

"Plant-Mediated Nanoparticles in Antimicrobial Therapy: A Review on Cissus quadranangularis-Derived CuNPs and AgNPs".

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"Plant-Mediated Nanoparticles in Antimicrobial Therapy: A Review on *Cissus quadrangularis*-Derived CuNPs and AgNPs".

Abstract

nanotechnology has ushered in a transformative era in antimicrobial research, with green synthesis of metal nanoparticles gaining increasing attention due to its sustainability, biocompatibility, and eco-friendliness. Among the various approaches, the use of plant-mediated synthesis offers a novel route to fabricate nanoparticles with potent antimicrobial properties. This review focuses on the comparative antimicrobial potential of copper (CuNPs) and silver nanoparticles (AgNPs) synthesized using *Cissus quadrangularis*, a medicinal plant rich in bioactive phytochemicals. Drawing on existing studies, including those utilizing UV-Vis spectroscopy, FTIR, and SEM for nanoparticle characterization, we explore how factors such as particle size, morphology, and phytochemical capping agents influence their biological activity. Evidence suggests that CuNPs exhibit enhanced antimicrobial and antifungal efficacy compared to AgNPs, particularly against strains like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Fusarium oxysporum*, and *Candida albicans*. Inhibition studies have consistently demonstrated the superior bioactivity of CuNPs, attributed to their unique redox properties and ion release mechanisms. This review underscores the promising potential of *Cissus quadrangularis*-mediated CuNPs in combating microbial resistance and highlights key areas for future research, including mechanistic studies, clinical integration, and large-scale production for medical and industrial applications.

Keywords: Antimicrobial, Nanoparticles, Pathogenic, *Cissus quadrangularis*

1. Introduction

The green synthesis of nanomaterials has emerged as a key focus within sustainable science, offering a promising route for the fabrication of functional materials with reduced environmental impact. Rooted in the principles of green chemistry, this approach emphasizes the use of non-toxic, renewable biological resources—particularly plant extracts—as eco-friendly alternatives to hazardous chemical reagents traditionally employed in nanoparticle production (Agarwal et al., 2018; Kumar et al., 2017). Among nanomaterials, metal nanoparticles such as copper (CuNPs) and silver nanoparticles (AgNPs) have garnered considerable attention due to their unique physicochemical properties, including high surface-area-to-volume ratios and potent antimicrobial activity against a broad spectrum of pathogens (Wang et al., 2017; Sánchez-López et al., 2020).

In recent years, there has been growing interest in utilizing medicinal plants for the green synthesis of nanoparticles, as their rich phytochemical profiles—including flavonoids, alkaloids, phenolics, and tannins—act both as reducing and capping agents (Pirsaheb et al., 2024; Shafey, 2020). Such biogenic synthesis not only improves the biocompatibility of the nanoparticles but also enhances their biological efficacy. Numerous plant species, including *Azadirachta indica*, *Withania somnifera*, and *Tinospora cordifolia*, have been successfully employed in nanoparticle synthesis, yielding particles with significant antibacterial, antifungal, and antioxidant properties (Stan et al., 2021; Pal et al., 2024).

Among these promising botanicals, *Cissus quadrangularis* L., a traditional medicinal plant from the family Vitaceae, has emerged as a valuable source for green synthesis. Commonly

used in Ayurvedic medicine for bone healing, antioxidant, and anti-inflammatory purposes, *C. quadrangularis* is rich in bioactive compounds such as quercetin, kaempferol, and stilbenes (Bafna et al., 2021). Its phytochemical composition makes it an ideal candidate for the green fabrication of nanoparticles with enhanced therapeutic potential.

Notably, comparative studies have indicated that CuNPs synthesized via green routes often exhibit stronger antimicrobial activity than their AgNP counterparts. This is frequently attributed to copper's superior ability to disrupt microbial membranes and generate reactive oxygen species (ROS), leading to oxidative stress in pathogens (Wahab et al., 2023; Nisar et al., 2019). Such findings have sparked further investigation into the advantages of plant-mediated CuNPs over AgNPs, particularly in the context of escalating microbial resistance.

This review consolidates current knowledge on the green synthesis of copper and silver nanoparticles using *Cissus quadrangularis* and evaluates their reported antimicrobial activities. By examining synthesis mechanisms, characterization techniques, and comparative efficacy data, this paper aims to provide a comprehensive overview of the potential of *C. quadrangularis*-mediated nanoparticles in biomedical and industrial applications. Special attention is given to the factors influencing nanoparticle performance, the biological mechanisms underpinning antimicrobial action, and future prospects for scale-up and clinical translation.

