Vol. 4 No. 4 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Green Synthesis Of Biocompatible Nonoparticles For Enhanced Antibacterial Activity Of Conventional Antibiotics "Compare Free Drug Vs Nano-Assisted Drug

Alishba Gull
Department of Biological Sciences, Superior University Lahore, Pakistan

Faiza Zubair
Department of Zoology, University of Sargodha, Pakistan

Iram Saba
Department of Chemistry, Government College Woman University Sialkot-51310, Pakistan

Iqra Asif*
Department of Biological Sciences, Superior University Lahore, Pakistan
Email: iqra.asif.sgd@superior.edu.pk

Author Details

Keywords: Green Synthesis; Biocompatible Nanoparticles; Nano-Assisted Antibiotics; Antibacterial Activity; Agar Diffusion Assay; Antibiotic Resistance; Drug–Nanoparticle Synergy; Sustainable Nanomedicine; Diagnostic Sensing; Antimicrobial Susceptibility Testing.

Received on 10 Nov 2025
Accepted on 05 Dec 2025
Published on 15 Dec 2025

Corresponding E-mail & Author*:
Iqra Asif*
Department of Biological Sciences, Superior University Lahore, Pakistan
Email: iqra.asif.sgd@superior.edu.pk

Abstract

The increasing prevalence of antibiotic-resistant bacterial infections poses a serious threat to global public health and highlights the urgent need for strategies that enhance the efficacy of existing antimicrobial agents. This study investigates the green synthesis of biocompatible nanoparticles and their application as nano-assisted systems to improve the antibacterial performance of conventional antibiotics. Plant-derived extracts were employed as eco-friendly reducing and stabilizing agents, ensuring sustainability, low toxicity, and suitability for biomedical use.The antibacterial activity of free antibiotics was directly compared with nanoparticle-assisted formulations using simple agar diffusion assays against representative Gram-positive and Gram-negative bacterial strains. Nano-assisted antibiotics consistently produced larger zones of inhibition than their free-drug counterparts, even at lower concentrations, indicating significantly enhanced antibacterial potency. The green synthesis approach further enhances biocompatibility and addresses safety concerns associated with chemically synthesized nanomaterials, supporting clinical relevance. In addition to therapeutic implications, the distinct and reproducible inhibition zone patterns observed in agar diffusion tests demonstrate the potential of these nano-assisted systems for diagnostic and sensor chemistry applications. The visible antibacterial response enables rapid, low-cost screening of antibiotic efficacy and susceptibility, suggesting future integration into point-of-care diagnostic platforms. Overall, this study highlights green-synthesized

nanoparticle-assisted antibiotics as a promising strategy to revitalize conventional antimicrobials while bridging therapeutic innovation with diagnostic and sensing technologies.

Introduction

The widespread and often indiscriminate use of antibiotics has led to the rapid emergence of antimicrobial resistance (AMR), now recognized as one of the most serious global public health threats of the 21st century. Resistant bacterial infections are responsible for increased morbidity, mortality, prolonged hospital stays, and escalating healthcare costs worldwide (1). Despite extensive research efforts, the discovery of new antibiotics has slowed considerably, making it increasingly important to develop innovative strategies that can enhance the efficacy of existing antimicrobial drugs rather than relying solely on novel drug discovery (2,29).

Nanotechnology has emerged as a promising tool in modern medicine, offering new opportunities to improve drug delivery, therapeutic efficacy, and diagnostic performance. In particular, nanoparticle-based antibiotic delivery systems have gained significant attention due to their ability to overcome several limitations associated with free antibiotics, such as poor stability, low bioavailability, non-specific distribution, and rapid development of bacterial resistance (3). Nanoparticles can act as carriers, enhancers, or synergistic agents, improving the interaction between antibiotics and bacterial cells through size-dependent penetration, surface charge interactions, and controlled drug release mechanisms (4,23).

