RESEARCH ARTICLE

Biogenic Synthesis and Characterization of Bimetallic CuO-ZnO Nanoparticles from Plant Extracts of *Ziziphus mauritiana*

Sarvaree Bano[1], Manisha Agrawal[2], Dharm Pal[3], Virendra Kumar Vaishnav[4*]

DOI: 10.18811/ijpen.v11i03.14

Abstract

The Ber plant, also known as *Ziziphus mauritiana*, is a medium-sized tropical fruit tree native to India and belongs to the Rhamnaceae family. The pharmacological and nutritional potential of *Z. mauritiana* has been extensively studied. It is rich in various minerals, including iron, copper, zinc, potassium, calcium, and magnesium. The edible parts of this plant are high in vitamin A, B-complex vitamins, and ascorbic acid (vitamin C). Several studies have reported that different parts of the plant exhibit anti-inflammatory, anti-allergic, and anticancer activities. Additionally, it has been investigated for its pharmacological effects in treating liver disorders, diabetes, tuberculosis, wound healing, asthma, and blood-related diseases. Unlike other parts of the plant, the leaves have been widely used by nanotechnologists as reducing and capping agents in the synthesis of nanoparticles. In this study, bimetallic copper-zinc nanoparticles were synthesized using the leaves of *Z. mauritiana*. The presence of bioactive compounds with antibacterial activity against pathogenic bacteria was confirmed. Copper and zinc nanoparticles are commonly used in food products as additives and in packaging due to their recognized safety. The formation of bimetallic Cu-Zn nanoparticles was confirmed through UV-visible spectroscopy and a simple visual color change. This is the first report on the synthesis of Cu-Zn bimetallic nanoparticles from *Z. mauritiana* leaves. Bimetallic nanoparticles are in greater demand than monometallic ones due to their enhanced antimicrobial activity and superior optical and magnetic properties.

Keywords: Antimicrobial, Phytochemical, Zone of inhibition, *Ziziphus mauritiana* Nanoparticles.

Highlights

- Green synthesis of CuO-ZnO bimetallic nanoparticles using *Ziziphus mauritiana* leaf extract.
- Eco-friendly and cost-effective approach to nanoparticle fabrication.
- Characterization confirms the formation, morphology, and crystallinity of CuO-ZnO NPs.
- Potential applications in the antimicrobial area against S. aureus, B. subtilis, E. coli, K. pneumoniae, P. aeruginosa

International Journal of Plant and Environment (2025); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Nanotechnology has emerged as a transformative force across various scientific disciplines, offering innovative solutions in materials science, electronics, catalysis, energy, and especially in biomedical and environmental applications (Ashraf *et al*., 2015; Abalaka *et al*., 2010). Among its various subfields, nanoparticle synthesis and engineering represent a significant area of interest due to the unique physical, chemical, and biological properties exhibited at the nanoscale. Nanoparticles, particularly metal and metal oxide nanoparticles, exhibit novel characteristics that differ markedly from their bulk counterparts, including enhanced surface area-to-volume ratio, quantum effects, and increased catalytic potential (Iravani, 2011).

Traditional methods of synthesizing nanoparticles, including physical and chemical approaches, often require high energy input and involve hazardous chemicals, leading to environmental concerns and limiting their biocompatibility and applicability in medical and ecological settings. To address these challenges, green synthesis methods have been developed as environmentally benign alternatives. Biogenic synthesis, in particular, utilizes natural reducing and capping agents present in biological organisms such as bacteria, fungi, algae, and especially plants (Kharissova *et al*., 2013). Plant-mediated synthesis offers advantages in simplicity, scalability, cost-effectiveness, and eco-friendliness. Additionally, plant extracts are rich in diverse phytochemicals—flavonoids, alkaloids, terpenoids, phenolics, and proteins—that function synergistically in reducing metal ions and stabilizing the resultant nanoparticles (Mittal *et al*., 2013).

In recent years, there has been growing interest in synthesizing bimetallic nanoparticles, which integrate two different metal components to produce hybrid nanomaterials with synergistic properties superior to their monometallic

[1]Department of Chemistry, Rungta College of Engineering & Technology, Bhilai-490024, Chhattisgarh, India,

[2]Department of Chemistry, Krishna's Vikash Institute of Technology, Raipur-492099, India

[3]Department of Chemical Engineering, National Institute of Technology Raipur, Raipur-492 010, Chhattisgarh, India

[4]Raipur Institute of Technology, Raipur, Chhattisgarh, India

***Corresponding author:** Virendra Kumar Vaishnav, Raipur Institute of Technology, Raipur, Chhattisgarh, India, Email: virendravaishnav7@gmail.com

How to cite this article: Bano, S., Agrawal, M., Pal, D.,Vaishnav, V. K. (2025). Biogenic Synthesis and Characterization of Bimetallic CuO-ZnO Nanoparticles from Plant Extracts of *Ziziphus mauritiana*. International Journal of Plant and Environment. 11(3), 566-574.

Submitted: 27/06/2025 **Accepted:** 23/7/2025 **Published:** 30/09/2025

counterparts. Bimetallic nanoparticles often demonstrate enhanced optical, catalytic, and antimicrobial properties due to interactions at the atomic level, such as alloying or core–shell structuring (Sánchez & Zhang, 2012). Among various bimetallic systems, copper oxide (CuO) and zinc oxide (ZnO) composites have gained considerable attention. CuO is a p-type semiconductor with excellent photothermal, catalytic, and antimicrobial properties, while ZnO is an n-type semiconductor renowned for its photocatalytic activity, UV absorption, and biocompatibility (Moezzi *et al*., 2012). The combination of CuO and ZnO in bimetallic nanoparticles offers enhanced functional performance, making them suitable for diverse applications, including biosensing, photocatalysis, wastewater treatment, and drug delivery (Padalia *et al*., 2015).

The current study focuses on the biogenic synthesis of CuO-ZnO bimetallic nanoparticles using aqueous leaf extracts of *Ziziphus mauritiana*, an underexplored but pharmaceutically significant medicinal plant. *Z. mauritiana*, commonly known as Indian jujube or "Ber," is a fast-growing plant widely distributed in arid and semi-arid regions of Asia and Africa. Traditionally, it has been utilized in ethnomedicine for treating various ailments such as fever, ulcers, inflammation, wounds, and microbial infections. Phytochemical investigations have revealed that *Z. mauritiana* leaves contain a rich repertoire of bioactive compounds, including saponins, tannins, flavonoids, and triterpenoids, all of which possess antioxidant and antimicrobial properties (Sharma *et al*., 2010). These bioactives are crucial not only for the biological activity of the synthesized nanoparticles but also for facilitating the reduction and stabilization processes during nanoparticle formation.