2. Green Synthesis of Metal Nanoparticles Using *Cissus quadrangularis*: Reported Methods

The synthesis of metal nanoparticles using *Cissus quadrangularis* has been widely investigated for its efficiency, simplicity, and eco-friendliness. Various studies have outlined protocols for preparing plant extracts and employing them in the green synthesis of silver (AgNPs) and copper nanoparticles (CuNPs), highlighting the role of the plant's rich phytochemical profile in facilitating reduction and stabilization.

2.1 Plant Material and Extract Preparation

- Cissus quadrangularis* L., a member of the Vitaceae family, is commonly sourced from tropical and subtropical regions across India. In several reports, fresh stems of the plant have been collected from botanical gardens or wild sources and authenticated through herbarium references or taxonomic verification at institutional botany departments (e.g., IIS University, Jaipur).
- For extract preparation, both fresh and dried stem materials have been used depending on the metal precursor. Typically, fresh stems are crushed to form a paste, whereas dried stems are cleaned, shade-dried, and powdered. The plant material (about 2–10 grams) is then boiled with distilled water (usually 100 mL) for a few minutes to activate bioactive compounds. After filtration to remove solid debris, the aqueous extract is stored under refrigerated conditions for nanoparticle synthesis (El-Sayyad et al., 2024; Ahmed et al., 2017).

2.2 Biosynthesis of Silver and Copper Nanoparticles

- In green synthesis protocols for silver nanoparticles (AgNPs), 10 mL of *Cissus quadrangularis* stem extract is typically mixed with 90 mL of 1 mM silver nitrate (AgNO₃) solution. The reaction mixture is stirred continuously, often using a

magnetic stirrer, at ambient temperature. A visible color change—usually from pale yellow or colorless to reddish-brown—indicates nanoparticle formation due to surface plasmon resonance, a characteristic of AgNPs. The mixture is then incubated and centrifuged at high speed (e.g., 10,000 rpm) to isolate the nanoparticles. The resulting pellet is washed with ethanol or water, oven-dried, and stored for further use (Pirsaheb et al., 2024).

- For copper nanoparticles (CuNPs), similar protocols involve the use of 1 mM copper sulfate (CuSO_4) solution and 2 grams of dried stem powder extract. After mixing, the solution often shows a change to greenish or bluish hues, confirming CuNP formation. These nanoparticles are likewise purified through centrifugation and drying steps.

These methods demonstrate the versatility of *Cissus quadrangularis* as a biological mediator in nanoparticle synthesis. The plant's secondary metabolites not only reduce metal ions but also serve as natural capping agents, enhancing nanoparticle stability and preventing agglomeration. Moreover, such green methods offer advantages over conventional chemical synthesis by eliminating toxic reagents and supporting environmental sustainability.



Figure 1. Schematic representation of the green synthesis of silver nanoparticles (AgNPs) using *Cissus quadrangularis* stem extract. In this biogenic process, phytochemicals present in the plant extract—such as flavonoids, phenolics, and alkaloids—act as natural reducing and stabilizing agents, converting Ag^+ ions into silver nanoparticles under ambient conditions.

2.3 Green Synthesis of Copper Nanoparticles

The green synthesis of copper nanoparticles (CuNPs) using plant extracts has been widely explored as an eco-friendly alternative to traditional chemical methods. In reported protocols, *Cissus quadrangularis* and related species such as *Cissus vitifolia* have been effectively used to mediate the biosynthesis of CuNPs. Typically, an aqueous plant extract (e.g., 10 mL) is mixed with a copper salt solution, such as 10 mM copper sulfate (CuSO_4), in a 1:9 ratio under continuous stirring at room temperature (Kumar et al., 2021). The reduction of Cu^{2+} ions by phytochemicals—such as flavonoids, tannins, and polyphenols—leads to the formation of CuNPs, indicated visually by a color change in the reaction mixture.

After synthesis, the nanoparticles are typically separated by centrifugation at high speed (e.g., 10,000 rpm), washed with ethanol to remove unreacted components, and oven-dried for further characterization. These green-synthesized CuNPs have demonstrated good stability

and bioactivity, attributed ² to the dual role of plant metabolites as both reducing and capping agents.



⁵⁰ **Figure 2.** Schematic representation of the green synthesis of copper nanoparticles (CuNPs) using *Cissus quadrangularis* stem extract. Bioactive phytochemicals present in the extract act as natural reducing and stabilizing agents, facilitating the conversion of Cu^{2+} ions into stable copper nanoparticles under mild, eco-friendly conditions.