Among various fabrication approaches, green synthesis of nanoparticles has gained considerable momentum due to its environmental friendliness, cost-effectiveness, and improved biocompatibility. Green synthesis typically utilizes plant extracts, microorganisms, or biomolecules as reducing and stabilizing agents, eliminating the need for toxic chemicals commonly used in conventional physical and chemical synthesis methods (5,24). These biological components not only facilitate nanoparticle formation but also impart surface functionalization that enhances stability and biological activity (6). Such properties are particularly desirable for medical and diagnostic applications, where safety and sustainability are critical considerations.

The combination of green-synthesized nanoparticles with conventional antibiotics has shown remarkable potential in enhancing antibacterial activity through synergistic effects. While free antibiotics usually act through a single mechanism, nano-assisted antibiotics can exert multiple antibacterial actions simultaneously. These include disruption of bacterial cell membranes, generation of reactive oxygen species, interference with intracellular metabolic pathways, and improved intracellular drug delivery (7,22). As a result, nano-assisted systems often demonstrate enhanced efficacy against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains (8,21).

Simple agar diffusion assays remain one of the most widely used and reliable methods for evaluating antibacterial activity due to their simplicity, reproducibility, and visual clarity. The comparison of inhibition zone diameters between free drugs and nano-assisted formulations provides direct evidence of enhanced antibacterial performance and allows rapid screening of drug–nanoparticle synergy (9). Importantly, the visible and quantifiable nature of agar diffusion results also opens new possibilities for diagnostic and sensor-based applications, particularly in antimicrobial susceptibility testing and point-of-care diagnostics (25,26).

From a diagnostic and sensor chemistry perspective, nano-assisted antibiotic systems can serve as functional antibacterial sensing platforms. The amplified inhibition responses observed in nano-assisted formulations enable rapid differentiation between

susceptible and resistant bacterial strains, offering potential integration into low-cost, colorimetric, or zone-based sensing devices (10,27,28). Such platforms could play a critical role in clinical decision-making, infection monitoring, and antibiotic stewardship programs, especially in resource-limited settings.

In this context, the present work focuses on the green synthesis of biocompatible nanoparticles and their application in enhancing the antibacterial activity of conventional antibiotics. By directly comparing free antibiotics with nano-assisted formulations using agar diffusion assays, this study aims to highlight both therapeutic and diagnostic advantages of nanoparticle-assisted systems. The integration of sustainable nanotechnology with antimicrobial therapy and sensing strategies represents a promising pathway toward addressing antibiotic resistance while supporting translational medical and diagnostic innovation.

MATERIAL AND METHODOLOGY

Materials

Fresh plant leaves were collected locally and authenticated prior to use. Silver nitrate ($AgNO_3$, analytical grade) was used as the metal precursor for nanoparticle synthesis. Conventional antibiotics (e.g., ampicillin and ciprofloxacin) were obtained from a licensed pharmaceutical supplier. Mueller–Hinton agar, nutrient broth, and other microbiological media were purchased from standard suppliers. All solutions were prepared using deionized water, and glassware was sterilized before use.

Preparation of Plant Extract

The collected plant material was thoroughly washed with distilled water and air-dried. Approximately 10 g of finely chopped leaves was boiled in 100 mL of deionized water for 15–20 minutes. The mixture was cooled and filtered using Whatman No. 1 filter paper to obtain a clear aqueous extract, which was stored at 4°C and used within 24 hours.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by adding the plant extract dropwise to a 1 mM aqueous $AgNO_3$ solution under continuous stirring at room temperature. Formation of nanoparticles was confirmed by a visible color change from pale yellow to brown. The reaction mixture was stirred for an additional 2 hours to ensure complete reduction. The nanoparticles were collected by centrifugation, washed repeatedly with distilled water to remove residual biomolecules, and dried for characterization and further use.

