Applied Physics A (2025) 131:153
https://doi.org/10.1007/s00339-025-08289-1

Applied Physics A
Materials Science & Processing

Bimetallic zinc-silver nanocomposites for superior antimicrobial efficacy

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Received: 8 October 2024 / Accepted: 20 January 2025

Abstract

Traditional treatments for microbial infections often have adverse effects and consequences, while chemically synthesized nanoparticles can produce harmful by-products. This study employed a green synthesis approach to produce zinc-silver bimetallic nanoparticles (Zn-Ag BMNPs) using the leaf extract of *Tinospora cordifolia* (TC). Characterization of the synthesized ZnNPs and Zn-Ag BMNPs revealed diverse structural and functional properties. FTIR analysis of these samples confirmed the anchoring of pytochmical groups on the surface of BMNPs, while XRD pattern revealed structural details with a crystallite size of approximately 19 nm. UV–Vis spectroscopy confirmed the formation of Zn-Ag BMNPs with absorption peaks in the range of 250–450 nm, and the optical energy gap was measured as 3.1 eV using Tauc plot analysis. Stability in aqueous suspensions was supported by zeta potential values of – 20.5 mV and – 22.7 mV, suggesting minimal aggregation. The potential antibacterial efficacy was assessed using disk diffusion and minimum inhibitory concentration (MIC) assays against *Escherichia coli* (*E. coli*). Zn-Ag BMNPs exhibited superior antimicrobial properties, achieving a maximum inhibition zone of 4 mm at optimal concentrations, while ZnNPs displayed comparatively weaker activity. These findings suggest that synthesized Zn-Ag BMNPs nanocomposites may have significant senergetic effect in treating chronic diseases and addressing the aforementioned issues.

Keywords Bimetallic nanocomposite · Bio-synthesis · Synergetic effect · Antimicrobial activity · Escherichia coli

1 Introduction

Modern society faces significant challenges related to microbial and fungal infections. These infections, caused by microorganisms such as bacteria and viruses, can lead to serious health issues if not properly addressed and resolved. Traditionally, chemically synthesized antibiotics have been used to treat these infections; however, long-term use of these agents can pose significant health risks. In recent years, bio-synthesized metal nanocomposites and their hybrid nanocomposites have gained considerable importance due to their unique multi-functional properties. They nanomaterials are engineered with specific functional characteristics that enhance their senergetic effect in eliminating microbes, offering a promising alternative to conventional antibiotics. They are utilized in pharmaceutical industry, drug delivery systems, energy sector, chemical and gas sensors, food & agriculture industry, and microbial infections [1–4]. Their multifunctional properties make them valuable in combating microbial infections and chronic diseases [5–12]. With the growing issue of bacterial resistance to traditional antibiotics, there is an urgent need for alternative antimicrobial strategies. In this context, green-synthesized

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metal nanocomposites emerge a promsing option for targeted antimicrobial applications, often outperforming conventional antibiotics.

The finding of nanomaterials that can effectvily address microbial activity represent a significant advancement. Simplyfying the treatment procedure and reducing cost are equally important, as these measures yield more benfical on the eco-system. Nanomaterials and nanocomposites have demonstrated great advantages in the field of biomedical applications. These nanomaterials can precisely engineered in such a way to anchor the bacteria cells, making these materials exstreamely useful for therapeutic applications. However, among the various types of nanomaterials, inorganic metal nanoparticles exhibit potential effects across the various industrial applications. These composite nanomaterials hold a great promises in the treatment of cancer and microbial infections. There are many synsthesis methods in developing sustainable and economically viable techniques for large-scale synthesis [13–16]. Currently, synthesis of metal nanoparticles use many methods such as chemical reduction processes, microwave-assisted techniques, electron irradiation methods, and biogenic approaches using plants extracts. [17–32, 78]. However, there are significant challenges exist in efficiently controlling the stability, scalability, and structural of the metal nanocomposites [33]. Chemical synthesis methods demand significant energy consumption and financial resources, leading to adverse environmental consequences, including pollution issue, ecological toxicity, and health risks [34]. Among the various synthesis methods available, biogenic synthesis approache has gained significant attention due to their eco-friendliness, low toxicity, cost-effectiveness, and simple procedure [35–37]. These methods employ plant extracts from sources like leaves, fruits, peels, and roots, as well as microorganisms, to both reduce and stabilize metal nanoparticles [38–41]. Plant extracts are rich in natural biomolecules and complex molecular structures with medicinal properties, making them effective capping and stabilizing agents for synthesizing metal nanocomposites which enhances the antibacterial efficacy of metal nanocomposites (MNC) [42–45]. Metal nanoparticles and their nanocomposites are highly valued in nanobiotechnology applications due to their multifunctional properties. Their rich multifunactional properties arise from their nanoscale dimensions and the resulting high surface area-to-volume ratio [46–50]. Bimetallic nanocomposites form core–shell nanostructures exhibit potential antimicrobial activity due to the synergistic interactions between the constituent metal ions and bacteria cell walls comared to mono-metal nanocomposites. Although Tinospora cordifolia leaf extract (TC) has been used for synthesizing mono-metallic nanoparticles, it has not yet been explored for the creation of zinc-silver bimetallic composites (Ag-Zn BMNPs) [51–58, 63, 69, 75–77]. The tinospora cordifolia plant is well-known for its rich medicinal properties, such as its anti-inflammatory and stress-reducing effects [57].

This study highlights the medicinal importance of TC plant leaf extract as a multifunctional agent, capable of reducing, capping, and stabilizing the synthesis of stable Zn-Ag BMNPs. Previous research has shown the strong antimicrobial properties of AgNPs and ZnNPs against *E. coli* bacteria strains [55, 56]. We hypothesize that these nanocomposites will display heightened antibacterial efficacy [57]. Both AgNPs and ZnNPs have been extensively studied for a range of biomedical technological applications, including their use as antimicrobial agents, drug delivery vehicles, components in water purification, and additives in the textile industry. Their exceptional stability and minimal toxicity to human cells have garnered significant recognition. Although numerous studies have focused on the synthesis and antimicrobial properties of various metal nanoparticles, the green synthesis of Zn-Ag BMNPs using TC plant extract, along with their antibacterial properties, is still an area that remains largely underexplored.