⁷ 2.4 Antibacterial Activity Assessment

The antibacterial efficacy of green-synthesized nanoparticles has been extensively evaluated using *in vitro* techniques, most commonly the agar well diffusion method. In this approach, test microorganisms are cultured on nutrient agar (NA) plates, and wells are created to introduce varying concentrations of nanoparticle suspensions. Typically, nanoparticle solutions are prepared in 10% dimethyl sulfoxide (DMSO) at concentrations ranging from 250 mg/L to 500 mg/L. After inoculating the agar plates with bacterial cultures and introducing the test samples, the plates are incubated at 37°C for 24 hours. The formation of zones of inhibition (IZ) around the wells serves as an indicator of antimicrobial activity.

⁷¹ Comparative studies often use a standard antibiotic—commonly streptomycin—as a positive control to benchmark the antimicrobial potential of nanoparticles. Results are usually expressed as the diameter of the inhibition zones (in mm), allowing for direct comparison of the efficacy of different nanoparticle types (e.g., AgNPs vs. CuNPs), concentrations, and plant-mediated formulations (Wang et al., 2017; Nisar et al., 2019).

2.5 Determination of Minimum Inhibitory Concentration (MIC)

⁴⁷ Several studies have also employed the agar well diffusion assay to estimate the minimum inhibitory concentration (MIC) of nanoparticles synthesized via green routes. In these assessments, bacterial cultures are pre-incubated in broth media at 37°C for 24 hours, after which standardized aliquots are spread over solidified agar plates. Wells are then punched into the agar medium, and serial dilutions of nanoparticles—commonly ranging from 25 to 200 µg/mL—are introduced.

Following incubation under controlled conditions, the MIC is inferred from the lowest concentration at which a measurable inhibition zone is observed. This approach not only evaluates bacteriostatic potential but also allows comparisons across various nanoparticle

formulations and microbial strains. Replicates and controls are typically employed to ensure reproducibility and statistical relevance.

These standardized microbiological assays collectively contribute to a growing body of evidence that supports the potent antibacterial effects of plant-mediated silver and copper nanoparticles, particularly when synthesized using phytochemically rich sources such as *Cissus quadrangularis*.

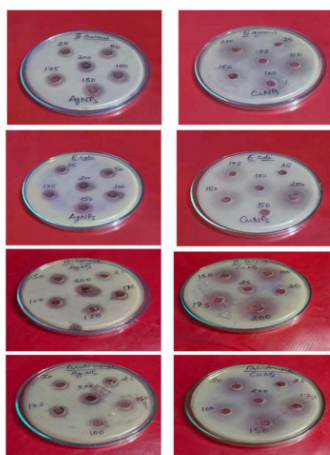


Figure 3. Representative results of minimum inhibitory concentration (MIC) assays evaluating the antibacterial efficacy of green-synthesized silver (AgNPs) and copper nanoparticles (CuNPs) against common pathogenic bacterial strains—*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. Nanoparticle suspensions were tested across a concentration gradient (25–200 $\mu\text{g/mL}$), with inhibition zones used to assess antimicrobial potency.

2.6 Antifungal Activity Assessment

The antifungal efficacy of green-synthesized nanoparticles has been widely investigated using the agar well diffusion method, a standard *in vitro* microbiological assay. In this method, test formulations—typically prepared at concentrations of 250 mg/L and 500 mg/L in 10% dimethyl sulfoxide (DMSO)—are introduced into potato dextrose agar (PDA) plates pre-inoculated with fungal strains. Wells of 6 mm diameter are punched into the agar, and 30 μL of nanoparticle suspensions or standard antifungal agents (e.g., ketoconazole) are added.

Plates are incubated at 37°C for 72 hours, and antifungal activity is assessed based on the diameter of inhibition zones (IZs) surrounding each well. This method allows for direct comparison of antifungal spectra between different nanoparticle formulations and conventional antifungal agents. Studies consistently report significant inhibition of fungal

pathogens such as *Fusarium oxysporum*, *Aspergillus niger*, and *Candida albicans* by both silver (AgNPs) and copper nanoparticles (CuNPs) synthesized using plant extracts like *Cissus quadrangularis*.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

To quantitatively determine the minimum inhibitory concentration (MIC) of green-synthesized nanoparticles against fungal pathogens, an extended agar well diffusion technique is commonly employed. Fungal strains are pre-cultured in potato dextrose broth (PDB) and incubated at 37°C for 72 hours. Once prepared, PDA medium is poured into sterile Petri dishes and solidified under UV light to ensure sterility.

