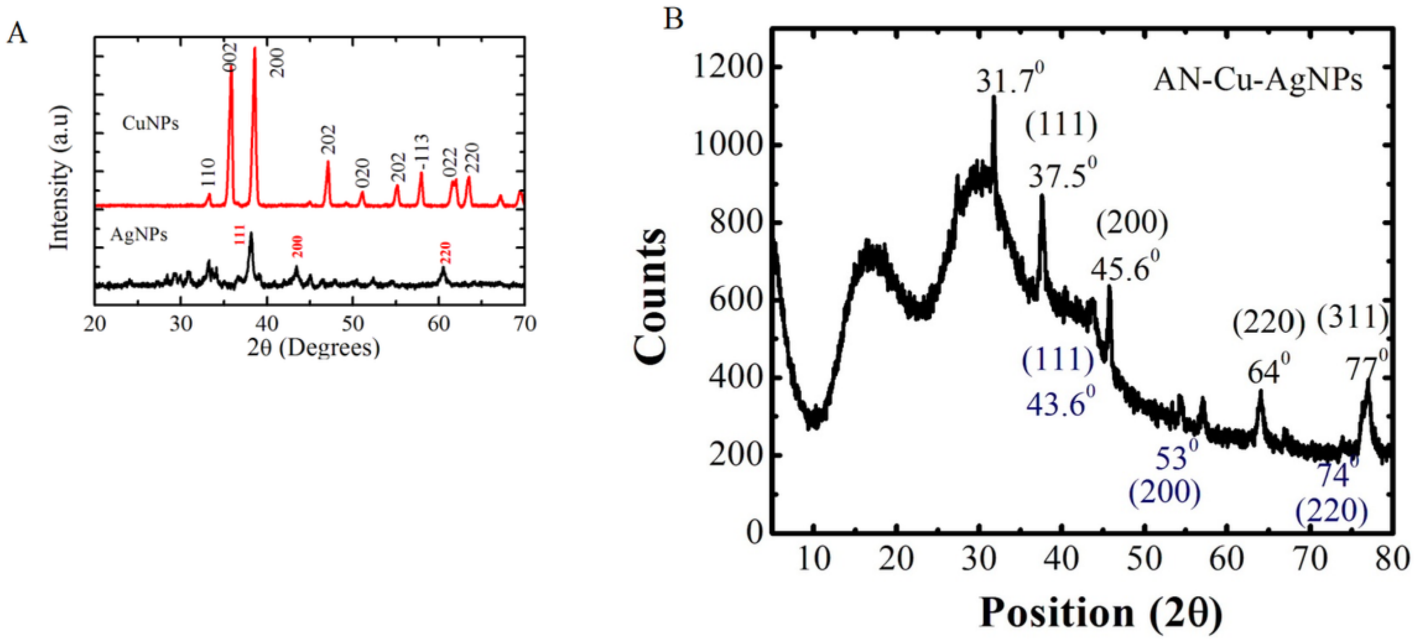

Figure 5

a. Stacked XRD spectrum of CuNPs and AgNPs

b. XRD spectrum of Cu-Ag BMNPs

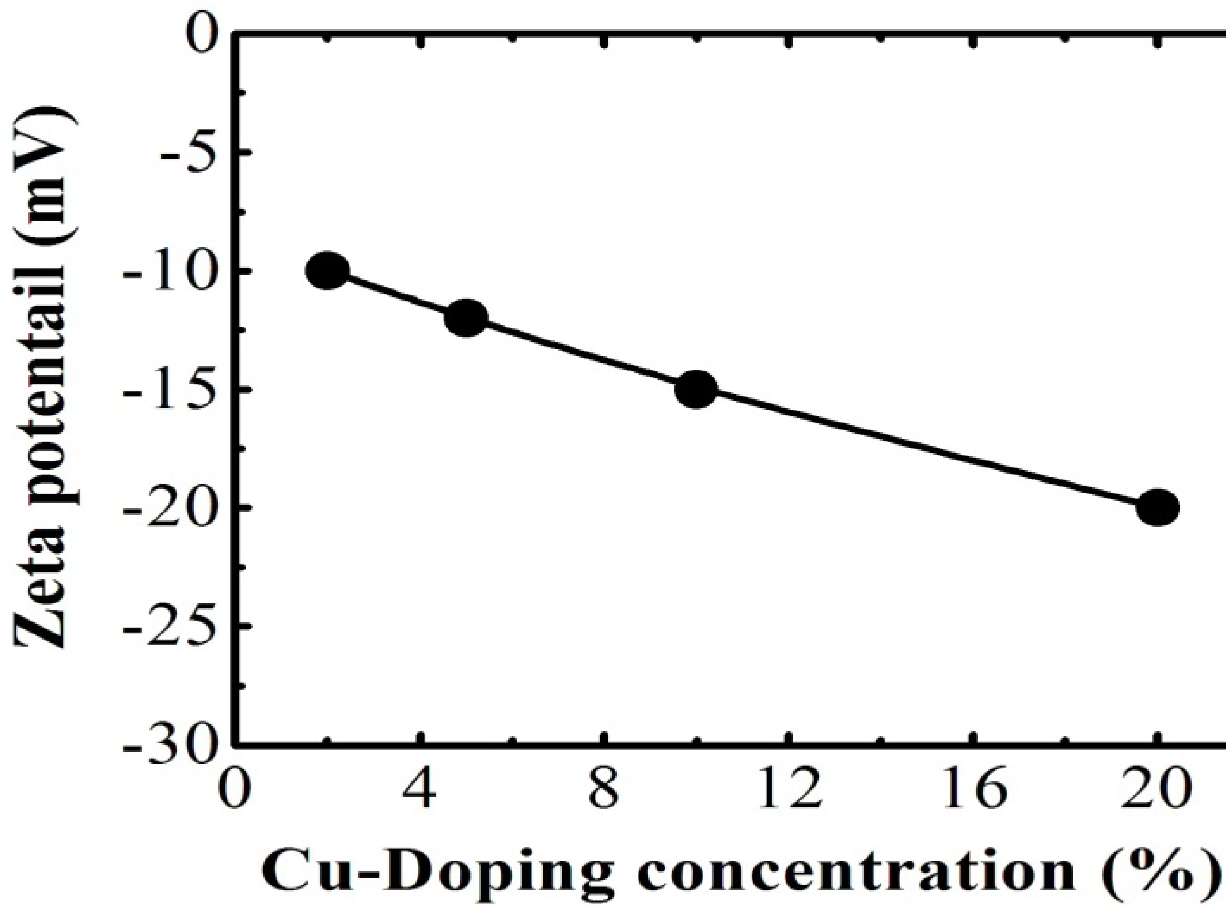