In this study, we successfully synthesized Zn-Ag BMNPs using TC plant leaf extract and assessed their antibacterial efficasy against *E. coli* bacteria strain through disc diffusion and minimum inhibitory concentration assays. Our findings highlight the potential of these environmentally friendly Zn-Ag BMNPs xomposite as powerful antibacterial agents. Moreover, we confirm that the green synthesis method employed, utilizing TC plant leaf extract, presents a promising, energy-efficient, and cost-effective strategy for large-scale production of Zn-Ag BMNPs, suitable for various biomedical industria applications.

2 Methods and characterizations

2.1 Materials

We obtained zinc nitrade ($Zn(NO_{3)2})$), silver nitrate ($AgNO_3$), and all standard solvents from Sigma-Aldrich in Bangalore, India. The TC plant leaves were carefully gathered from the herbal garden at enivoronmental protection and training institute (EPTRI) in Hyderabad.

2.2 Synthesis of zinc-silver bimetallic nanocomposites (Zn-Ag BMNCs)

The synthesis of Zn-Ag BMNPs were carried out usig a biosynthesis approach with TC plant extract, using on our previous study [13–16]. Briefly, the process involved adding of 50 mL of the TC extract from a burette into bimetallic pre-cursor salt solution. The mixture solution was prepared by adding of 200 mL of 0.3 M $Zn(NO_{3)2})$ (5 g) and 50 mL of $AgNO_3$ at concentration ranging from 0.005 M to 0.01 M ($AgNO_3$ concentrations of 0.5% and 1% in zinc nitrade ($Zn(NO_{3)2})$,) in a 300 mL beaker. The combined salt solution was heated to 90 °C for 15–20 min while being stirred at 800 rpm. The TC leaf extract solution was then added at a controlled rate into into bimetallic salt solution at our lab standard procedure [16]. This process is continued on a hot plate until a visible color change occurred, indicating the formation of BMNPs and then obtained powderd of BMNPs through drying process [16]. A schematic diagram of the Zn-Ag BMNPs synthesis process is shown in Fig. 1. All samples were repeated twice to ensure consistency in the measuremnets.

2.3 Characterization of synthesized Zn-Ag BMNPs

In line with our previous study [13, 14, 16], we characterized the Zn-Ag bimetallic nanoparticles (BMNPs) using various techniques. UV–visible spectroscopy assessed optical properties and band gap energy, while Fourier-transform infrared spectroscopy (FTIR) identified functional groups involved in stabilization. Field-emission scanning electron microscopy (FE-SEM) provided insights into surface morphology and particle size distribution, and powder X-ray diffraction (XRD) analyzed crystal structure. Finally, zeta potential measurements using a Zeta-sizer Nano-ZS instrument evaluated the surface charge and stability of the Zn-Ag BMNPs.

2.4 Antibacterial activity

The antimicrobial properties of ZnNPs and Zn-Ag BMNPs were measured against *E-coli* bacateria. All equipment and glassware used for monitoring antimicrobial activity were sterilized through autoclaving. To evaluate the antibacterial properties of the green synthesized ZnNP and Zn-Ag BMNPs, we repeated experiments 3times with time gap of one month involving EPEC, in comparison with a reference standard antibiotic-*Gentamicin*. In the subsequent sections, we provide a brief overview of the two methods employed to evaluate the antibacterial activity of ZnNP and Zn-Ag BMNPs.

2.4.1 Bacterial culture

Using on our previous procedure[16], We estimated the antibacterial properties of the green syntheszised ZnNP and Zn-Ag BMNPs using the disc diffusion and minimum inhibition concentration. We cultured *EPEC* media from glycerol stocks stored at – 80 °C and the bacteria were grown in Luria Bertani (LB) broth at 37 °C for 12nhours with shaking at 200 rpm. *EPEC* was streaked on LB agar plates and the overnight culture with an optical density (OD600nm) of 1 was used for disc diffusion and minimum inhibitory concentration assays. This approach ensured

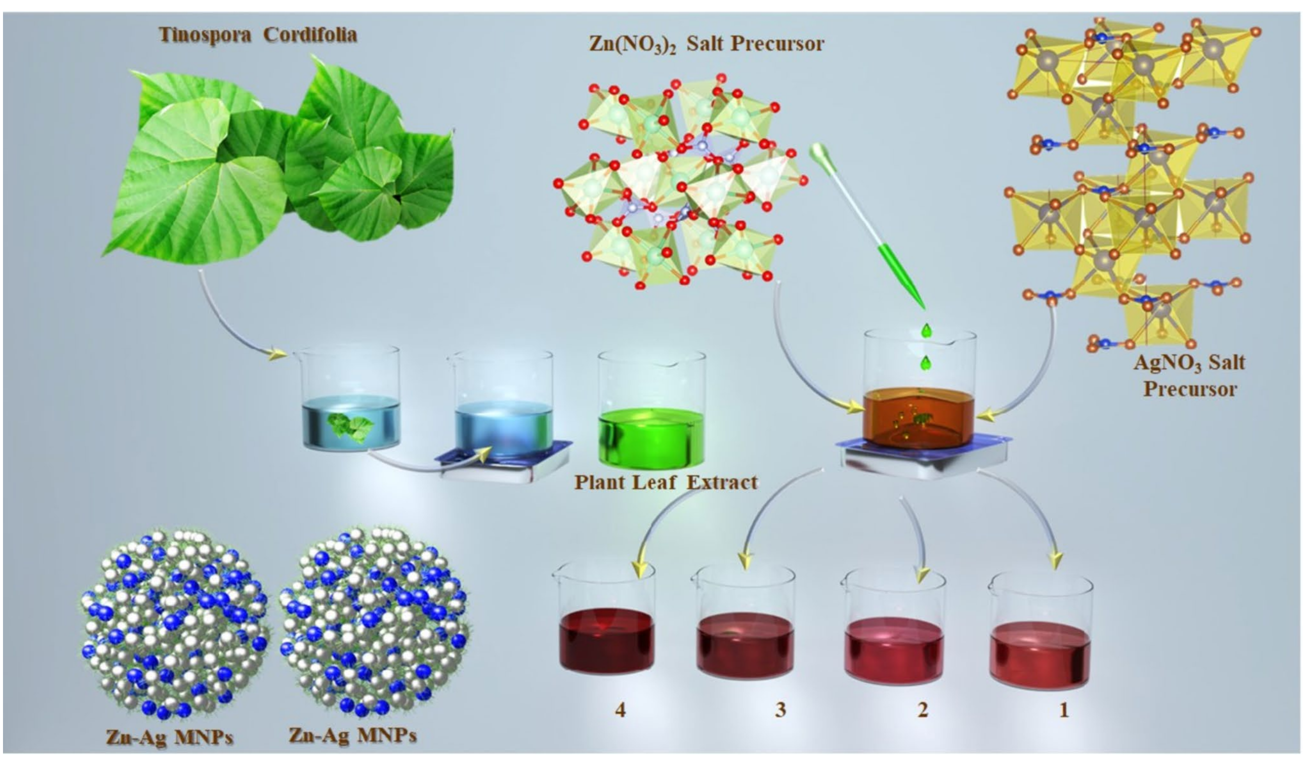


Fig. 1 Synthesis process of TC leaf extract mediated Ag-Zn BMNPs

consistent bacterial growth and standardized conditions for estimating antibacterial properties of our samples.