Wells are then bored into the medium, and nanoparticle suspensions are added at serial concentrations ranging from 25 to 200 µg/mL. After incubation under the same conditions, the MIC is determined as the lowest concentration that produces a measurable inhibition zone, indicating effective antifungal activity.

Replicates are typically included to ensure reproducibility, and results are compared with those from commercial antifungal controls. This method has proven useful in differentiating the potency of AgNPs and CuNPs, with many studies reporting CuNPs as slightly more effective, likely due to their enhanced capacity for disrupting fungal cell walls and generating reactive oxygen species (ROS).

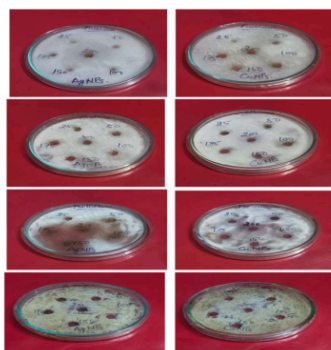


Figure 4. Representative minimum inhibitory concentration (MIC) assay results demonstrating the antifungal efficacy of green-synthesized silver (AgNPs) and copper nanoparticles (CuNPs) against pathogenic fungal strains—*Fusarium oxysporum*, *Aspergillus niger*, *Candida albicans*, and *Penicillium chrysogenum*. Nanoparticles were tested across a concentration range (25–200 µg/mL), and antifungal potency was assessed by measuring the diameter of inhibition zones.

3.1 Characterization of Green-Synthesized Nanoparticles

3.1.1 UV-Visible Spectrophotometric Analysis

UV-Visible spectrophotometry is widely employed as a primary analytical technique to monitor the formation and stability of metallic nanoparticles synthesized via green methods. In numerous studies involving *Cissus quadrangularis*-mediated nanoparticle synthesis, absorption spectra are typically recorded in the range of 300–700 nm, using deionized water as a reference blank.

Silver nanoparticles (AgNPs) generally exhibit a characteristic surface plasmon resonance (SPR) peak around 420 nm, while copper nanoparticles (CuNPs) demonstrate a corresponding peak around 470 nm. These SPR bands are indicative of nanoparticle formation, as they arise due to collective oscillation of conduction electrons on the nanoparticle surface in response to incident light.

The position and intensity of SPR peaks are highly sensitive to particle size, shape, dispersion, and dielectric environment. In general, larger nanoparticles show a red shift (towards longer wavelengths), while smaller nanoparticles produce a blue shift. The consistent appearance of distinct peaks at 420–470 nm in UV-Vis spectra strongly supports the successful synthesis of AgNPs and CuNPs using plant-based reducing agents.

These findings reinforce the applicability of UV-Vis spectroscopy as a rapid, non-destructive method to confirm nanoparticle formation and provide preliminary insights into their physicochemical properties.

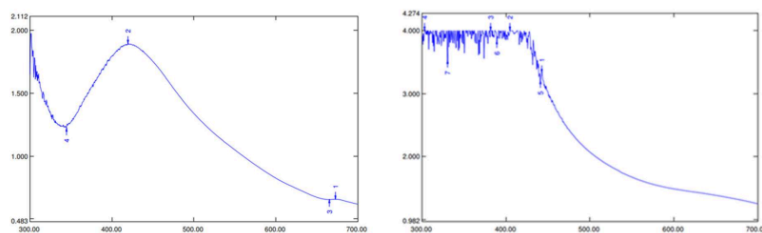


Fig.5 UV-Vis spectra of synthesized silver nanoparticles (a) and copper nanoparticles (CuNPs) (b), showing surface plasmon resonance peaks at 420 nm and 470 nm, respectively.

3.1.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy serves as a powerful tool in characterizing the functional groups involved in the synthesis and stabilization of green-synthesized nanoparticles. When utilizing *Cissus quadrangularis* stem extracts, FTIR analysis provides evidence of the bioactive compounds responsible for reducing metal ions and capping the resultant nanoparticles.

For silver nanoparticles (AgNPs), characteristic absorption bands are typically observed at:

- **2921 cm^{-1}** , corresponding to C–H stretching vibrations of aliphatic hydrocarbons,
- **1601 cm^{-1}** , indicating C=C stretching of unsaturated compounds,
- **1362 cm^{-1}** , attributed to aliphatic C–H bending,
- and **1040 cm^{-1}** , representing C–O–C stretching vibrations of alkyl aryl ethers.