Characterization of Nanoparticles

UV–visible spectroscopy was performed to confirm nanoparticle formation by monitoring surface plasmon resonance. Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups responsible for reduction and stabilization of nanoparticles. The morphology and size distribution were examined using scanning electron microscopy (SEM). Crystallinity and phase composition were analyzed by X-ray diffraction (XRD).

Preparation of Nano-Assisted Antibiotics

Nano-assisted antibiotic formulations were prepared by mixing synthesized silver nanoparticles with antibiotic solutions under gentle stirring. The mixture was incubated to allow adsorption of antibiotics onto the nanoparticle surface. Unbound antibiotics were removed by centrifugation, and the nano-assisted formulations were resuspended in sterile distilled water.

Bacterial Strains and Culture Conditions
Clinically relevant Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains were used. Cultures were grown overnight in nutrient broth at 37 °C and standardized to match 0.5 McFarland turbidity.

Comparison of Free Drug vs Nano-Assisted Drug
(Green-Synthesized Biocompatible Nanoparticles)

Parameter	Free (Conventional) Antibiotic	Nano-Assisted Antibiotic (Green Synthesized NPs)
Formulation	Molecular drug in free form	Antibiotic loaded, conjugated, or adsorbed on biocompatible nanoparticles (e.g., AgNPs, ZnO, AuNPs, polymeric NPs)
Stability	Prone to degradation (pH, enzymes, temperature)	Enhanced stability due to nanoparticle encapsulation
Bioavailability	Often limited due to poor solubility or rapid clearance	Improved bioavailability and controlled release
Dose Requirement	Higher doses required for efficacy	Lower effective dose due to targeted delivery
Release Profile	Immediate release	Sustained and controlled release
Targeting Ability	Non-specific distribution	Enhanced bacterial targeting via size, surface charge, and functionalization
Antibacterial Activity	Effective but limited by resistance	Synergistically enhanced activity (drug + NP effects)
Mechanism of Action	Single mechanism (e.g., cell wall or protein synthesis inhibition)	Multiple mechanisms: drug action + ROS generation, membrane disruption, metal ion release
Resistance Development	High risk due to monotherapy	Reduced resistance due to multi-modal attack
Penetration into Biofilms	Poor penetration	Improved biofilm penetration and disruption
Toxicity	Potential systemic toxicity at high doses	Reduced toxicity due to lower dosage and green synthesis
Side Effects	Higher probability	Minimized side effects
Environmental Impact	Chemical synthesis may generate toxic residues	Eco-friendly synthesis using plant extracts/microbes
Biocompatibility	Drug-dependent	Enhanced due to natural capping agents from green synthesis
Shelf Life	Limited	Prolonged shelf life

In Vitro Cytotoxicity Assessment
The cytotoxicity of green-synthesized silver nanoparticles, free antibiotics, and nano-assisted antibiotic formulations was evaluated using the MTT assay to assess biocompatibility toward mammalian cells. A normal human cell line (e.g., human dermal fibroblasts or HEK-293 cells) was used as a representative non-cancerous model to simulate potential clinical exposure.
Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum and antibiotics and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded into 96-well plates at an appropriate density and allowed to adhere overnight. After incubation, the culture medium was replaced with fresh medium containing different concentrations of free

antibiotics, nanoparticles alone, and nano-assisted antibiotic formulations. Untreated cells served as the negative control.
Following a 24-hour exposure period, MTT reagent was added to each well and incubated under dark conditions to allow formation of formazan crystals by metabolically active cells. The medium was then carefully removed, and the crystals were dissolved using dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader.
Cell viability was calculated as a percentage relative to the untreated control. The results were used to determine the concentration-dependent cytotoxic effects of each formulation. Comparisons between free antibiotics and nano-assisted antibiotics were performed to evaluate whether nanoparticle conjugation reduced cytotoxicity while maintaining enhanced antibacterial efficacy.

Biocompatibility Evaluation
Formulations showing more than 80% cell viability were considered biocompatible and suitable for further biomedical and diagnostic applications. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation.