2.4.2 Disc diffusion and minimum inhibition concentration (MIC)

Following our previous methodology [13, 14, 16], we evaluated the antibacterial properties of ZnNP and Zn-Ag BMNPs against *EPEC* bacteria strain. The disc diffusion assay was employed to estimate the zone of inhibition at various Zn-Ag BMNPs concentrations. We then assessed MIC tests to determine the lowest effective concentration against *EPEC*. Time-kill kinetics were analyzed to understand the rate of bacterial elimination, while biofilm inhibition assays explored. Lastly, cytotoxicity assessments were performed to evaluate the safety profile of the Zn-Ag BMNPs, providing a comprehensive understanding of their antimicrobial efficacy and potential biomedical applications.

3 Results

3.1 UV–VIS spectroscopy

In this study, ZnNP and Zn-Ag BMNPs were sysnthesized using a sun dried green leaf extract of TC as natural green precursor. This extract include several biomolecules and phytochemicals which act as reducing, stabilizing, and capping agents in the fabrication of ZnNPs and Zn-Ag BMNPs. The nanoparticles formation has been identified visually as a mixure of various colors from yellow color to brown indicating the formation of Zn-Ag BMNPs. In this fabrication method, temperature, reaction rate, drop rate, and stirring play important role during the synthesis of these nanocomposites. Controlling the above experimental parameters can accerlerate the reduction of metal nanocomposites and help the formation of nanocompounds. The UV–Vis spectroscopy was used to estimate the optical properties of Zn-Ag BMNPs fabricated by using green sysnthesis. The analysis was conducted over a wavelength range of 250–800 nm, and the observed data were presented as absorption as a function of wavelength. The absorption peak of these BMNPs and ZnNPs showed similar trend and where BMNPs showed sharp peaks, indicative of complex AgNP anchoring within the structure of ZnNPs. This spectral profile suggests enhanced interparticle interactions, possibly driven by silver complexation. These findings highlight the intricate relationship between surface characteristics and optical behavior in Zn-Ag BMNPs [49, 59, 61] (Fig. 2).

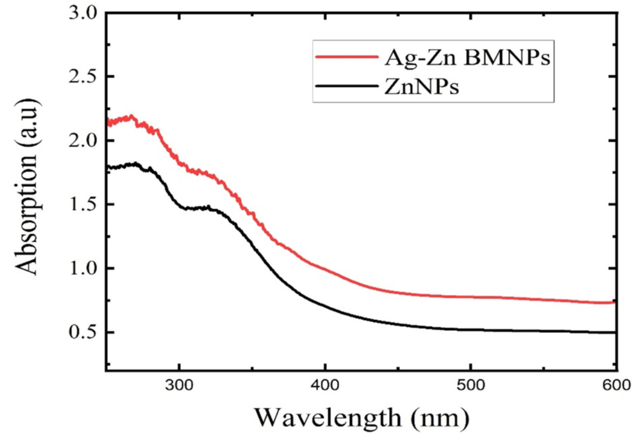


Fig. 2 UV–Vis Absorption Spectra of ZnNP and Ag-Zn BMNPs showing a broader absorption peak

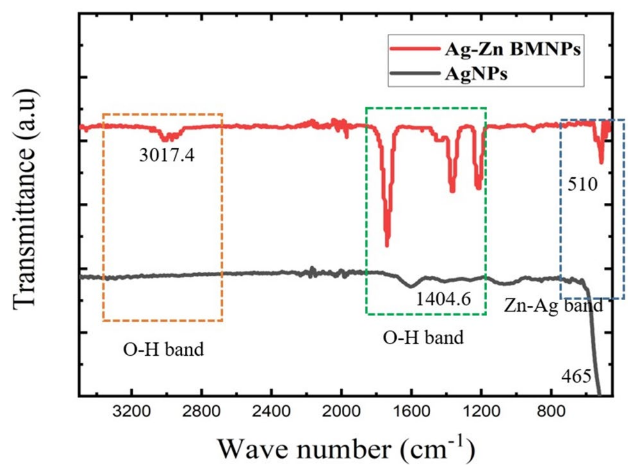


Fig. 3 FTIR spectra of TC leaf extract mediated ZnNPs and Ag-Zn BMNCs

3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra analysis of ZnNPs and Zn-Ag BMNPs were measured in a scanning ranges from 4000 to 400 cm^{-1} and revealed key insights into their chemical composition and surface functionalization (Fig. 3). The spectra exhibited characteristic peaks at 1404.6 and 3017.4 cm^{-1} for Zn-Ag BMNPs where as ZnNPs was shown only 1404.6 cm^{-1}. These characterization peaks indicate bending vibration of the O–H band caused by the presence of water molecules. Characterization peaks at 510 and 465 cm^{-1} were aboserved due to the stretching vibration of ZnNPs and Zn-Ag BMNPs corresponding the existence of of both metal nanoparticles and their complex structure. The peak intensity decresed in Zn-Ag BMNPs owing to the anchoring of AgNPs on the interior of the ZnNPs. The other characterization peaks confirm the presence of biomolecules and phytochemicals from the plant extract, which likely facilitated the reduction of metal ions and stabilization of the nanocomposite [49]. This spectroscopic evidence supports the successful formation of Zn-Ag BMNPs and elucidates the surface chemistry governing their stability.