In the case of copper nanoparticles (CuNPs), FTIR spectra often reveal:

- A **broad peak at 3211 cm^{-1}** , indicative of N–H stretching from amino groups (likely derived from proteins or amino acids),
- **Peaks at 2886 cm^{-1} and 2819 cm^{-1}** , associated with C–H stretching and conjugated C=C or C=C bonds,
- A distinct band at **1648 cm^{-1}** , corresponding to amide I (C=O stretching),
- **1407 cm^{-1}** , signifying inorganic carbonate groups (C=O),
- and a strong absorption at **1099 cm^{-1}** , suggestive of C–O–C linkages, likely from polysaccharides.

These functional groups, originating from phytochemicals such as flavonoids, proteins, and polysaccharides, not only participate in the reduction of metal ions but also act as stabilizing agents, capping the nanoparticles and preventing aggregation.

3.1.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is commonly utilized to assess the surface morphology and approximate size of biosynthesized nanoparticles. Studies involving *Cissus quadrangularis*-mediated nanoparticle synthesis reveal that SEM imaging provides detailed insights into particle shape and aggregation patterns.

Silver nanoparticles typically exhibit **spherical to oval morphologies with a size distribution ranging from 30 to 74 nm**, indicating uniformity in biosynthesis and effective stabilization by plant-derived metabolites.

In contrast, copper nanoparticles synthesized via similar green protocols are often reported to have **average diameters near 100 nm**, although their morphology may vary depending on reaction conditions and plant extract composition. The larger size of CuNPs relative to AgNPs may be attributed to the difference in reduction kinetics and capping efficiency of the phytochemicals involved.

These morphological observations through SEM further support the efficacy of *Cissus quadrangularis* extracts in controlling nanoparticle shape and size, a critical factor influencing their antimicrobial potential.

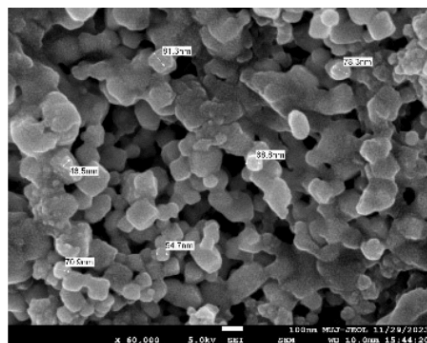


Fig.6 Scanning electron microscopy (SEM) images of silver nanoparticles (AgNPs), showing their morphology and size distribution (30–74 nm).

3.1.4 Antimicrobial Activity

The antimicrobial efficacy of biosynthesized silver (AgNPs) and copper nanoparticles (CuNPs) was evaluated against selected Gram-positive and Gram-negative bacterial strains, including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The assessment was conducted using the agar well diffusion method at two concentrations—250 mg/L and 500 mg/L—to observe the dose-dependent response.

The results revealed a significant antibacterial effect exhibited by both types of nanoparticles across all tested strains. Notably, AgNPs demonstrated comparatively greater zones of inhibition than CuNPs, indicating a higher degree of bactericidal activity, particularly at the higher concentration of 500 mg/L. Among the tested microorganisms, *S. aureus* and *E. coli* showed considerable susceptibility to AgNPs, while CuNPs also exhibited effective antibacterial activity, albeit to a slightly lesser extent.

These findings were benchmarked against streptomycin, a commonly used standard antibiotic. In most cases, nanoparticle treatments at higher concentrations produced inhibition zones comparable to or greater than those observed with the standard drug.

Table 1 summarizes the comparative antibacterial performance of AgNPs and CuNPs at both tested concentrations, emphasizing their potential application as alternative antimicrobial agents in the context of rising antibiotic resistance.

S.no	Organism	Standard	Silver NP'S				Cu NP'S			
			250		500		250		500	
			AI	IZ	AI	IZ	A I	IZ	AI	IZ
1	<i>P.aeruginosa</i>	32	0.5	16+ _{-0.25}	0.485	17+ _{-0.35}	0.343	11+ _{-1.02}	0.571	20+ _{-1.05}
2	<i>B.cereus</i>	35	0.428	15+ _{-0.52}	0.486	18+ _{-0.69}	0.742	28+ _{-1.55}	0.756	26+ _{-1.33}
3	<i>S.aureus</i>	35	0.428	15+ _{-0.85}	0.555	20+ _{-0.78}	0.542	19+ _{-1.03}	0.777	28+ _{-0.22}
4	<i>E.coli</i>	32	0.468	15+ _{-0.62}	0.5	17+ _{-0.95}	0.281	9+ _{-0.66}	0.735	25+ _{-1.05}



Figure 7. Comparative antibacterial activity of biosynthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. The inhibition zones (IZ) were measured using the agar well diffusion method at nanoparticle concentrations of 250 mg/L and 500 mg/L, highlighting the dose-dependent antimicrobial efficacy of both nanoparticle types.