Figure 6

Zeta potential of Cu-Ag BMNPs

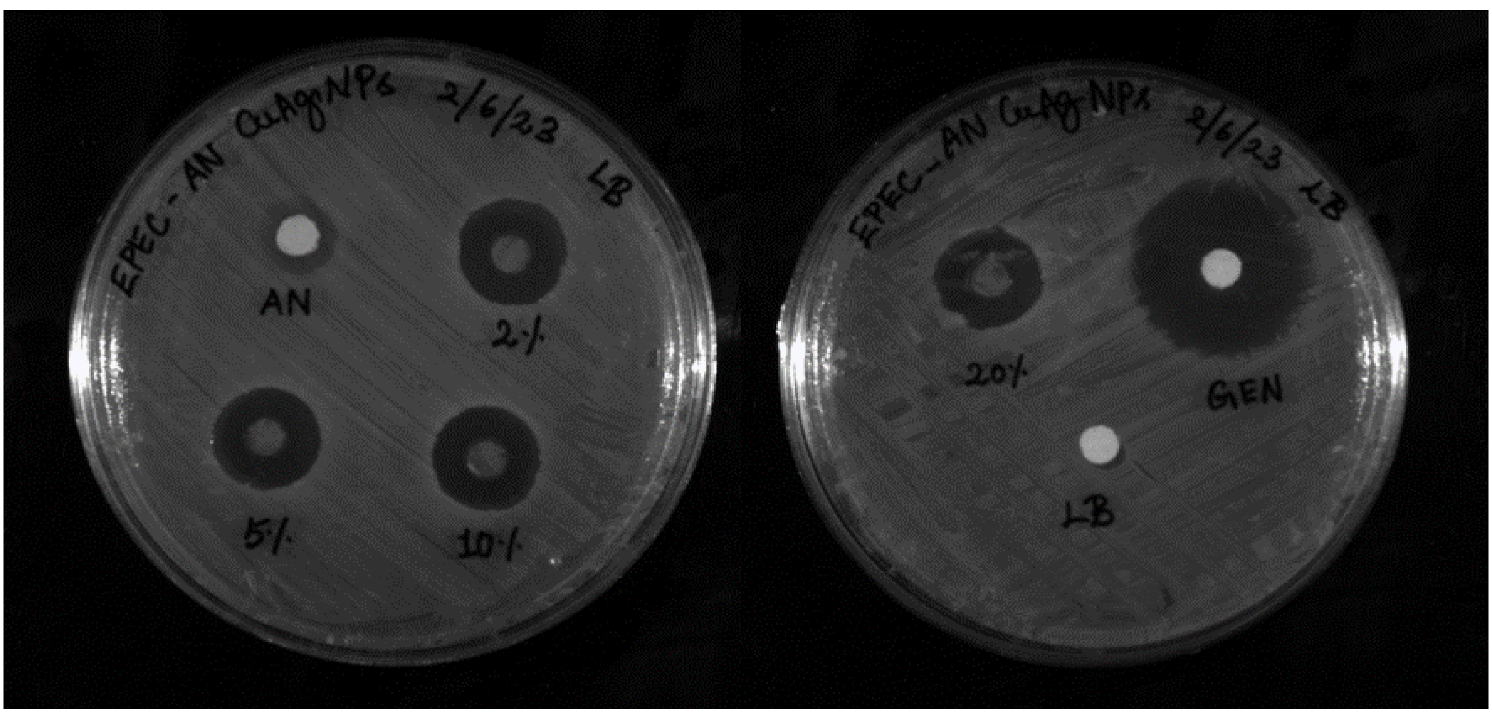

Figure 7

Antibacterial activity of AN-Cu-Ag BMNPs on EPEC via disc diffusion assay