Previous research has demonstrated the potential of *Z. mauritiana* in the green synthesis of monometallic nanoparticles such as silver and gold, showing promising antibacterial and antioxidant properties (Ibrahim, 2015). However, there is a significant research gap in exploiting this plant extract for the synthesis of bimetallic nanomaterials, particularly CuO-ZnO nanoparticles (Upadhyay *et al*., 2025; Chandrakar *et al*., 2023; Chauhan *et al*., 2019). The novelty of this study lies in using *Z. mauritiana* leaf extract for the first time biogenic synthesis of CuO-ZnO bimetallic nanoparticles, accompanied by comprehensive characterization and evaluation of their physicochemical and potential biological properties (Dwivedi *et al., 2024;* Chaudhari and Dwivedi, 2023; Pansambal *et al*., 2019).

The synthesis process adopted in this study is straightforward and scalable, involving the mixing of copper and zinc precursor salts with the plant extract under controlled conditions. The color change of the reaction mixture and pH modulation were preliminary indicators of nanoparticle formation. The synthesized nanoparticles were then subjected to detailed characterization using various analytical techniques: UV–vis spectroscopy to monitor optical properties, Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups involved in stabilization, and X-ray diffraction (XRD) for crystallographic structure.

In addition to physicochemical characterization, this study also aims to explore the antimicrobial efficacy of the synthesized CuO-ZnO nanoparticles against pathogenic bacterial strains (Datta *et al*., 2012; El-Sayyad, 2024; Al-Habeeb and Al-Bishri, 2024). The antimicrobial mechanism of metal oxide nanoparticles is multifactorial, involving reactive oxygen species (ROS) generation, cell membrane disruption, and interference with microbial metabolic pathways (Raghunath & Perumal, 2017). The incorporation of bioactive compounds from *Z. mauritiana* may further enhance these effects, leading to synergistic antimicrobial activity. Such findings are of immense relevance in the context of growing antibiotic resistance and the search for novel antimicrobial agents from natural sources.

Furthermore, the study contributes to the growing body of sustainable nanotechnology by promoting the use of renewable biological resources for nanoparticle synthesis. The valorization of *Z. mauritiana*, an easily accessible and resilient plant species, highlights the potential for rural and low-resource settings to adopt green nanotechnological practices. This approach aligns with the principles of green chemistry, which emphasize the minimization of hazardous substances and the use of renewable feedstocks (Anastas & Warner, 1998).

The present research proposes an innovative, eco-friendly method for the synthesis of CuO-ZnO bimetallic nanoparticles using *Z. mauritiana* leaf extract. It addresses the dual objectives of green synthesis and functional enhancement, combining the advantages of bimetallic nanotechnology with the therapeutic potential of plant-based bio compounds. The study is expected to open new avenues in the development of sustainable nanomaterials with applications in medicine, environmental remediation, and material science.

Materials and Methods

Chemicals

Nanoparticles were synthesized using a green synthesis route, which requires a minimal amount of chemicals. During the experiment, triple-distilled water was used. The chemicals employed were E. Merck A.R. grade copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) and zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), both with 99% purity, along with absolute alcohol (95%) from LOBA Chemie.

Plant Collection

Fresh *Z. mauritiana* leaves were collected in cotton bags from the Sarona region of Raipur, Chhattisgarh, India. The collected plant material was authenticated and then processed for analysis. To ensure cleanliness and eliminate surface contaminants such as dust and debris, the leaves were thoroughly washed two to three times using running tap water. This preparatory step is essential to maintain the integrity of phytochemical and biological analyses by minimizing contamination (Sasidharan *et al*., 2011). The cleaned leaves were then air-dried at room temperature for further experimental procedures.

Preparation of Plant Leaf Extract

Z. mauritiana leaves were carefully cleaned with tap water, followed by washing with deionized water. Afterward, they were dried in an oven set between 50 and 65°C until all moisture was removed. The dried leaves were then crushed into a fine powder. Ten grams of this powder were soaked in 100 mL of deionized water and heated to 70 to 80°C for half an hour. According to Shah *et al*., (2018), with a few minor modifications, the extract

Table 1: Phytochemicals analysis of Z. mauritiana extract

S. N.	*Phytochemical*	*Test*
1	Alkaloids	2 mL of HCl was added to 5 mL of plant extract. Dragendroff's reagent (1-mL) was then added.
2	Flavonoids	10% lead acetate was added to 1-mL of the plant extract.
3	Polyphenols	1-mL of plant extract was mixed with a few drops of a 5% lead acetate solution.
4	Phenols	3 mL of a 10% lead acetate solution was added to 5 mL of plant extract and gently stirred.
5	Tannins	A few drops of a neutral 5% ferric chloride solution were added to 5 mL of plant extract.
6	Amino acids	1-mL of the plant extract was mixed with a few drops of the ninhydrin reagent (10 mg of ninhydrin in 200 mL of acetone). A few drops of nitic acid were added to 2 mL of plant extract.

was then filtered through Whatman filter paper No. 1 and stored at 4°C until it was used for nanoparticle synthesis within a week.

Phytochemical Analysis of *Z. mauritiana* Extract

Phytochemical screening of the aqueous leaf extracts of *Z. mauritiana* was conducted to identify the presence of various bioactive constituents using standardized procedures as outlined by Balamurugan *et al*., (2019). This qualitative analysis aimed to detect primary and secondary metabolites, including alkaloids, flavonoids, polyphenols, phenols, tannins, amino acids, and other phytochemicals that contribute to the medicinal potential of the plant. These compounds are known to possess a wide range of pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and cytoprotective properties, which support the traditional uses of *Z. mauritiana* in folk medicine. Each class of phytochemical was tested using specific reagents and protocols described in the referenced method to ensure accuracy and reproducibility. The outcomes of this screening provide a scientific basis for further pharmacological studies and are summarized in Table 1. Such analyses are essential for establishing the therapeutic relevance of medicinal plants and guiding future drug discovery efforts.

Preparation of bimetallic Cu-Zn nanoparticles solution

Approximately 20 mM of $CuSO_4{\cdot}5H_2O$ and 20 mM of $ZnSO_4{\cdot}7H_2O$ were dissolved in 90 mL of deionized water, and the mixture was allowed to equilibrate at room temperature in a water bath until a homogeneous solution was formed. Subsequently, 10 mL of plant extract was added drop by drop to the 90 mL copper-zinc solution. The resulting mixture was then incubated in a water bath at 70°C for 1 hour. The synthesized solution was subjected to further analysis using various analytical techniques to confirm the formation of nanoparticles.