3.3 Morphology

The FE-SEM analysis provided the surface morphology of the micrographs of Zn-Ag BMNPs samples. Figure 4 reveal diverse irregular shapes and sizes ranging from 30 to 300 nm for the synthesized Zn-Ag BMNPs. In contrast, the AgNPs, ZnNPs, and their complex nanostructures display distinct shapes [59–62]. At higher magnification, the particle distribution and their suspensions reveals an aggregated structure with multiple shapes and sizes. Aggregation at the larger scale (Fig. 4a) suggests a tendency of the nanoparticles to cluster, which might limit their surface area and reactivity. However, at smaller scales (Fig. 4b–d), the particles appear well-defined and retain much of their individuality, suggesting that aggregation might be mitigated under optimized dispersal conditions. The combination of granular and rod-like morphologies, observed in medium magnifications, suggests anisotropic growth processes, which can enhance directional properties such as electron mobility in photocatalysis. The Zn-Ag BMNPs exhibit irregular shapes with smooth surfaces and these different irregular shapes can be attributed to the varying crystallization growth patterns of ZnNPs and AgNPs at different concentrations. The irregular shapes can be tuned by optimizing precursor concentrations and external synthesis conditions such as temperature, stirring, and extract concentration. The degree of BMNPs dispersion improved compared to previous studies [49], likely due to optimized precursor metal salt ratios and extract concentrations. The EDX analysis revealed the elemental composition of the Zn-Ag BMNPs, as shown in Fig. 5. This EDAX spectrum revealed the presence of only Ag and Zn, while no other elements were detected. The EDX analysis (Fig. 5) confirms the presence of zinc (Zn) as the predominant element, with minimal traces of silver (Ag), as indicated by the weight and atomic percentages. This suggests the nanomaterial primarily consists of Zn-based compositions, with Ag incorporation likely below the detection threshold. To prevent complex nanostructure formation, future work should focus on modifying surface strategies, exploring alternative formulation strategies, and optimizing surface functionalization approaches.

3.4 X-ray diffraction (XRD)

The structural properties an dpurity of the crystals of AgNP, ZnNps, and Zn-Ag BMNPs were analysized with the help of XRD analysis. The XRD patterns (Fig. 6) confirm the crystalline nature of the synthesized nanoparticles. The Zn-Ag BMNPs show prominent diffraction peaks corresponding to both zinc oxide and silver, indicating the successful formation of a Zn-Ag BMNPs. Compared to ZnNPs and AgNPs, the Zn-Ag BMNPs exhibit a combination of peaks with slightly modified intensities, suggesting possible synergistic effects and slight lattice distortions due to metal incorporation. Strong peaks were observed at 2θ values of 31.48°, 34.08°, 35.9°, 47°, 56.3°, 62.6°, 65.8°, 67.5°, 68.6°, 72.2°, and 76.5°, corresponding to reflection planes (100),

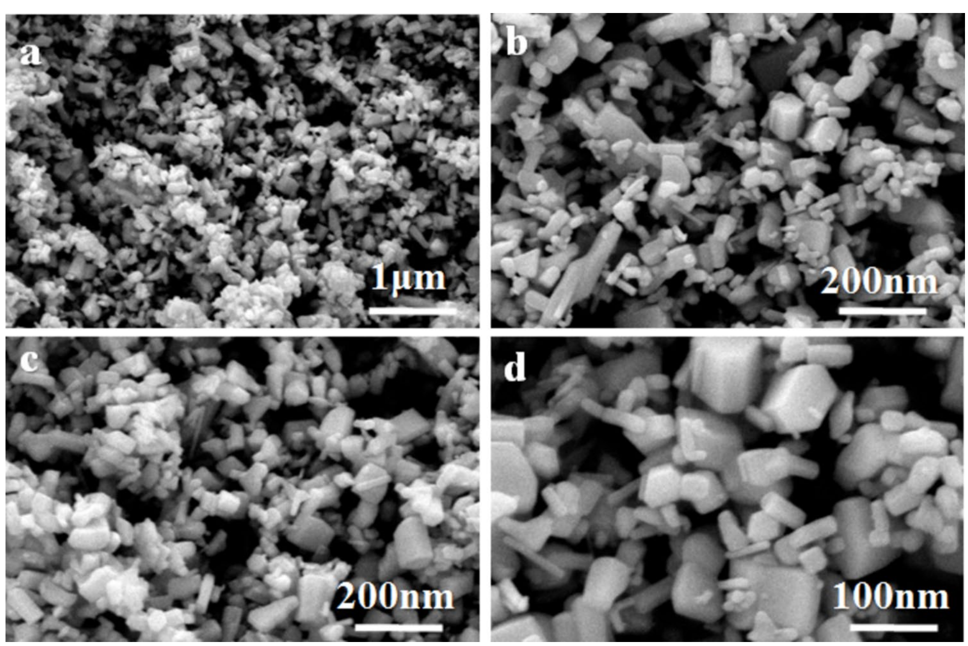
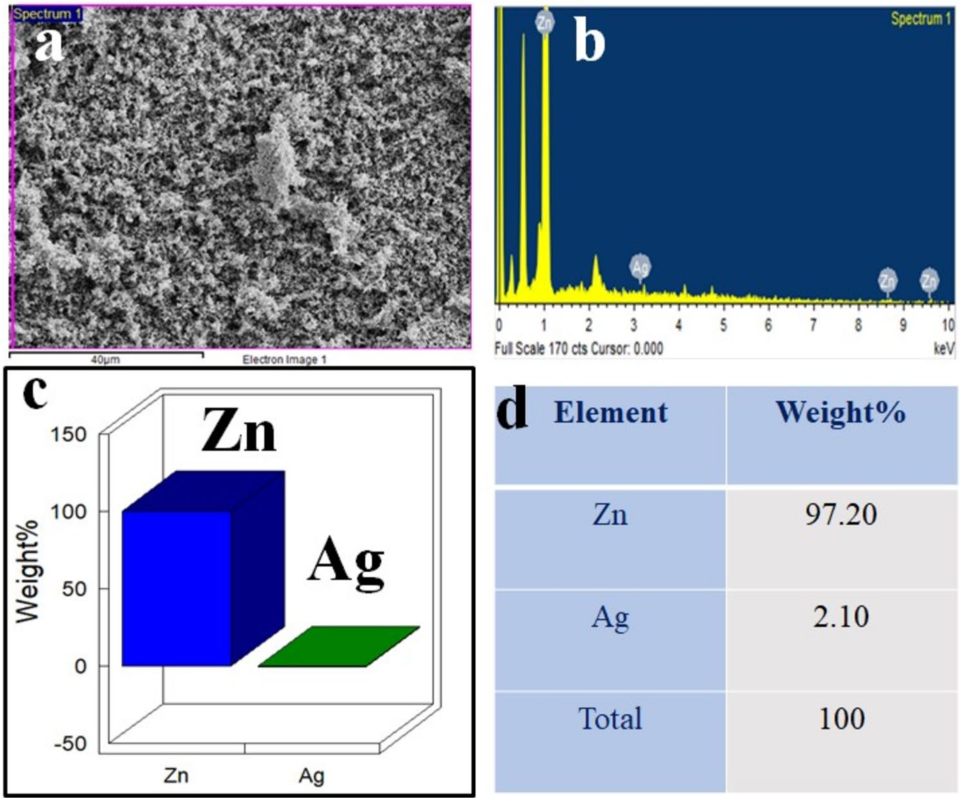