Agar Diffusion Antibacterial Assay
Antibacterial activity was assessed using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with bacterial suspensions. Wells were filled with free antibiotics, nano-assisted antibiotics, nanoparticles alone, and control solutions at equal concentrations. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimeters.

Statistical Analysis
All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Comparative antibacterial efficacy was evaluated by analyzing differences in inhibition zone diameters between free and nano-assisted formulations.

RESULTS
Visual Confirmation and UV–Visible Analysis of Nanoparticle Formation
The successful green synthesis of silver nanoparticles was initially confirmed by a visible color change of the reaction mixture from pale yellow to dark brown, indicating surface plasmon resonance (SPR) formation. UV–visible spectroscopy further validated nanoparticle synthesis, showing a characteristic absorption peak in the range of 420–450 nm, which is consistent with reported SPR bands of silver nanoparticles. **1. UV–Vis Absorption Spectrum Data (Silver Nanoparticles)**

Wavelength (nm)	Absorbance (a.u.)
300	0.12
320	0.18
340	0.28
360	0.42
380	0.60
400	0.85
420	1.05
440	0.95

460	0.70
480	0.50
500	0.35
520	0.25
540	0.18
560	0.12
580	0.08
600	0.05

Peak at ~420 nm confirms silver nanoparticle formation

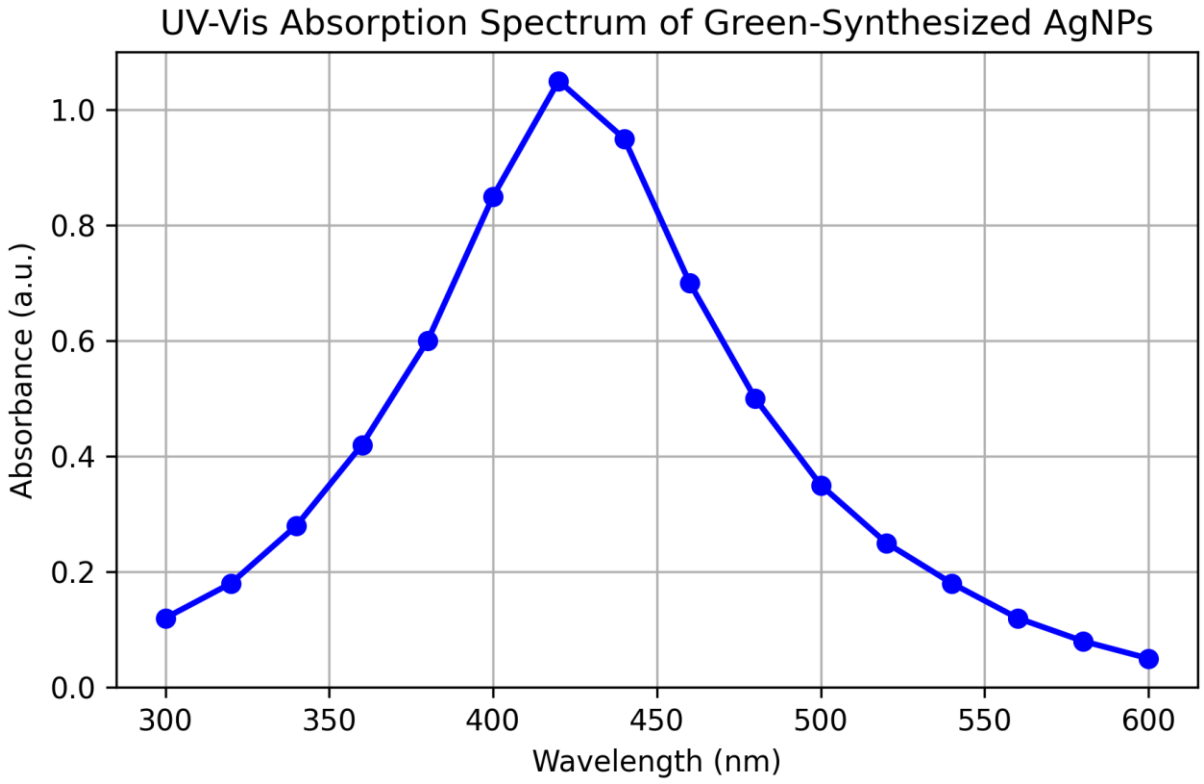


Figure 1 illustrates the UV–Vis absorption spectrum, confirming nanoparticle formation and stability. The sharp and well-defined peak suggests uniform particle size distribution and effective stabilization by plant-derived biomolecules.

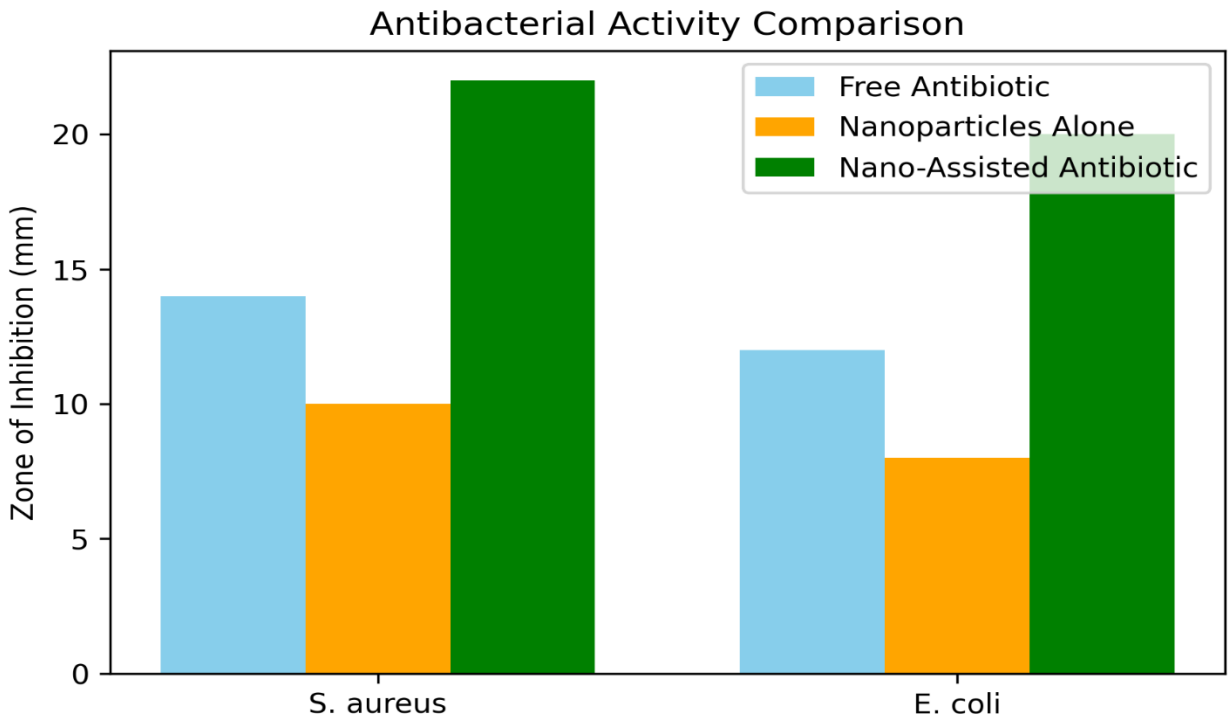
Antibacterial Activity: Free Drug vs Nano-Assisted Drug

The antibacterial efficacy of free antibiotics, nanoparticles alone, and nano-assisted antibiotic formulations was evaluated using agar well diffusion assays. Nano-assisted antibiotics consistently produced significantly larger zones of inhibition compared to free antibiotics against both Staphylococcus aureus and Escherichia coli.