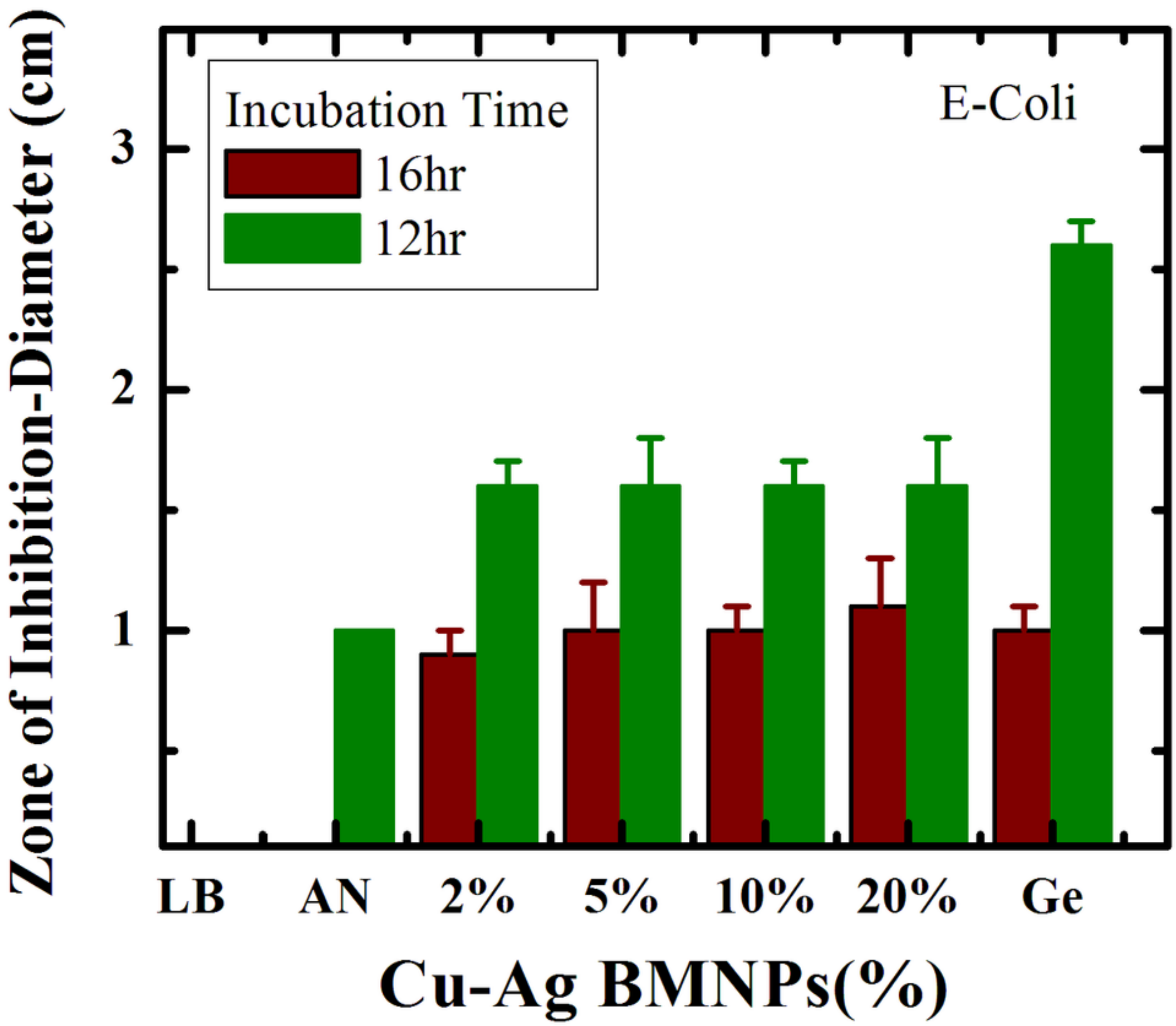

Figure 8

Histogram showing zone of inhibition against EPEC as the function of Cu doping in Cu-Ag BMNPs