3.1.5 Antifungal Activity

The antifungal efficacy of green-synthesized silver (AgNPs) and copper nanoparticles (CuNPs), derived from *Cissus quadrangularis* stem extract, has been evaluated against a spectrum of clinically and agriculturally significant fungal pathogens, including *Penicillium chrysogenum*, *Fusarium oxysporum*, *Aspergillus niger*, and *Candida albicans*. These findings contribute to the growing body of evidence supporting the potential of plant-mediated nanoparticles as broad-spectrum antifungal agents. The activity is typically quantified through agar well diffusion assays, with inhibition zones indicating the extent of fungal growth suppression. Such studies consistently demonstrate that both AgNPs and CuNPs exhibit dose-dependent antifungal activity, with variations in sensitivity observed among different fungal strains.

Table 2: Antifungal activity (Inhibition Zone in mm and Activity Index) of Silver and Copper Nanoparticles Synthesized from *Cissus quadrangularis* against Selected Fungal Strains

Sr. No.	Organism	Standard (IZ mm)	Silver NPs (250 mg/L) AI	IZ (mm)	Silver NPs (500 mg/L) AI	IZ (mm)	Copper NPs (250 mg/L) AI	IZ (mm)	Copper NPs (500 mg/L) AI	IZ (mm)
1	<i>Penicillium chrysogenum</i>	15	1.00	15 ± 1.02	0.89	17 ± 0.95	0.86	13 ± 0.84	0.84	16 ± 0.45
2	<i>Fusarium oxysporum</i>	17	1.11	19 ± 0.65	1.05	21 ± 1.05	0.88	15 ± 0.78	0.95	19 ± 0.95
3	<i>Aspergillus niger</i>	15	1.20	18 ± 0.99	1.11	20 ± 0.82	0.93	14 ± 0.46	1.00	18 ± 0.84
4	<i>Candida albicans</i>	20	0.85	17 ± 1.01	0.68	20 ± 0.94	0.80	16 ± 0.94	0.72	21 ± 1.11

Note: AI = Activity Index; IZ = Inhibition Zone (in mm); ± values represent standard deviation.

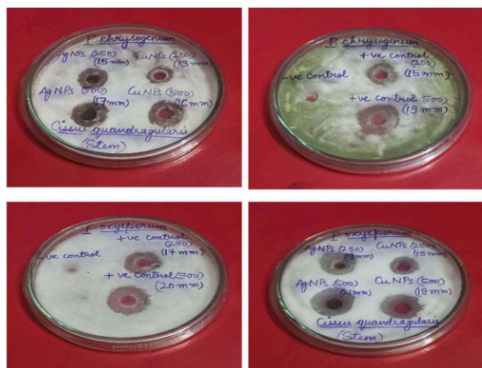


Fig. 8. Comparative antifungal efficacy of biosynthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against *Fusarium oxysporum* and *Penicillium chrysogenum*. The inhibition zones (IZ) were recorded at two concentrations—250 mg/L and 500 mg/L—demonstrating dose-dependent antifungal activity.

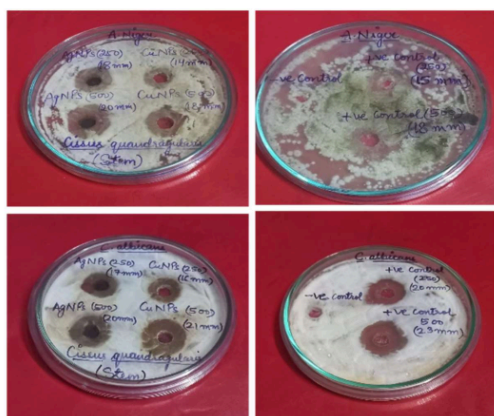


Fig. 9. Antifungal potential of green-synthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against *Candida albicans* and *Aspergillus niger*. Inhibition zone (IZ) diameters were measured at two different concentrations (250 mg/L and 500 mg/L), highlighting the concentration-dependent response of the fungal strains to the nanoparticles.