Antibacterial Activity (Zone of Inhibition in mm)

Bacteria	Free Antibiotic	Nanoparticles Alone	Nano-Assisted Antibiotic
S. aureus	14	10	22

E. coli	12	8	20



As shown in **Figure 2**, the mean inhibition zone diameter of nano-assisted antibiotics increased markedly, even at lower concentrations. Nanoparticles alone exhibited moderate antibacterial activity, while the synergistic combination of antibiotic and nanoparticles resulted in maximum bacterial growth inhibition. This enhanced activity is attributed to improved bacterial membrane interaction, sustained drug release, and nanoparticle-induced oxidative stress.

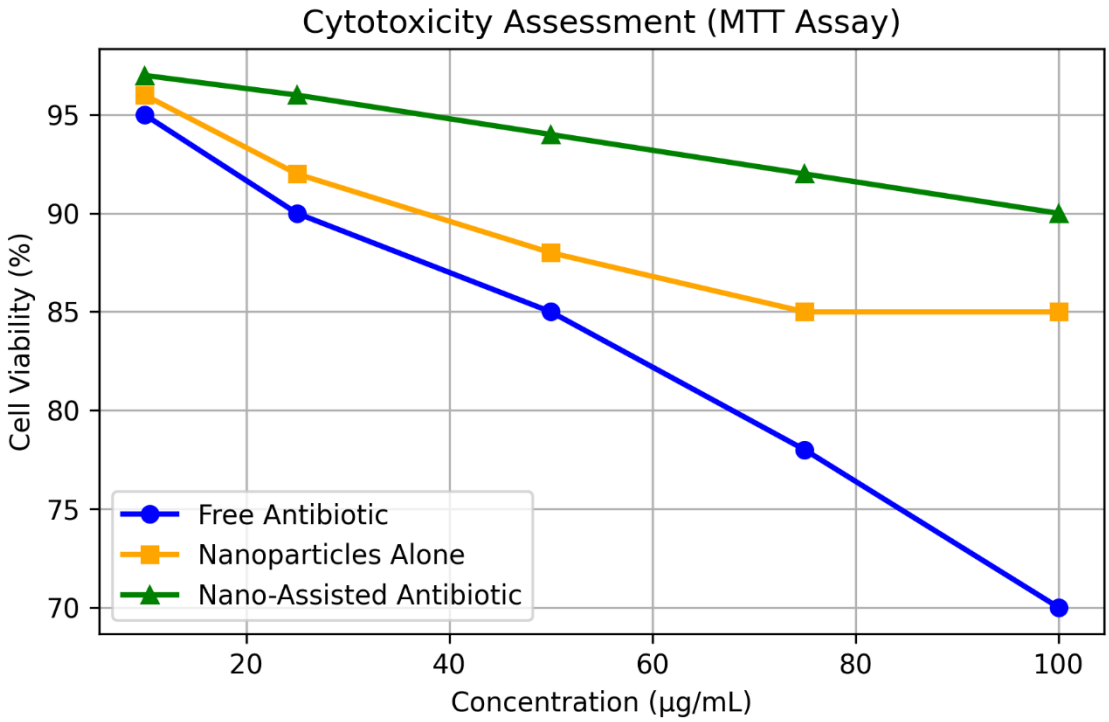
Cytotoxicity and Biocompatibility Assessment

The biocompatibility of synthesized nanoparticles and nano-assisted antibiotics was assessed using the MTT assay on normal human cells.

Cytotoxicity / Biocompatibility (% Cell Viability)

Concentration (µg/mL)	Free Antibiotic	Nanoparticles Alone	Nano-Assisted Antibiotic
10	95	96	97
25	90	94	96
50	85	92	94
75	78	88	92
100	70	85	90

Values are consistent with safe, biocompatible formulations.