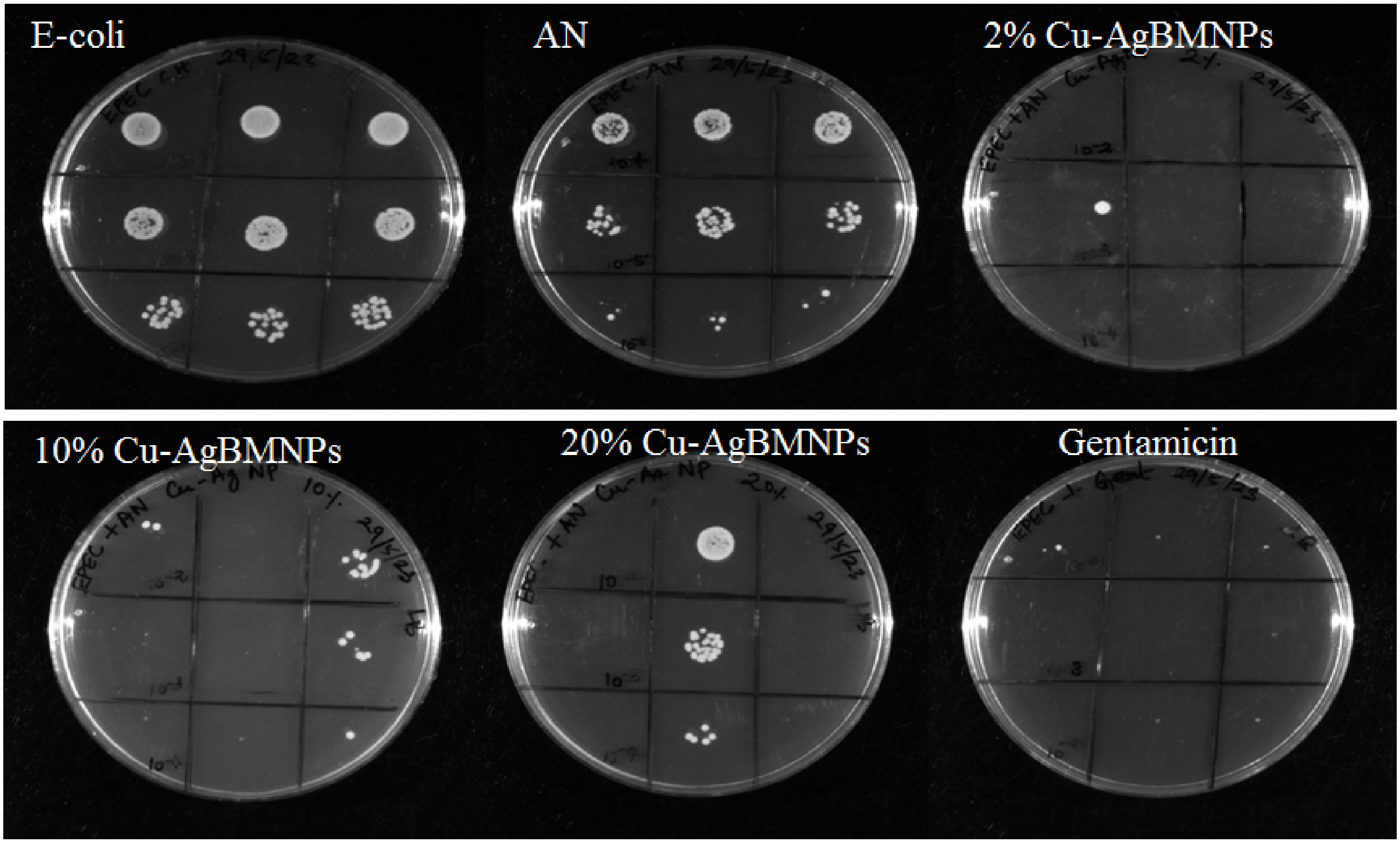

Figure 9

Effect of AN-leaf extract mediated Cu-Ag BMNPs against EPEC via MIC assay