5. Discussion

The present study demonstrates the successful green synthesis of silver (AgNPs) and copper nanoparticles (CuNPs) using stem extracts of *Cissus quadrangularis*, and evaluates their antimicrobial efficacy against a broad spectrum of pathogenic bacteria and fungi. The use of

4
plant-derived phytochemicals as reducing and stabilizing agents underscores the eco-friendly and sustainable nature of this synthesis approach.4 These findings are consistent with prior studies advocating plant-mediated nanoparticle synthesis due to its cost-effectiveness, reduced environmental impact, and biocompatibility (Hussain et al., 2016; Dubey et al., 2024).

The antimicrobial assays revealed that CuNPs exhibited superior inhibitory effects compared to AgNPs across all tested microbial strains. Notably, at 500 µg/mL, CuNPs produced inhibition zones of 28 mm against *Bacillus cereus* and 25 mm against *Escherichia coli*, while AgNPs yielded zones of 18 mm and 17 mm, respectively (Alavi & Moradi, 2022). The enhanced antimicrobial activity of CuNPs is likely due to their higher surface reactivity and their ability to generate reactive oxygen species (ROS), which disrupt microbial membranes and cellular processes (Mammari et al., 2024). These trends were mirrored in antifungal assays, where CuNPs demonstrated strong activity, with inhibition zones of 21 mm for *Candida albicans* and 19 mm for *Fusarium oxysporum* (Parveen et al., 2023).

Such outcomes are in line with previous research involving CuNPs synthesized from other medicinal plants like *Azadirachta indica* and *Withania somnifera*, which also reported potent antimicrobial effects attributed to cellular membrane disruption and interference with microbial metabolism (Kashyap et al., 2022; Sarkar et al., 2021).

Characterization analyses confirmed the successful synthesis and physicochemical stability of the nanoparticles. UV-Visible spectroscopy indicated prominent surface plasmon resonance (SPR) peaks at 420 nm for AgNPs and 470 nm for CuNPs, which is a characteristic signature of metal nanoparticles (El-Sayyad et al., 2024; Shah & Lu, 2018). The nanoparticles exhibited size ranges between 30–74 nm for AgNPs and approximately 100 nm for CuNPs, which aligns with existing evidence suggesting that smaller particle sizes enhance surface area and antimicrobial potency (Aminzai et al., 2024). FTIR spectroscopy revealed key functional groups such as C-H, C=C, and NH₂, supporting the role of bioactive phytoconstituents in the reduction and capping of metal ions during nanoparticle formation (Ishak et al., 2019).

The superior efficacy of CuNPs over AgNPs also holds clinical relevance in addressing multidrug-resistant (MDR) microbial infections. CuNPs exert a dual mechanism of action involving direct interaction with microbial membranes and oxidative stress through ROS generation, enabling them to effectively target both Gram-positive and Gram-negative bacteria, as well as fungal pathogens (Wahab et al., 2019; Badoni & Prakash, 2024). While AgNPs also possess antimicrobial capabilities, their primary mechanism—disruption of membrane integrity and interference with cellular respiration—may be less effective against certain resistant strains (Dakal et al., 2016).

In comparison with other botanical sources previously used for nanoparticle synthesis, such as *Tinospora cordifolia* and *Withania somnifera*, the use of *Cissampelos quadrangularis* has shown comparable or enhanced antimicrobial performance, particularly in the case of CuNPs (Puri et al., 2024; Tortella et al., 2021). The phytochemical richness of *C. quadrangularis*, including flavonoids, phenolic compounds, and alkaloids, likely contributes to the effective synthesis and stabilization of bioactive nanoparticles, further enhancing their antimicrobial spectrum (Ovais et al., 2018).

6. Conclusion and Future Perspectives

This study demonstrates the successful green synthesis of silver (AgNPs) and copper nanoparticles (CuNPs) using stem extracts of *Cissus quadrangularis*, reinforcing the plant's potential as an effective biogenic resource [21] sustainable nanomaterial production. The synthesized nanoparticles exhibited notable antimicrobial activity against a spectrum of Gram-positive and Gram-negative bacteria, as well as pathogenic fungi, with CuNPs consistently showing superior efficacy over AgNPs. These findings emphasize the utility of *C. quadrangularis*-mediated nanoparticles as promising alternatives to conventional antimicrobial agents, particularly in the context of rising multidrug-resistant (MDR) pathogens.

The characterization techniques, including UV-Visible spectroscopy, FTIR, and scanning electron microscopy (SEM), confirmed the formation, stability, and nanoscale morphology of the nanoparticles. The distinct surface plasmon resonance peaks, functional group signatures, and nanoscale dimensions underscore the successful biosynthesis and structural integrity of the nanoparticles.