Fig. 4 SEM micrographs of the Zn-Ag BMNCs **a** 1 μm, **b** & **c** 200 nm, **d** 100 nm



Element	Weight%
Zn	97.20
Ag	2.10
Total	100

Fig. 5 EDX micrographs of the Ag-Zn BMNPs: **a** 40 µm. **b** Spectrum showing both Zn and Ag composition. **c**, & **d** Weight fraction of Zn and Ag composition in BMNPs

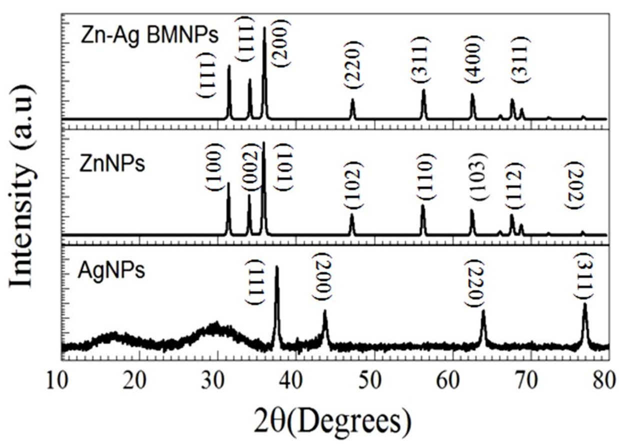


Fig. 6 Stacked XRD pattern of **a** AgNPs, **b** ZnNPs, **c** Zn-Ag -BMNPs

(001), (101), (102), (110), (103), (112), and (201). These patterns match the hexagonal wurtzite structure of green sysnthesized ZnNPs (JCPDS card No. 89–0510 & JCPDS, card No. 04–0783). In Zn-Ag BMNPs, the Ag exhibit two low intensity peaks at the 2θ values of 37.5°& 44.4° with respective lattice planes (111) & (200).

High-intensity peaks at 31.48°, 34.08°, and 35.9° suggest the presence of bio-organic phases on particle surfaces. A characteristic Ag^+ signal was detected at $2\theta = 62.45°$, confirming silver incorporation in the nanocomposite. The average crystallite sizes, calculated using the Debye–Scherrer equation, were approximately 19.04 nm, 18.8 nm, and 19 nm for ZnNPs, AgNPs, and Zn-Ag BMNPs, respectively. These findings provide crucial insights into the crystalline nature and composition of the synthesized Zn-Ag bimetallic nanocomposites [64].

3.5 Zeta potential

The zeta potential of dispersed ZnNPs & Zn-Ag BMNPs were measured and this would give crucial insights into the surface charge interactions and colloidal stability of nanomaterials. The zeta potential values estaimated as -24 to -12 mV for Zn-Ag BMNCs and – 20 to – 10 mV for ZnNPs when dispersed in deionized water (Fig. 7). These values fall within the range of + 25 mV to – 25 mV, indicating high degree of dispersion stability. The observed zeta potential range suggests that the Zn-Ag BMNPs maintain sufficient electrostatic repulsion to prevent significant aggregation. This stability is essential for preserving the unique properties of the nanocomposites in solution form. However, values closer to zero may indicate a tendency towards aggregate formation due to reduced inter-particle repulsion. These results suggest significant repulsion among the Ag and ZN nanoparticles. Our findings helps to understand the surface characteristics and predicting the long-term stability of metal hybrid nanppartciles in aqueous environments.

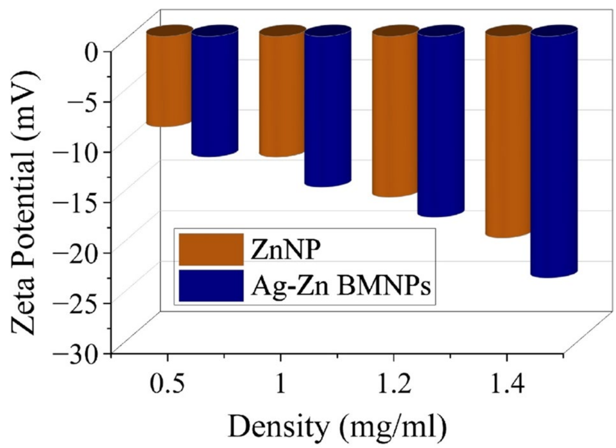


Fig. 7 Variation Zeta potential of ZnNPs and Ag-Zn BMNPs as the function off density

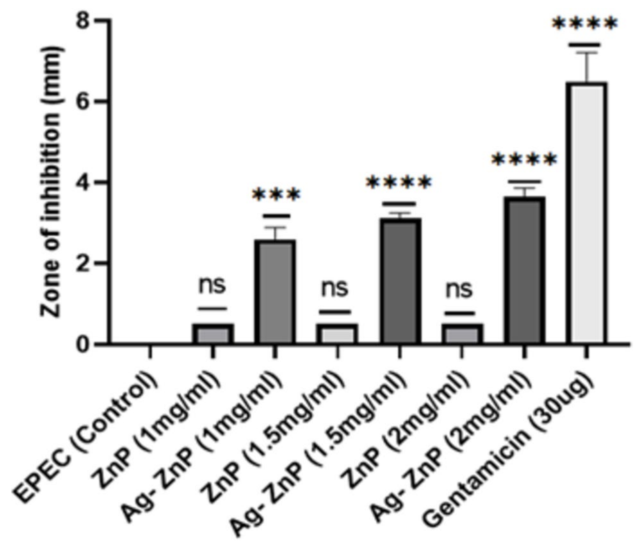


Fig. 9 Antibacterial activity of ZnNPs and Zn-Ag BMNPs at different concentrations against *EPEC* via *disc diffusion assay*