Confirmation of bimetallic Cu-Zn nanoparticles

Visual confirmation of the synthesized Cu-Zn nanoparticles was achieved by observing a color change from the original bluish solution to a dark green one, along with the recording of absorption maxima in the wavelength range of 200–450 nm.

Fourier transform infrared spectrometric analysis.

Fourier transform infrared spectrometry (FTIR 7800A) was used to identify the functional groups on the cell surface, which was responsible for the adsorption of heavy metals. The absorbance of the IR spectrum was recorded within 500 to 4000 cm^{-1} using an FTIR (François *et al*., 2012).

X-ray Diffraction

The study the crystalline nature of extracted cellulose the samples were characterized by XRD analysis, diffraction patterns of the cellulosic materials were investigated at room temperature using an Advanced Bruker AX D8 diffractometer in the range 2θ=10 - 90º, equipped with nickel-filtered Cu Kα radiation (λ = 1.542 Å) at 40 kV and 40 mA. The scan speed was 0.5 sec/step (Mzimela *et al*., 2018; Feng *et al*., 2018).

Antimicrobial Analysis through Well Diffusion Method

Green-synthesized copper and zinc nanoparticles were examined for antibacterial activity using agar diffusion. After cultivating bacteria in LB media, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were dispersed on petriplates to test for growth inhibition (Gopikrishna *et al*., 2018). Comparing it against streptomycin yielded the zone of inhibition in percentage (%). The inhibitory zones of bimetallic nanoparticles and streptomycin were compared.

Results

Phytochemical Screening

Phytochemical analysis serves as a critical preliminary step in identifying the bioactive constituents present in medicinal plants, which may contribute to their pharmacological potential. In the present study, a comprehensive phytochemical screening was conducted on the aqueous leaf extract of *Z. mauritiana*. The screening results revealed the presence of various bioactive compounds, including alkaloids, flavonoids, polyphenols, phenols, tannins, and amino acids in significant concentrations.

Alkaloids are nitrogen-containing compounds known for their broad spectrum of pharmacological effects, including analgesic, antimalarial, antiasthmatic, and antibacterial activities (Sasidharan *et al*., 2011). The detection of alkaloids in the aqueous extract suggests potential therapeutic properties and aligns with traditional uses of *Z. mauritiana* in folk medicine for treating gastrointestinal disorders and inflammation (Maji *et al*., 2010). Flavonoids, another major group identified in the extract, are polyphenolic compounds known for their antioxidant, anti-inflammatory, and cardioprotective effects (Panche *et al*., 2016). Their presence further underscores the potential of *Z. mauritiana* in combating oxidative stress-related diseases.

Flavonoids can scavenge free radicals, chelate metal ions, and modulate cellular antioxidant defenses, thereby offering protective effects at the molecular level. Phenolic compounds, including both polyphenols and simple phenols, were also found in considerable amounts. These compounds are primarily responsible for the antioxidant properties of many plant species. Phenolics exert their effects through hydrogen donation, radical scavenging, and metal chelation, playing a vital role in preventing oxidative damage to biomolecules such as DNA, lipids, and proteins (Dai & Mumper, 2010).

The presence of tannins, a subclass of polyphenols, is particularly noteworthy due to their astringent properties and ability to precipitate proteins. Tannins exhibit antimicrobial, antiviral, and antiparasitic activities and have been widely used in the treatment of diarrhea and wounds (Haslam, 1996). Their identification in the leaf extract of *Z. mauritiana* supports its ethnopharmacological relevance and potential for further therapeutic investigation. Additionally, amino acids were detected in the aqueous extract. While not phytochemicals in the strict sense, amino acids are essential for protein synthesis and may contribute to the nutritional value of the plant. Some amino acids also act as precursors for bioactive secondary metabolites and may influence the pharmacodynamic properties of plant-based preparations (Borrelli & Izzo, 2000).

Overall, the presence of these bioactive compounds highlights the therapeutic potential of *Z. mauritiana* leaves. A summary of the phytochemical constituents identified in the aqueous extract is provided in Table 02. These findings are consistent with previous studies that have documented the rich phytochemical profile of *Z. mauritiana*, supporting its traditional use and suggesting its suitability for further pharmacological and clinical investigations (Singh *et al*., 2013).

UV–Visible Spectroscopy

In the present study, UV–Visible spectral analysis in the 200–800 nm range was used to monitor the role of *Z. mauritiana* extract as a reducing and stabilizing agent in the synthesis of Cu/Zn nanoparticles. The dual peaks observed in the spectra at 264.4 nm and 334.9 nm are attributed to the absorption by Cu/Zn nanoparticles. Similar results have been reported for Cu/Zn nanoparticles synthesized using *Eryngium foetidum*, toddy palm, *C. longirostrata*, *Azadirachta indica*, and *Ocimum sanctum*. These nanoparticles exhibited dual peaks at 401 nm (CuNPs) and 408.5 nm (ZnNPs), along with other absorption peaks at 344 nm, 338 nm, 370 nm, and 400 nm, respectively (Merugu *et al*., 2021; Mendez-Trujillo *et al*., 2022; Minal and Prakash, 2020; Elemike *et al*., 2019). Although the biogenic synthesis of Cu/Zn nanoparticles is accompanied by a visible color change, the precise mechanism by which *Z. mauritiana* extract facilitates the bio-reduction of Cu^{2} and Zn^{2} ions remains unclear. Some studies suggest that the presence of natural phytochemicals—such as alkaloids, flavonoids, polyphenols, phenols, tannins, and amino acids—contributes to the plant's biological activity. In green synthesis, these compounds can act as both reducing and stabilizing agents (Larrañaga-Tapia *et al*., 2024; Suresh *et al*., 2024; Dabbeta *et al*., 2025; Bahnasawy *et al*., 2022). The biosynthesis of Cu/Zn nanoparticles using *Z. mauritiana* likely proceeds through two major phases: A reduction and nucleation phase, where phytochemicals in the extract reduce metal ions to form small nuclei and another is a growth phase, during which these nuclei aggregate into larger nanoparticles, thereby increasing the thermodynamic stability of the green-synthesized particles. Fig. 1 shows the UV–Visible spectrum with the corresponding absorption peaks.