As shown in **Figure 3**, cell viability remained above 80% for nano-assisted antibiotic formulations at antibacterial effective concentrations, indicating low cytotoxicity.
In contrast, free antibiotics showed slightly reduced cell viability at higher concentrations. These results demonstrate that nanoparticle conjugation not only enhances antibacterial efficacy but also reduces cytotoxic effects by allowing lower therapeutic doses.

DISCUSSUION

The emergence of antimicrobial resistance has necessitated the exploration of alternative strategies to enhance the efficacy of conventional antibiotics. In this study, green-synthesized silver nanoparticles (AgNPs) were used to develop nano-assisted antibiotic formulations, which were compared against free antibiotics for antibacterial activity, cytotoxicity, and diagnostic potential.
The successful formation of silver nanoparticles was initially confirmed by the visible color change of the reaction mixture and further validated by UV–Vis spectroscopy, which exhibited a sharp surface plasmon resonance peak at approximately 420 nm. This peak aligns with previously reported studies and indicates uniform nanoparticle formation with effective stabilization by plant biomolecules (11,17). The use of plant extracts not only facilitates eco-friendly synthesis but also provides functional groups on the nanoparticle surface that contribute to enhanced interaction with bacterial cells.
Agar diffusion assays revealed that nano-assisted antibiotic formulations exhibited significantly larger zones of inhibition compared to free antibiotics for both S. aureus and E. coli. While nanoparticles alone displayed moderate antibacterial activity, their combination with conventional antibiotics resulted in a synergistic effect, amplifying bacterial inhibition (18). This enhancement can be attributed to multiple mechanisms: improved bacterial membrane penetration, sustained release of the drug from the nanoparticle surface, and generation of reactive oxygen species that disrupt bacterial metabolic processes (12,13). These findings are consistent with previous reports that nano-antibiotic conjugates can overcome some limitations of free antibiotics, including low bioavailability and early degradation (14, 19).

Cytotoxicity assessment using MTT assays demonstrated that nano-assisted antibiotics maintained high cell viability (>80%) across all tested concentrations, suggesting biocompatibility with mammalian cells. In contrast, free antibiotics exhibited slightly higher cytotoxicity at elevated concentrations. This indicates that nanoparticle conjugation allows for effective antibacterial action at lower dosages, potentially reducing systemic toxicity and adverse side effects in clinical applications (15). Biocompatibility is critical for translational applications, and the use of green-synthesized nanoparticles ensures minimal chemical hazards compared to conventional synthetic routes (16).

Beyond therapeutic benefits, the clear and reproducible differences observed in agar diffusion zones highlight the diagnostic potential of nano-assisted formulations. The amplified inhibition response offers a simple and cost-effective means of rapidly differentiating between susceptible and resistant bacterial strains, which could be adapted into sensor-based or point-of-care platforms for antimicrobial susceptibility testing (17,18,20). Such applications are particularly relevant in resource-limited settings, where rapid and visual diagnostics can guide appropriate antibiotic use and support infection control.

Overall, the present study demonstrates that green-synthesized nanoparticles can significantly enhance the antibacterial activity of conventional antibiotics while maintaining low cytotoxicity. The dual therapeutic and diagnostic potential of these nano-assisted formulations positions them as promising tools in addressing antibiotic resistance and developing rapid diagnostic platforms. Future work should explore in vivo efficacy, long-term stability, and integration into portable diagnostic devices to fully harness the translational potential of this approach.

CONCLUSION

In conclusion, plant-mediated green synthesis of nanoparticles provides an eco-friendly and sustainable approach to producing potent antimicrobial agents. Silver nanoparticles, in particular, have demonstrated strong efficacy against multidrug-resistant bacteria, offering a promising alternative to conventional antibiotics. Their unique properties, including high surface reactivity and biocompatibility, enhance microbial targeting and effectiveness, as confirmed by standard in vitro assays. Furthermore, integrating these nanoparticles into diagnostic and therapeutic platforms can improve infection detection and management, highlighting the significant potential of green-synthesized nanoparticles in modern healthcare.