3.6 Antibacterial activity of Ag-Zn BMNPs

3.6.1 Disc diffusion assay

Our study investigated the antibacterial efficacy of TC-mediated ZnNPs and Ag-Zn bimetallic nanopartciles (BMNPs) against *E. coli* & control antibiotic-*Gentamicin*, (Figs. 8 and 9) using the agar well disc diffusion technique. The observed reults of the culture plates suggest that Ag-Zn BMNPs (2 mg/ml) exhibited high antibacterial activity at 12 h of incubation, rather than 16 h. Notably, the TC extract alone failed to produce a clear inhibition zone after 16 h, in contrast to its effect at 12 h. Comparative analysis of ZnNPs and Ag-Zn BMNPs at different concentrations revealed significant differences in inhibition zones ($P < 0.05$). The Zn-Ag BMNPs showed highest zone of inhibitaion (4 mm) at 2 mg/ml. Zn-Ag BMNPs demonstrated superior antibacterial activity compared to ZnNPs (1 mm) alone, which showed minimal inhibitory effects. A positive correlation was observed between Zn-Ag BMNPs at different concentration and inhibition zone size. The enhanced antibacterial efficacy of Zn-Ag BMNPs against *E. coli* may be attributed to their ability to bind, interact, and penetrate bacterial cells through hydrophobic and van der Waals forces, thereby disrupting intracellular activities. In our study, The green sysnthesized bimetal nanopartciles exhibited superior bacterial activity compared to monometal nanoparticles against all the pathogens. In future, these nanomaterials may be potentially serve as an effective alternative to traditional chemical antibiotics in combating antibiotic-resistant for different bacteria strains.

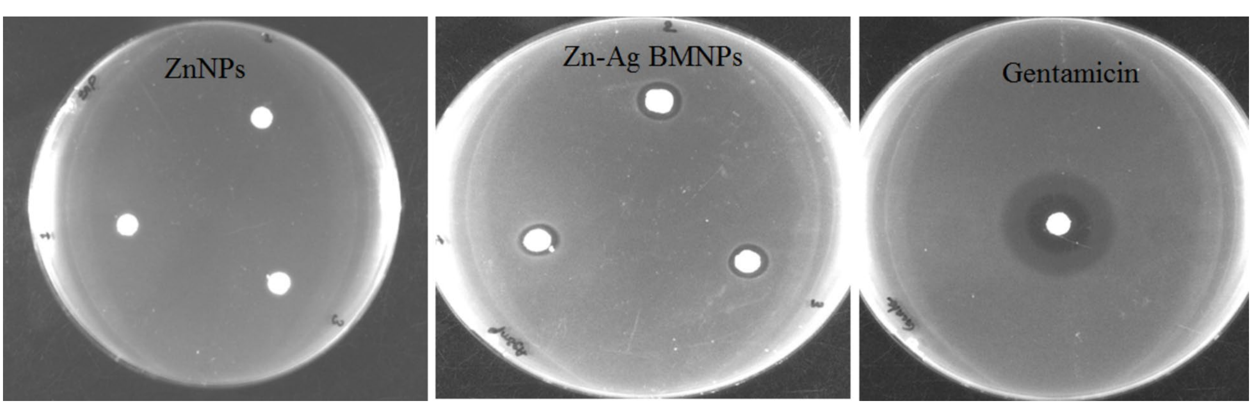


Fig. 8 Antibacterial activity of ZnNPs and Ag-Zn BMNPs on EPEC via disc diffusion assay

3.6.2 Minimum inhibition concentration (MIC) assay

Figure 10 demonstrates the antimicrobial activity of ZnNP and Zn-Ag BMNPs against the *E.coli* pathogen, confirmed through the minimum inhibitory concentration (MIC) assay. In Fig. 11, a histogram offers a comparative analysis of the antibacterial activity of ZnNPs & Ag-Zn BMNPs against *EPEC* bacteria at various concentrations. The antibacterial effect of ZnNPs and Zn-Ag BMNPs against the *E.coli* strain was evaluated using MIC at different concentrations: Zn-Ag BMNPs (0.5 mg/ml), ZnNPs (1 mg/ml), Zn-Ag BMNPs (1 mg/ml), ZnNPs (1.2 mg/ml), and Zn-Ag BMNPs (1.2 mg/ml). ZnNPs at 0.5 mg/ml did not significantly reduce pathogen growth, whereas the Zn-Ag BMNPs material exhibited a strong antibacterial effect. A significant reduction in pathogen growth was observed with increasing concentrations of Zn-Ag BMNPs compared to the control *E.coli.* The 1.2 mg/ml concentration of Zn-Ag BMNPs showed the highest antibacterial activity. Our study demonstrates that the Zn-Ag BMNPs composite material outperforms antibacterial efficacy against the *E.coli* strain compared to monometallic nanomaterial.

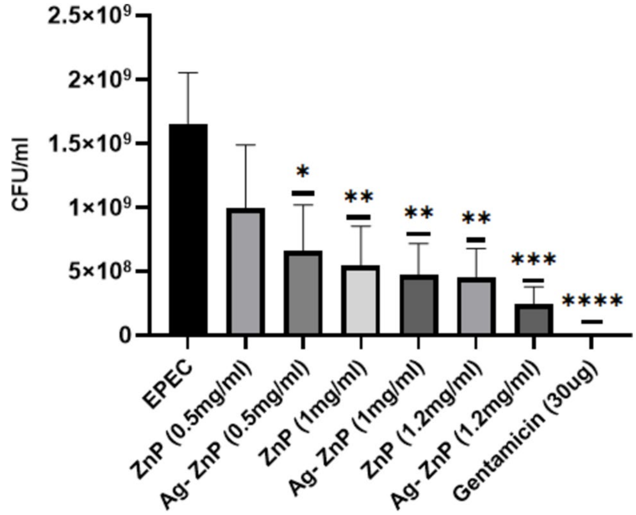


Fig. 11 Effect of TC leaf extract mediated ZnNPs and Zn-Ag BMNPs against E.coli via MIC assay