Table 2: Qualitative phytochemical screening of the aqueous leaf extract of *Z. mauritiana*

S. No.	*Phytochemical tests*	*Observation*	*Results*
1.	Alkaloids	Orange or red precipitate	++
2.	Flavonoids	Yellow precipitate	+++
3.	Polyphenols	Yellow precipitate	+++
4.	Phenols	White precipitate	+++
5.	Tannins	Dark green colour	++
6.	Amino acids	Purple colour	-

Note: + = low concentration, ++ = moderate concentration, +++ = high concentration

FTIR Analysis

The Fourier Transform Infrared (FTIR) spectroscopy is an essential analytical technique to determine the functional groups present on the surface of nanoparticles and the biomolecules involved in their synthesis and stabilization. The FTIR spectrum provided (see image) of *Z. mauritiana*-mediated CuO-ZnO bimetallic nanoparticles reveals various characteristic absorption bands that confirm the presence of phytochemicals acting as reducing and capping agents. Broad Peaks around 3242.7 cm^{1} and 3663 cm^{1} are indicative of –OH stretching vibrations, which are typical for hydroxyl groups present in alcohols and phenols. These groups are primarily responsible for the reduction of metal ions during nanoparticle formation. Their broad nature suggests strong hydrogen bonding. Strong Peaks at 1607.06 and 1019.76 cm^{1} are crucial regions representing C=C stretching of aromatic rings and C–N/C–O vibrations, respectively. Their prominence implies a substantial role of aromatic phytochemicals such as flavonoids and tannins in both reducing and stabilizing the nanoparticles. Peak at 2898.90 cm^{1} attributed to C–H stretching of alkanes, possibly indicating the presence of long hydrocarbon chains or aliphatic compounds in the extract that might aid in capping and preventing aggregation. Metal-Oxide

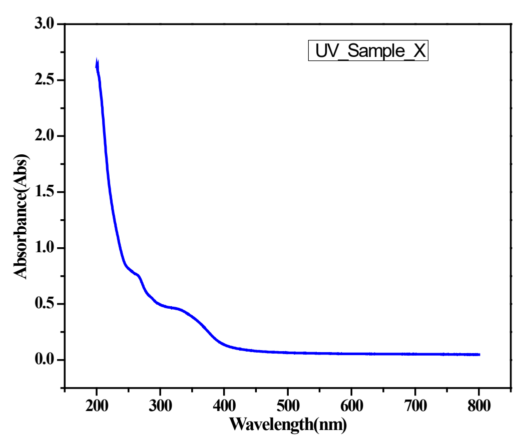

Fig 1: Peaks of Cu-Zn solution in the UV spectrophotometer

Confirmation (558.64 cm^1) is a definitive indicator of Cu–O and Zn–O bond formation, verifying successful synthesis of bimetallic CuO-ZnO nanoparticles. This region is characteristic of stretching vibrations of metal-oxygen bonds in metal oxides. Minor Peaks (2160.59 and 1984.67 cm^{-1}) may correspond to C≡C or C≡N triple bonds, and overtone/combination bands, respectively (Khatak *et al*., 2021), suggesting the complex nature of the biomolecular environment provided by *Z. mauritiana* extract (Fig. 2). The FTIR analysis validates the presence of various functional groups from the *Z. mauritiana* leaf extract, such as hydroxyl, amine, carboxyl, and aromatic groups, which actively participate in the reduction and capping of copper and zinc precursors during nanoparticle synthesis. The peaks observed in the lower wavenumber region, particularly around 558 cm^1, confirm the successful formation of metal oxide (CuO-ZnO) bonds (Table 3). The strong interaction between the phytochemicals and the nanoparticle surface further suggests a stable and biofunctional nanomaterial system.

XRD (X-ray diffraction)

The formation of the nanostructure synthesized via the plant extract was confirmed by the characteristic peaks observed in the XRD pattern. Figure 3 shows the XRD spectrum of the fabricated Cu-Zn nanoparticles. The peaks were observed at 12.32°, 21.24°, 26.73°, 31.59°, 33.31°, 35.59°, 38.62°, 41.13°, 46.30°, 49.91°, 54.28°, 60.02°, 62.74°, 64.46°, and 68.07°. These peaks were analyzed to determine their intensity, position, and width. All diffraction peaks correspond to the characteristic lines of a face-centered cubic (FCC) structure. Additionally, distinct diffraction lines observed at 2θ values of 32.27°, 34.93°, 38.80°, and 57.31° further support the crystalline nature of the synthesized Cu-Zn nanoparticles. The analysis confirms the successful formation of bimetallic nanoparticles with a crystalline structure (Suresh *et al*., 2024; Elemike *et al*., 2019). Figure 3 illustrates the XRD analysis of Cu-Zn bimetallic nanoparticles synthesized from *Z. mauritiana*.

Antimicrobial Study

Due to bacterial resistance and the high cost of sophisticated antimicrobials, scientists are seeking effective, economically viable, and broadly applicable medicines (Aslam, 2018). We investigated the well diffusion method of CuONPs, ZnONPs, and CuO-ZnO bimetallic NPs against gram-positive and gram-negative bacteria to find a new treatment. Table 4 shows the zone of inhibition and antibacterial efficacy of synthesised silver and copper nanoparticles at 20 mg against *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumonia*. Compared to streptomycin at 10 mg, it inhibited *B. subtilis, S. aureus, E. coli, K. pneumoniae, and P. aeruginosa* by 79, 81, 78, 86, and 82%,