Looking ahead, future research should aim to scale up the green synthesis protocols to evaluate their commercial feasibility and environmental sustainability. Further mechanistic studies focusing on nanoparticle-microbe interactions, including the role of reactive oxygen species (ROS) generation and disruption of microbial metabolic pathways, would provide deeper insights into their antimicrobial mode of action. Additionally, the potential application of CuNPs in biomedical domains such as wound healing, targeted drug delivery, and medical coatings, as well as in agriculture and environmental remediation, presents promising avenues for exploration.

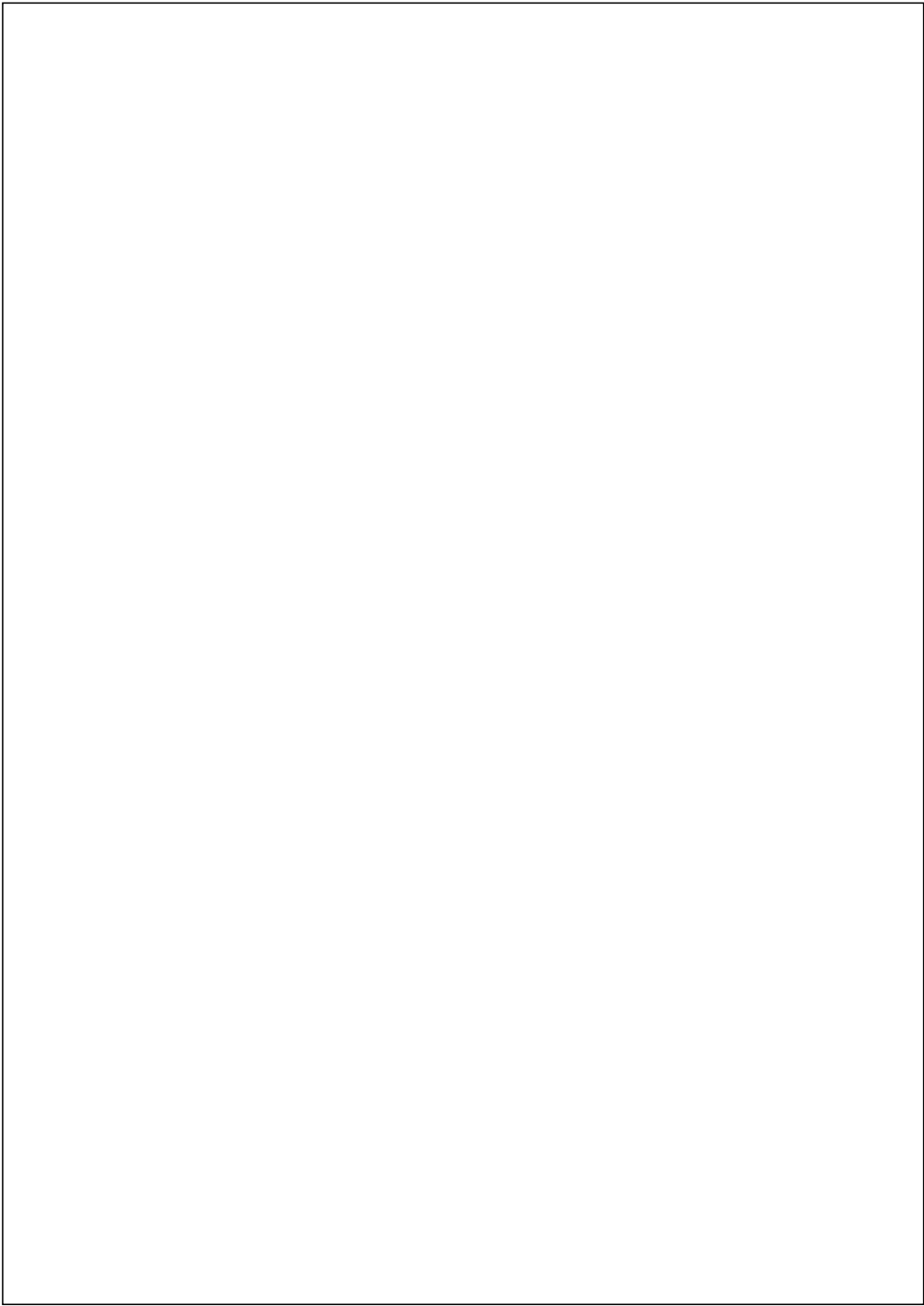
This work contributes to the evolving field of green nanotechnology, highlighting the relevance of plant-based approaches in the design and development of eco-friendly, bioactive nanomaterials with broad-spectrum utility.

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