4 Discussion

Metallic nanoparticles, ranging from mono- to multi-metallic compositions, offer promising solutions for diagnosing and treating chronic infections and diseases. By integrating multiple metal ions into hybrid nanostructures, we can

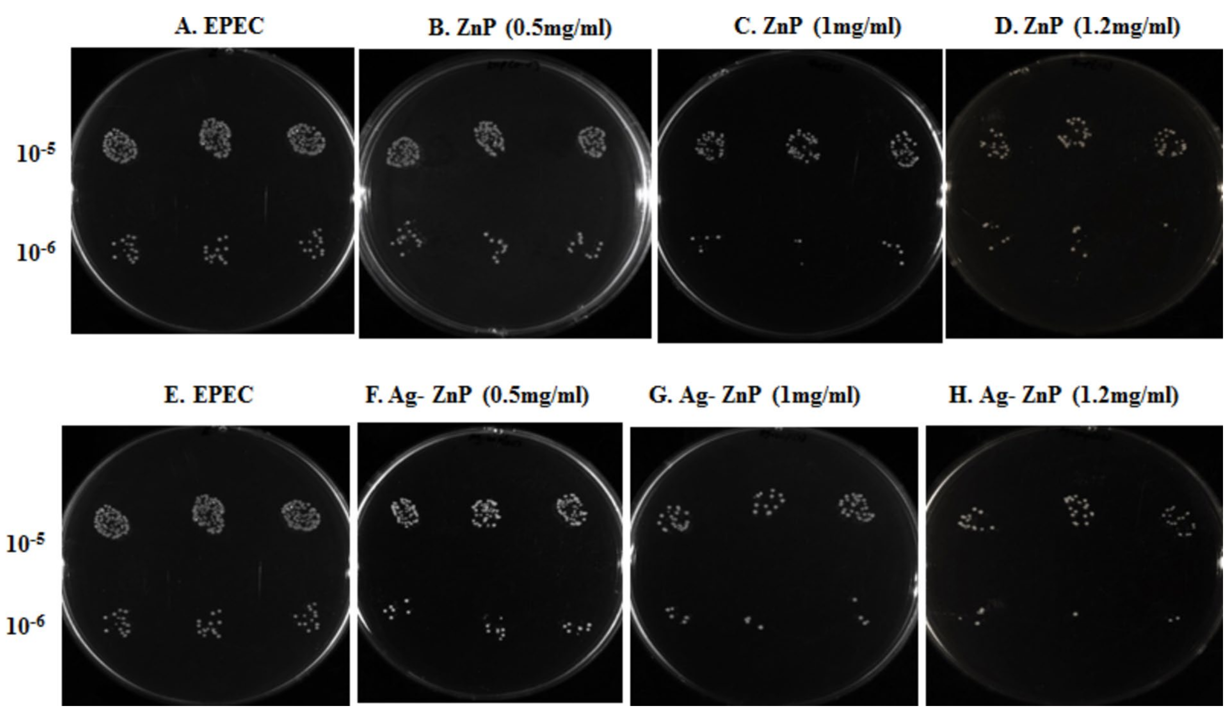


Fig. 10 In-vitro antimicrobial effect of ZnNPs and Zn-Ag BMNPs against the growth of pathogen was analysed using MIC assay. Significant inhibitory effect of different concentrations of ZnNPs and Zn-Ag BMNps against the growth of the pathogen E.coli. Statistical bar represents the mean ± SD for n = 3, One-way ANOVA ***P = 0.0006, **P = 0.0026,*P = 0.0111

explore synergistic effects that enhance physicochemical properties and antimicrobial activity. These hybrid nano composites demonstrate improved efficacy against *E-coli* bacteria strain, potentially revolutionizing treatment approaches for complex medical challenges. In our study, we successfully synthesized ZnNPs & Zn-Ag BMNCs using a biogenic approach with medicinal plant leaf extract serving as a multifunctional agent. The controlled green sysnthesis method employed as a reducing, capping, and stabilizing. Comprehensive characterization of the Zn-Ag BMNPs was performed using various analytical techniques. UV–Vis spectroscopy confirmed the formation of the nanocomposites through characteristic absorption peaks. XRD and FTIR analyses provided crucial insights into the crystalline structure and nano-structural details of the ZnNPs and Zn-Ag BMNPs. Notably, the plant leaf extract played a key role in facilitating the formation of complex BMNPs nanostructures. Our findings demonstrated that this biogenic synthesis approach significantly enhanced the antibacterial activity of the Zn-Ag BMNPs, highlighting the potential of this eco-friendly method for developing effective antimicrobial nanomaterials.

The antibacterial properties of ZnNPs and Zn-Ag BMNPs were evaluated against *E. coli*, using *Gentamicin* as a standard reference antibiotic. The results showed that Zn-Ag BMNPs at 2 mg/ml concentration had significantly higher antimicrobial activity over the ZnNPs against *E. coli* bacteria strain, highlighting Zn-Ag BMNPs as a potential antibacterial agent. These findings align with previous studies that demonstrate the enhanced physicochemical properties and increased antimicrobial efficacy of BMNPs[16]. The superior antibacterial activity of Zn-Ag BMNPs is attributed to the synergistic effects of incorporating different metal ions, which enhances their overall antimicrobial effectiveness.

To elucidate the potential mechanisms behind the enhanced antibacterial activity, Fig. 12 illustrates various possible interactions between Zn-Ag BMNPs material and *E. coli* strain. These mechanisms include: 1. Zn-Ag BMNPs adhering to cell and then disrupting & damage the bacterial cell membrane; 2. Infiltrating the cell membrane and deactivate the functioning cell activity; 3. Producing more reactive oxygen species (ROS) within the cell, leading to complete structural damage of cell; 4.Modulating the cellular signaling system, ultimately deactivating the cells functions and causing cell death.