Table 3: FTIR peak analysis and functional group assignment

Wavenumber (cm^{-1})	Functional Group/ Assignment	Description/Significance	Reference
3855.84	O–H stretching (free hydroxyl)	Indicates free hydroxyl groups, possibly from alcohols or phenols present in the plant extract.	Mittal et al., 2013
3721.67	O–H stretching	Corresponds to hydrogen bonding of hydroxyl groups from polyphenols.	Kharissova et al., 2013
3663.00	O–H stretching (intra-molecular hydrogen bonded)	Often attributed to alcohol or phenolic groups, suggests active participation of plant metabolites.	Iravani, 2011
3620.39	O–H or N–H stretching	Likely due to hydroxyl or amine groups, indicating the presence of proteins/alkaloids.	Padalia et al., 2015
3242.70	Broad O–H stretching	Confirms the presence of alcohol or phenolic compounds aiding nanoparticle reduction and stabilization.	Ibrahim, 2015
2898.90	C–H stretching (alkanes)	Indicates saturated hydrocarbon chains from phytochemicals (terpenoids, lipids).	Sharma et al., 2010
2160.59	C≡C or C≡N stretching	Might be associated with alkyne/nitrile groups, often derived from flavonoids or alkaloids.	Raghunath & Perumal, 2017
1984.67	Combination/overtone bands	These may arise from complex overtones or combinations of lower-energy vibrations.	Moezzi et al., 2012
1607.06	C=C stretching (aromatic/ alkene)	Strong and broad indicates flavonoids or aromatic rings in phenolics. Likely linked to nanoparticle capping.	Mittal et al., 2013
1380.49	C–N or C–H bending (aliphatic nitro or methyl)	Suggests thecc presence of amines or methyl groups from proteins or amino acids in the extract.	Iravani, 2011
1229.45	C–O stretching (esters or phenols)	Points to flavonoids, tannins, or esters in the extract acting as capping agents.	Kharissova et al., 2013
1019.76	C–N or C–O stretching	Often due to alcohols or amines; also indicates involvement of proteins in nanoparticle stabilization.	Padalia et al., 2015
558.64	M–O stretching (Cu–O, Zn–O)	Confirms formation of metal oxide bonds (CuO and ZnO nanoparticles).	Moezzi et al., 2012

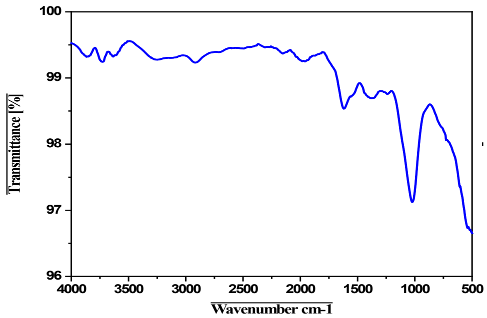

Fig 2: FTIR spectra of Cu/Zn nanoparticles.

respectively. Cu–Zn bimetallic NPs' synergistic antibacterial action in complex matrices, like bacterial cultures, may be non-linear. A synergy test like the Bliss independence model that accounts for the effect of the antimicrobial agent on the bacterial culture is better for analysing this type of response than the isobologram and FIC index interpretations, which assume all antimicrobial agents and their combinations have a linear dose-response curve. Thus, we examined whether the Bliss Independence Model's synergistic effect in Cu–Zn bimetallic nanoparticles was preserved or altered after combining independently synthesised CuONPs and ZnO NPs. All bimetallic nanoparticle interactions were synergistic (Table 4). Additionally, the combination of CuONPs and ZnONPs showed synergistic antibacterial effectiveness against E. coli. Remember that employing bimetallic nanoparticles instead of single metal nanoparticles makes their manufacture greener and delivery easier. This approach also greatly decreases toxic waste from nanoparticle production (Roy *et al*., 2013). Cu–Cu-Zn bimetallic NPs' antibacterial effect appears to depend on the microorganism cell wall or membrane composition. The antibacterial mechanism of Cu–Zn bimetallic nanoparticles is unknown. However, core–shell nanostructures have a greater influence than monometallic nanoparticles. Porins may transport nanoparticles to and from cells (Neal *et al*., 2008). Silver nanoparticles can permeate bacterial cell walls. Once inside, they produce free radicals that cause intracellular oxidative stress and cell death (Prabhu and Poulose, 2012). Iron may interact with cysteine-SH –SH groups in bacterial cell wall proteins, according to recent research. The oxidising species' electrons are most likely to reach cysteine's thiol side chain (Al-Asfar *et al*., 2018).

Table 4: Antibacterial activity of the synthesized copper and zinc nanoparticles

S. N.	*Name of organism*	*Inhibitory zones diameter in mm*	
		Copper and zinc nanoparticles (20 mg/L)	Streptomycin (10 mg/L)
1.	*S. aureus*	18.12 ± 1.24	22 ± 2.65
2.	*B. subtilis*	17.20 ± 2.01	21 ± 2.15
3.	*E. coli*	16.80 ± 1.87	22 ± 1.25
4.	*K. pneumoniae*	15.34 ± 1.20	17 ± 1.45
5.	*P. aeruginosa*	14.60 ± 1.05	17 ± 0.89

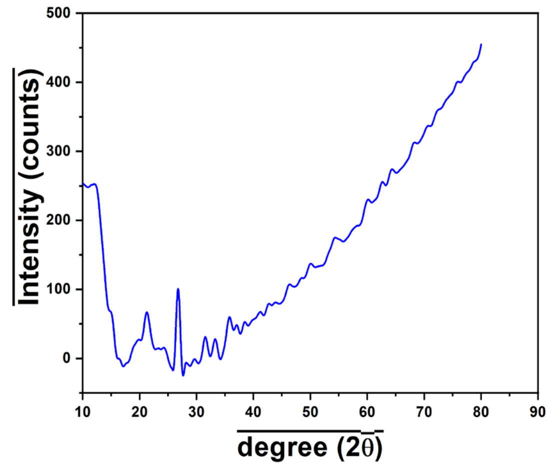

Fig 3: XRD analysis showing zinc Zn-Cu Nanoparticle peaks of Z. mauritiana
* Intensity (Counts –X ray Photons)

Discussion

The growing interest in nanotechnology-based antimicrobial strategies has led to the exploration of various metallic and bimetallic nanoparticles as alternative or synergistic agents to traditional antibiotics. In this study, we investigated the synergistic antibacterial efficacy of copper–zinc (Cu–Zn) bimetallic nanoparticles (NPs) using the Bliss Independence Model, a statistical synergy test that provides a more accurate depiction of interaction effects compared to conventional methods like the fractional inhibitory concentration (FIC) index or isobologram analysis. Traditional methods of assessing antimicrobial synergy, such as the FIC index, assume a linear dose-response relationship between antimicrobial agents and bacterial cultures. However, this assumption can be problematic, especially when the pharmacodynamics of the agents or nanoparticles are inherently non-linear (Tang *et al*., 2018). The Bliss Independence Model overcomes this limitation by accounting for the individual and interactive contributions of each agent to the observed antibacterial effect. Our findings demonstrated that all tested interactions between CuONPs and ZnONPs exhibited synergistic behavior, reinforcing the model's appropriateness in nanoparticle synergy assessments. The combination of independently synthesized CuONPs and ZnONPs resulted in a robust synergistic antibacterial effect against *Escherichia coli*. These findings are in line with previous research by Roy *et al*., (2013), which highlighted the environmental advantages and enhanced antibacterial performance of bimetallic nanoparticles over their monometallic counterparts. Notably, bimetallic NPs often exhibit properties that are not simply the sum of their components. Instead, they can display novel physicochemical behaviors due to the interaction between the two metal ions at the nanoscale, such as altered redox potentials, enhanced ion release, and increased surface reactivity (Rai *et al*., 2016).