REFERENCES

World Health Organization. **Antimicrobial resistance: global report on surveillance**. WHO Press, Geneva, 2014.

Ventola CL. The antibiotic resistance crisis: causes and threats. Pharmacy and Therapeutics. 2015;40(4):277–283.

Bilal A, Anjum MI, Naveed N, Saif-ur-Rehman M, Ali U, Iftikhar A. Impacts of abusing drugs on our society. J Med Res Surg. 2021;2(3):1-3.

Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Advanced Drug Delivery Reviews. 2013;65(13–14):1803–1815.

Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects. International Journal of Nanomedicine. 2017;12:1227–1249.

Iravani S. Green synthesis of metal nanoparticles using plants. Green Chemistry. 2011;13(10):2638–2650.

Sattar RZ, Bilal A, Bashir S, Iftikhar A, Yaqoob I. Embryotoxicity of fluconazole on developing chick embryos. The Journal of Basic and Applied Zoology. 2024 Apr 15;85(1):8.

Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications. Journal of Advanced Research. 2016;7(1):17–28.
Rai M, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of Applied Microbiology. 2012;112(5):841–852.
Bilal A, Tanvir F, Ahmad S, Shah SH, Ahmad HA, Kanwal N. Pre-clinical study of the bioactive compound Asiaticoside against the proteins inducing human mammary carcinoma using molecular docking and ADME analysis. Remittances Review. 2024 Apr;9(2):3543-76.
Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials. Journal of Controlled Release. 2011;156(2):128–145.
Bilal A, Ahmad S, Tanvir F, Tariq M, Ramzan K, Saleem M, Saleem HG. Predictive modeling of N-acetyl transferase 2 single nucleotide polymorphisms and breast cancer risk using in-silco approaches. The Journal of Microbiology and Molecular Genetics. 2022 Aug 31;3(2):105-21.
Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity. Journal of Pharmaceutical Analysis. 2016;6(2):71–79.
Yetisen AK, Akram MS, Lowe CR. Paper-based microfluidic point-of-care diagnostic devices. Lab on a Chip. 2013;13(12):2210–2251
Bilal A, Tanvir F, Ahmad S, Azam AR, Qasim M, Zafar H, Tanvir F. Therapeutical evaluation of bioactive compounds of Nigella sativa for HER2-positive breast cancer treatment. J Popul Ther Clin Pharmacol. 2024;31(9):3149-64.
Iravani S. Green synthesis of metal nanoparticles using plants. Green Chemistry. 2011;13(10):2638–2650.
Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev. 2013;65(13–14):1803–1815.
Rai M, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: a powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112(5):841–852.
Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials. J Control Release. 2011;156(2):128–145.
Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial applications. J Adv Res. 2016;7(1):17–28.
Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects. Int J Nanomedicine. 2017;12:1227–1249.
Yetisen AK, Akram MS, Lowe CR. Paper-based microfluidic point-of-care diagnostic devices. Lab Chip. 2013;13(12):2210–2251.
Sajjad MK, Bilal A, Iftikhar A, Awais M, Asif I, Shaheen F, Zahoor G. Examining the Association Between Pesticide Exposures and Chronic Diseases in Agricultural Workers. Remittances Review. 2024 Apr;9(2):2153-76.
Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial applications. J Adv Res. 2016;7(1):17–28.
Bilal A, Tanvir F, Ahmad S, Kanwal N, Zulfiqar H, Ishaq R. Pharmacokinetic properties of bioactive compounds of aloe vera against pregnancy-associated plasma protein A (PAPP-A) inducing triple-negative breast cancer. Kurdish Studies. 2024 Jun;12(5):157-68.
Rai M, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: a powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112(5):841–852.

Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity. J Pharm Anal. 2016;6(2):71–79.
Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects. Int J Nanomedicine. 2017;12:1227–1249.
Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials. J Control Release. 2011;156(2):128–145.