Our study demonstrates the successful biosynthesis of Zn-Ag BMNPs using TC medicinal plant leaf extract as a multifunctional agent. This eco-friendly approach yielded high-quality nanoparticles with enhanced antibacterial properties. UV–Vis spectroscopy, XRD, and FTIR analyses confirmed the formation and characteristics of the Zn-Ag BMNPs. The plant extract's role in facilitating complex

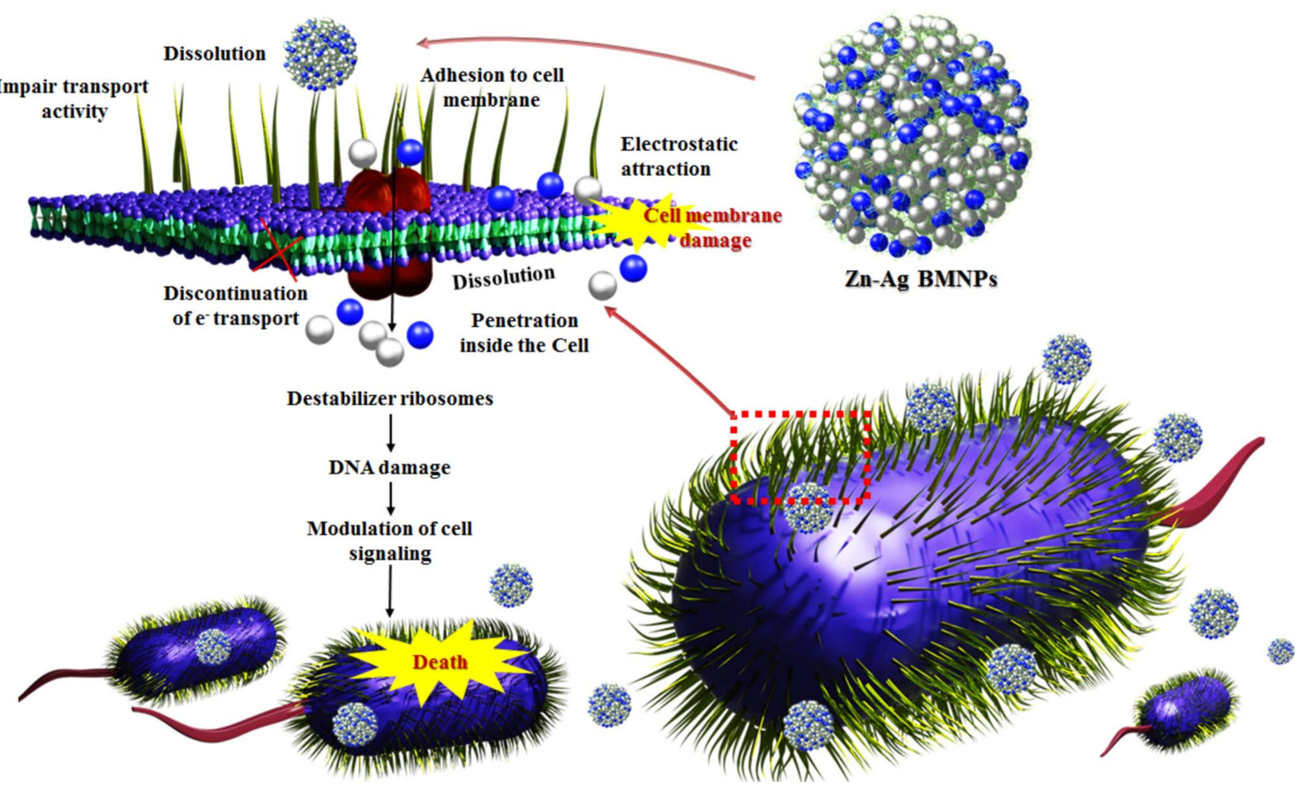


Fig. 12 The possible mechanism routes of antibacterial action of Zn-Ag BMNPs nanocomposites

nanostructure formation was evident and contributed to the improved antibacterial activity. Further research into molecular interactions and pathways could elucidate the BMNPs' mode of action against bacterial cells, potentially leading to more effective treatments for chronic infections. This work highlights the promise of biogenic BMNPs combined with phytochemicals as sustainable alternatives to conventional antibacterial agents.

The exponential growth of multi-drug resistant microbes and superbugs calls for safer alternatives to combat microbial infections compared to the tradition antibiotics[70–72], it is an urgent need for the potential antibacterial agents that are non-toxic to patients and do not promote microbial resistance. From mono to tri, and multi-metallic nanoparticles are being investigated for their potent antibacterial applications. Recent advances in the biogenic synthesis of both mono-metallic and bimetallic nanoparticles (BMNPs) have been recognized for their sustainability [73, 74]. The synthesized Zn-Ag BMNPs show potentaial antibacterial activity in treating chronic infections and microbial diseases, emphasizing the need for further in vitro and in vivo studies [65–68].

Our study adds valuable insights into the application of bimetallic nanocomposites for treating chronic infections, paving the way for advancements in nano-biotechnology. Future research should include a life cycle assessment to evaluate the environmental impact of Zn-Ag BMNPs, from production to disposal, and compare their performance with other nanoparticles regarding physicochemical properties, scalabilty, stability, and efficacy [60, 61]. Although scaling up production in a industrial scale, present industrial scal up challenges, optimizing extract consumption, metal composition concentrations, and synthesis time may address these issues.

5 Conclusion

This study highlights the successful green synthesis of Zn-Ag bimetallic nanoparticles using *Tinospora cordifolia* leaf extract for the first time as a dual role of biogenic reducing and stabilizing agent. The comprehensive analysis confirmed their stable structural, morphological, and functional characteristics. The Zn-Ag BMNPs exhibited enhanced antibacterial efficacy against *Escherichia coli*, outperforming their mono-metallic counterparts due to the synergistic effect of their bimetallic composition of Zn-Ag BMNPs. This eco-friendly synthesis process offers a sustainable solution to develop nanoparticles with superior therapeutic potential for treating microbial infections. By elucidating their optical characteristics and potential mechanisms of action, our study lays the basic study for broader investigations into their therapeutic potential in biomedical applications. Future studies can also investigate biological and environmental applications of these BMNPs, including studying their anticancer and antibacterial effects on in vivi animal models.

Acknowledgements The authors would like to thank the Department of Physics, Government City College (A), Osmania University, for providing the facilities necessary to carry out this work.

Author contributions Parvathalu Kalakonda: Supervision, writing-original draft and editing, visualization of the project. Greeshma Sunkari, Naveen Kumar Madipoju: Designing experiment, and Data collection, Sai Madhuri Vasamsetti & Vijay Morampudi: Designing experimental procedure and investigation antibacterial activity. Rami Nayudu Savu, Murali Banavath, Sarvani Jowhar Khanam, & Sreenivas Banne: Designing optical experiments and data interpretation. Note: All authors read and approved the final submission.

Funding No funding was received for this project.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interest.

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

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