Furthermore, our results suggest that the antibacterial potency of Cu–Zn bimetallic nanoparticles may be linked to cell wall composition, a hypothesis previously proposed by Neal *et al*., (2008) in their study on nanoparticle transport mechanisms.

Porin channels in gram-negative bacteria such as *E. coli* are potential pathways for nanoparticle uptake. Once internalized, nanoparticles can disrupt membrane potential, generate reactive oxygen species (ROS), and interfere with intracellular components, eventually causing cell lysis and death.

In a study by Prabhu and Poulose (2012), silver nanoparticles (AgNPs) were shown to penetrate bacterial membranes and generate free radicals, leading to oxidative stress and cellular damage. Although AgNPs are widely regarded as potent antimicrobials, their toxicity profile and environmental persistence remain concerns. Comparatively, Cu–Zn bimetallic nanoparticles present a more biocompatible and sustainable alternative, as both copper and zinc are essential trace elements with relatively lower cytotoxicity when properly formulated (Sirelkhatim *et al*., 2015). A study by Al-Asfar *et al*., (2018) showed that iron nanoparticles interact with bacterial cell wall proteins through thiol (-SH) groups, leading to protein inactivation. A similar mechanism could be speculated for Cu–Zn NPs, as copper ions are known to form stable complexes with thiol-containing biomolecules, potentially disrupting bacterial enzymes and structural proteins. This highlights a multi-targeted antibacterial action, which may reduce the likelihood of resistance development.

Moreover, our use of the Bliss Independence Model, compared to the more commonly used Loewe additivity or FIC-based synergy evaluation, offers a more realistic approach for nanoparticle interactions, as demonstrated in recent combinatorial drug studies (Berenbaum, 1989; Yeh *et al*., 2006). Given that nanoparticle uptake, diffusion, and intracellular behavior are non-linear and context-dependent, our choice of analytical model provides more reliable predictions of synergy and facilitates a deeper understanding of nanoparticle-based antimicrobial strategies. Although the precise antibacterial mechanism of Cu–Zn bimetallic nanoparticles remains elusive, several plausible hypotheses emerge from the current literature. First, the formation of core–shell nanostructures in bimetallic systems may influence their interactions with bacterial cells. In such structures, one metal forms the core while the other forms a shell, allowing for controlled ion release and optimized redox behavior. This architecture can enhance stability and provide a sustained antibacterial effect, as demonstrated in studies involving Au–Ag and Cu–Ag nanoparticles (Zhang *et al*., 2018).

Second, both copper and zinc are capable of generating ROS within microbial cells, such as superoxide anions and hydroxyl radicals, which damage DNA, proteins, and lipids (Ruparelia *et al*., 2008). The dual action of copper and zinc could amplify this oxidative damage, making it more difficult for bacteria to employ standard resistance mechanisms. Additionally, zinc ions are known to inhibit key bacterial enzymes by replacing essential metal cofactors, thereby impairing metabolic function (Huang *et al*., 2010).

Third, nanoparticle interaction with bacterial membrane lipids may lead to increased permeability or leakage of cytoplasmic contents. This has been confirmed in TEM imaging studies where CuO and ZnO NPs caused membrane disintegration in both Gram-negative and Gram-positive bacteria (Padmavathy & Vijayaraghavan, 2008). The enhanced surface activity and smaller particle size observed in Cu–Zn bimetallic NPs may contribute to these effects, as smaller particles have higher surface-to-volume ratios, enabling more efficient bacterial binding. One of the most significant advantages of using bimetallic Cu–Zn NPs lies in their green synthesis potential and reduced environmental impact. Unlike silver or gold nanoparticles, whose synthesis often involves hazardous chemicals or costly reagents, Cu–Zn NPs can be synthesized through eco-friendly methods, including plant-mediated or microbial routes. This aspect aligns with the principles of green chemistry and supports sustainable nanotechnology development (Ahmed *et al*., 2016).

In addition, the production of bimetallic nanoparticles can reduce toxic byproducts, as the synergistic action of the metals allows for lower effective doses, minimizing metal leaching and associated toxicity. From a pharmaceutical perspective, these findings suggest the potential of Cu–Zn bimetallic NPs as adjuvants or replacements for traditional antibiotics, especially in an era where antibiotic resistance is a major global health concern.

While our results demonstrate synergistic antibacterial effects using the Bliss Independence Model, several avenues remain for further investigation. First, mechanistic studies using advanced imaging techniques (e.g., SEM, TEM, and confocal microscopy) and molecular tools (e.g., proteomics, transcriptomics) are needed to elucidate the exact pathways involved in Cu–Zn NP-mediated bacterial killing. Second, it is necessary to examine the cytotoxic effects of Cu–Zn bimetallic NPs on mammalian cells, as biocompatibility is critical for any clinical or therapeutic application. Although copper and zinc are essential nutrients, nanoparticle forms may exhibit different toxicity profiles, and dosage optimization is crucial. Third, comparative studies involving other bimetallic combinations (e.g., Cu–Ag, Zn–Fe, Zn–Ag) using the same analytical model would provide a broader understanding of nanoparticle synergy and potentially identify superior antibacterial formulations. Finally, incorporating these nanoparticles into delivery systems such as hydrogels, coatings, or encapsulated materials could open up novel biomedical and industrial applications, from wound dressings and food packaging to water purification and medical device coatings.

Conclusion

The present study successfully demonstrates the biogenic synthesis of bimetallic CuO-ZnO nanoparticles using plant extracts of *Z. mauritiana*, providing a sustainable and eco-friendly approach for nanoparticle fabrication. The phytochemicals present in the plant extract acted as reducing and stabilizing agents, enabling the formation of well-dispersed and stable CuO-ZnO nanoparticles. Characterization techniques, including UV-vis spectroscopy, FTIR, and XRD, confirmed the formation, crystalline nature, and bimetallic composition of the nanoparticles. The synthesized nanoparticles exhibited nanoscale size, high surface area, and distinct morphological features, indicating their potential applicability in various fields. The synergistic effect of copper and zinc oxides imparts enhanced physicochemical properties, making the bimetallic nanoparticles more effective than their monometallic counterparts. The green synthesis route not only reduces the environmental impact associated with conventional chemical methods but also adds value by incorporating medicinally significant plant-based biomolecules. Overall, the study establishes a reliable and scalable method for synthesizing multifunctional nanoparticles using *Z. mauritiana* extracts. Future work can explore their application in antimicrobial, photocatalytic, and environmental remediation fields, further unlocking the potential of plant-mediated bimetallic nanoparticles in nanotechnology and sustainable materials science.