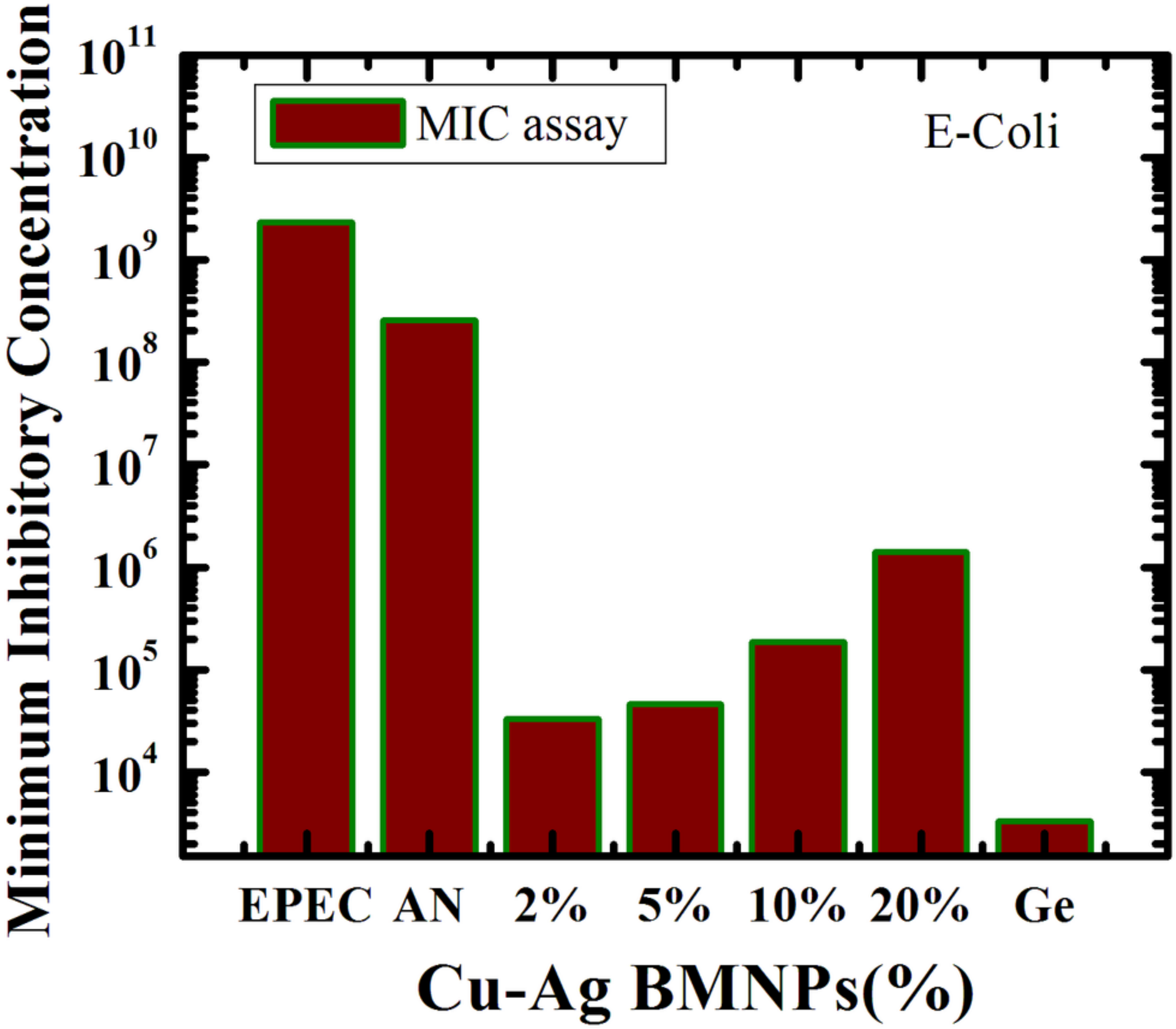

Figure 10

Effect of AN leaf extract mediated Cu-Ag BMNPs against EPEC via MIC assay