Acknowledgments

Sarvaree Bano did all the experiments, and the data collection was included in this work along with paper drafting. Dr. Manisha Agrawal, being a PhD guide of Sarvaree Bano helped her in conducting experiments and drafting papers and Dr. Dharm Pal and Virendra Kumar Vaishnav also helped in drafting the paper and helped in designing the experiment.

Conflict of Interest

We, the authors of this research article, declare no conflict of interest.

References

Aslam, B. (2018). Antibiotic resistance: a rundown of a global crisis. *Infect. Drug Resist*. 11, 1645–1658.

Anastas, P. T., & Warner, J. C. (1998). *Green Chemistry: Theory and Practice*. Oxford University Press.

Al-Asfar, A., Zaheer, Z. & Aazam, E. S. (2018). Eco-friendly green synthesis of Ag@Fe bimetallic nanoparticles: antioxidant, antimicrobial and photocatalytic degradation of bromothymol blue. *J. Photochem. Photobiol. B Biol.* 185, 143–152.

Abalaka, M. E., Daniyan, S. Y., & Mann, A. (2010). Evaluation of the antimicrobial activities of two Ziziphus species (*Ziziphus mauritiana* L. and Ziziphus spina Christi L.) on some microbial pathogens. *African Journal of Pharmacy and Pharmacology*, *4*(4), 135-139.

Ashraf, A., Sarfraz, R. A., Anwar, F., Shahid, S. A., & Alkharfy, K. M. (2015). Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. *Pak. J. Bot*, *47*(1), 367-376.

Al-Habeeb, R. S., & Al-Bishri, W. M. (2024). Synthesis of Bimetallic Copper oxide-silver Nanoparticles by Gum Arabic: Unveiling Antimicrobial, and Antibiofilm Potential against some Pathogenic Microbes Causing Wound Infection. *Journal of Cluster Science*, *35*(7), 2311-2327.

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extracts mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of Advanced Research*, 7(1), 17–28.

Balamurugan, V., Fatima, S., & Velurajan, S. (2019). A guide to phytochemical analysis. *International Journal of Advance Research and Innovative Ideas in Education*, *5*(1), 236-245.

Borrelli, F., & Izzo, A. A. (2000). The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research*, 14(8), 581–591.

Bahnasawy, N., Elbanna, A. M., Ramadan, M., & Allam, N. K. (2022). Fabrication of polyhedral Cu–Zn oxide nanoparticles by dealloying and anodic oxidation of German silver alloy for photoelectrochemical water splitting. *Scientific Reports*, *12*(1), 16785.

Berenbaum, M. C. (1989). What is synergy? *Pharmacological Reviews*, 41(2), 93–141.

Chauhan, S., & Upadhyay, L. S. B. (2019). Biosynthesis of iron oxide nanoparticles using plant derivatives of Lawsonia inermis (Henna) and its surface modification for biomedical application. *Nanotechnology for Environmental Engineering*, *4*, 1-10.

Chandrakar, V., Tapadia, K., & Gupta, S. K. (2023). Greener approach for gold nanoparticles synthesis from fruit peel extract of Manilkara zapota: a fluorometric assay for determination of thiourea. *Inorganic and Nano-Metal Chemistry*, *53*(9), 923-931.

Chaudhari, A. K., & Dwivedi, S. K. (2023). Green synthesis of silver nanoparticle by *Ziziphus mauritiana* leaf extract and its antifungal activity against Aspergillus species. *Biochemical & Cellular Archives*, *23*(1).

Dwivedi, S. D., Singh, D., & Singh, M. R. (2024). A Piper nigrum based zinc oxide nanoparticles for anti-arthritic and antioxidant activity. *Journal of Applied Pharmaceutical Research*, *12*(5), 51-59.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Datta, A., Patra, C., Bhardwaj, H., Kaur, S., Dimri, N., Khajuria, R.C. (2012). Green synthesis of zinc oxide nanoparticles using Parthenium hysterophorus leaf extracts and evaluation of their antimicrobial properties. J of Biotechnology and biomaterials.

Dabbeta, N. K., Kante, M., Konda, R., Devarala, A., Kalakonda, P. B., Khanam, S. J., ... & Kalakonda, P. (2025). Eco-inspired Cu-Zn bimetallic nanoparticles for Enhanced Water Purification Technologies. *Materials Chemistry and Physics: Sustainability and Energy*, *2*, 100013.

El-Sayyad, G. S. (2024). The need for smart bimetallic nanoparticles in the battle against pathogenic multi-drug-resistant bacteria: a brief communication. *Discover Bacteria*, *1*(1), 2.

Elemike, E. E., Onwudiwe, D. C., Nundkumar, N., Singh, M., & Iyekowa, O. (2019). Green synthesis of Ag, Au and Ag-Au bimetallic nanoparticles using Stigmaphyllon ovatum leaf extract and their in vitro anticancer potential. *Materials Letters*, *243*, 148-152.

François, F., Lombard, C., Guigner, J.M., Soreau, P., Brian-Jaisson, F., Martino, G., Vandervennet, M., Garcia, D., Molinier, A.L., Pignol, D. (2012). Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. Appl. Environ. Microbial, 78, 1097–1106.

Feng, Y.H., Cheng, T.Y., Yang, W.G., Ma, P.T., He, H.Z., Yin, X.C. and Yu, X.X. (2018). Characteristics and environmentally friendly extraction of cellulose nanofibrils from sugarcane bagasse. *Industrial Crops and Products*, 111, pp.285-291. DOI: 10.1016/j.indcrop.2017.10.041

Gopikrishna, P., Nageshwari, B., Rao, K.K., Vasavi, D., Merugu, R. (2018), Int. J. Green Herbal Chem. 8, 099–108.

Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *Journal of Natural Products*, 59(2), 205–215.

Huang, Y., He, L., Liu, W., Fan, C., Zheng, W., Wong, Y. S., & Chen, T. (2010). Selective cellular uptake and induction of apoptosis of cancer-targeted selenium nanoparticles. *Biomaterials*, 31(5), 730–737.

Ibrahim, H. M. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*, 8(3), 265–275.

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

Khatak, S., Wadhwa, N., & Jain, P. (2021). Monometallic zinc and bimetallic Cu-Zn nanoparticles synthesis using stem extracts of Cissusquadrangularis (Haddjod) and Proneness as alternative antimicrobial agents. *Biosciences Biotechnology Research Asia*, *17*(4), 763-774.

Kharissova, O. V., Dias, H. R., Kharisov, B. I., Pérez, B. O., Pérez, V. M. (2013). The greener synthesis of nanoparticles. *TrAC Trends in Analytical Chemistry*, 50, 51–59.

Larrañaga-Tapia, M., Betancourt-Tovar, B., Videa, M., Antunes-Ricardo, M., & Cholula-Díaz, J. L. (2024). Green synthesis trends and potential applications of bimetallic nanoparticles towards the sustainable development goals 2030. *Nanoscale Advances*, *6*(1), 51-71.

Merugu, R., Gothalwal, R., Deshpande, P. K., De Mandal, S., Padala, G., & Chitturi, K. L. (2021). Synthesis of Ag/Cu and Cu/Zn bimetallic nanoparticles using toddy palm: Investigations of their antitumor, antioxidant and antibacterial activities. *Materials Today: Proceedings*, *44*, 99-105.

Maji, A. K., Banerji, P., & Banerjee, D. (2010). Phytochemistry and pharmacology of *Ziziphus mauritiana* Lam.: A review. *International Journal of Pharmaceutical and Biomedical Research*, 1(2), 80–87.

Mendez-Trujillo, V., Valdez-Salas, B., Curiel-Alvarez, M., Beltran-Partida, E., Alfaro-Corres, A., Ruiz-Sanchez, E., ... & González-Mendoza, D. (2022). Insecticidal effect of green bimetallic nanoparticles from Crotalaria longirostrata on cotton mealybug, Phenacoccus solenopsis. *Journal of Renewable Materials*, *10*(10), 2543-2552.

Minal, S. P. and Prakash, S. (2020). Laboratory analysis of Au-Pd bimetallic nanoparticles synthesized with citrus limon leaf extract and its efficacy on mosquito larvae and non-target organisms. Scientific Reports, 10(1), 21610. DOI 10.1038/s41598-020-78662-y.

Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 31(2), 346–356.

Moezzi, A., McDonagh, A. M., & Cortie, M. B. (2012). Zinc oxide particles: Synthesis, properties and applications. *Chemical Engineering Journal*, 185, 1–22.

Mzimela, Z. N.T., Linganiso, L. Z., Revaprasadu, N., Motaung, T. E. (2018). Comparison of cellulose extraction from sugarcane bagasse through alkali. *Materials Research*, 21, p.e20170750. DOI: http://dx.doi.org/10.1590/1980-5373-MR-2017-0750

Neal, A. L. (2008). What can be inferred from bacterium–nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? *Ecotoxicology* **17**, 362–371.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5, e47.

Pansambal, S., Ghotekar, S., Oza, R., Deshmukh, K. (2019). Biosynthesis of CuO nanoparticles using aqueous extract of *Ziziphus mauritiana* L. leaves and their Catalytic performance for the 5-aryl-1, 2, 4-triazolidine-3-thione derivatives synthesis. *Int. J. Sci. Res. Sci. Technol*, *5*(4), 122-128.

Padalia, H., Moteriya, P., & Chanda, S. (2015). Green synthesis of silver nanoparticles from *Psidium guajava* leaf extract and its antibacterial activity. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 6(3), 035009.

Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. *Science and Technology of Advanced Materials*, 9(3), 035004.

Prabhu, S. & Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int. Nano Lett.* **2**, 32 (2012).

Roy, N., Gaur, A., Jain, A., Bhattacharya, S. & Rani, V. (2013). Green synthesis of silver nanoparticles: an approach to overcome toxicity. *Environ. Toxicol. Pharmacal.* https ://doi.org/10.1016/j.etap.2013.07.005.

Rai, M., Yadav, A., & Gade, A. (2016). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83.

Raghunath, A., & Perumal, E. (2017). Metal oxide nanoparticles as antimicrobial agents: A promise for the future. *International Journal of Antimicrobial Agents*, 49(2), 137–152.

Ruparelia, J.P., Chatterjee, A.K., Duttagupta, S.P., Mukherji, S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater;4(3):707-16. doi: 10.1016/j.actbio.2007.11.006. Epub 2007 Nov 26. PMID: 18248860.

Suresh, P., Doss, A., Praveen Pole, R. P., & Devika, M. (2024). Green synthesis, characterization and antioxidant activity of bimetallic (Ag-ZnO) nanoparticles using Capparis zeylanica leaf extract. *Biomass Conversion and Biorefinery*, *14*(14), 16451-16459.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1), 1–10.

Sánchez, A. & Zhang, K. (2012). Bimetallic nanoparticles: Synthesis, characterization, and applications. *Frontiers of Physics*, 7, 1–16.

Sirelkhatim, A. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, 7(3), 219–242.

Sharma, P. C., Yelne, M. B., & Dennis, T. J. (2010). *Database on Medicinal Plants Used in Ayurveda*, Central Council for Research in Ayurveda and Siddha, Vol. 5.

Shah, M. T., Balouch, A., Panah, P., Rajar, K., Mahar, A. M., Khan, A., Jagirani, M. S. (2018). Synthesis of Magnetite Nanoparticles and Its Application as Electrode Material for the Electrochemical Oxidation of Methanol. Journal of Electronic Materials, 47, 5321–5333.

Singh, A., Singh, S. K., & Rai, P. K. (2013). Phytochemical and pharmacological properties of *Ziziphus mauritiana*: A review. *International Journal of Advanced Research*, 1(7), 122–129.

Tang, S. S., Zheng, J. S., Antanasijevic, A., & Roder, H. (2018). Linear and non-linear dose-response relationships in pharmacological studies. *Journal of Pharmacology and Experimental Therapeutics*, 366(2), 379–387

Upadhyay, L. S. B., Tirkey, A., Bhagat, P., Singh, S. K., Mishra, A. (2025). Green Synthesis of Copper Oxide Nanoparticles Using Solanum tuberosum Extract to Mediate Photocatalytic Degradation of Methylene Blue. *Plasmonics*, 1-16.

Yeh, P. J., Hegreness, M. J., Aiden, A. P., & Kishony, R. (2006). Drug interactions and the evolution of antibiotic resistance. *Nature Reviews Microbiology*, 4(12), 556–562.

Zhang, L., Pornpattananangkul, D., Hu, C. M. J., & Huang, C. M. (2018). Development of nanoparticles for antimicrobial drug delivery. *Current Medicinal Chemistry*, 17(6), 585–594.