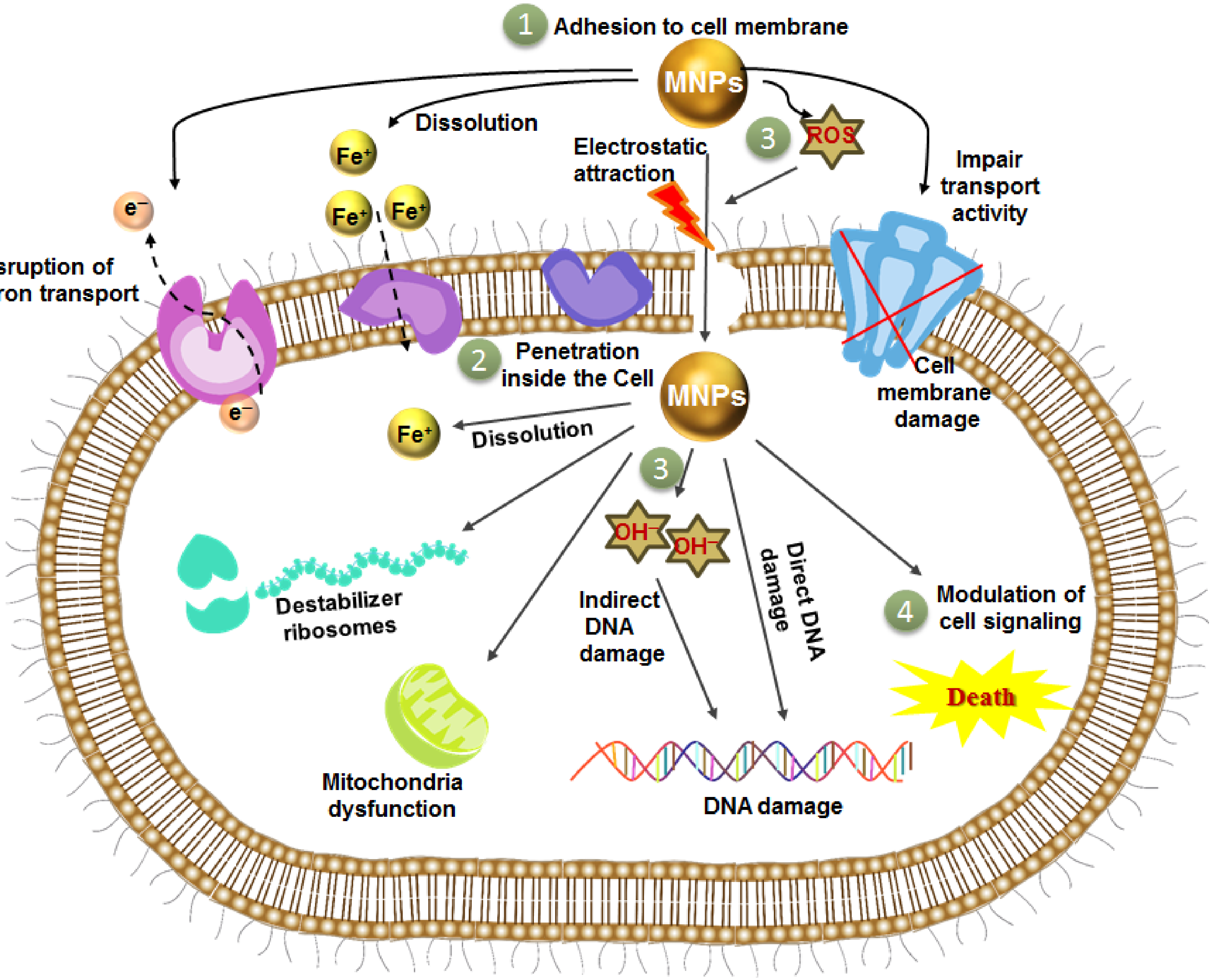

Figure 11

The possible mechanism routes of antibacterial action of Cu-AgNPs nanoparticles.