ISSN: 2640-5571

Research Article

Advances in Nanoscience and Nanotechnology

Comparative Study of Copper and Silver Nanoparticles Synthesized from Extract of *Cissus Quandanularis* l. Stem and their Antimicrobial Activity

Ruchika Khatri[1], Rohit Jain[2], Abhishek Dadhich[2] and Smita Purohit[1*]

[1]*IIS (deemed to be university), Jaipur*

[2]*Department of Biosciences, Manipal University Jaipur*

[*]Corresponding Author
Sumita Purohit, IIS (deemed to be university), Jaipur.

Submitted: 2025, Jan 02; **Accepted:** 2025, Jan 28; **Published:** 2025, Feb 07

Citation: Khatri, R., Jain, R., Dadhich, A., Purohit, S. (2025). Comparative Study of Copper and Silver Nanoparticles Synthesized from Extract of Cissus Quandanularis l. Stem and their Antimicrobial Activity. *Adv Nanoscie Nanotec, 9*(1), 01-10.

Abstract

Nanotechnology has revolutionized antimicrobial strategies, with green synthesis of nanoparticles emerging as a sustainable and eco-friendly alternative to conventional methods. This study explores the synthesis of copper (CuNPs) and silver nanoparticles (AgNPs) using stem extracts of Cissus quadrangularis, a medicinal plant known for its rich phytochemical profile. The objective was to compare the antimicrobial efficacy of CuNPs and AgNPs against Gram-positive and Gram-negative bacteria, including Escherichia coli and Staphylococcus aureus, and fungal strains such as Fusarium oxysporum and Aspergillus niger. The nanoparticles were synthesized using green methods and characterized by UV-Vis spectroscopy, FTIR, and SEM, confirming their stability, nanoscale size (30–74 nm for AgNPs and approximately 100 nm for CuNPs), and morphology. The results indicated that CuNPs displayed superior antimicrobial activity, with inhibition zones measuring up to 28 mm for Bacillus cereus and 25 mm for E. coli, compared to AgNPs, which achieved inhibition zones of 18 mm and 17 mm, respectively, at 500 µg/mL. Similarly, CuNPs demonstrated significant antifungal activity, with inhibition zones of 21 mm for Candida albicans and 19 mm for Fusarium oxysporum. These findings underscore the enhanced efficacy of CuNPs over AgNPs, highlighting their potential as effective antimicrobial agents. Cissus quadrangular is mediated synthesis of CuNPs offers a promising pathway for sustainable nanotechnology applications. Future studies should focus on scaling up production, elucidating antimicrobial mechanisms, and exploring CuNPs' integration into medical and industrial antimicrobial systems.

Keywords: Antimicrobial, Nanoparticles, Pathogenic, Cissus Quandrangularis

1. Introduction

Currently, green fabrication of synthetic compounds or materials plays a key part in the betterment of humanity and for the sustainment of nature. 'Green Chemistry' is a better source of production of non-toxic nanomaterials in contrast to harmful chemical compounds [1,2]. Nanomaterials are materials that have at least one dimension (1–100 nm) in the nanometer scale range or whose basic unit in the three-dimensional space is in this range. NPs in particular have demonstrated broad-spectrum antibacterial properties against both Gram-positive and Gram-negative pathogenic bacteria [3]. In the reported study done against E.*Coli* ,*S.Aureus* ,*bacillus* ,*Psuedomonas* and fungus strain i.e, Fusarium ,*Aspergillus* ,*candida* ,*Penicillium* on *Cissus Quandrangularis L. Cissus quandrangularis* L. belongs to family Vitaceae and is an traditional medicinal plant of India. *C. quadrangularis* is a tendril-climbing shrub with stout and tendrils , fleshy and muscular quadrangular stems that is found throughout India and nearby countries [4]. The synthesis of nanoparticles using eco-friendly methods has garnered significant attention in recent years, as it offers a sustainable and cost-effective alternative to conventional chemical synthesis technique [5]. Among various nanomaterials, metal nanoparticles such as copper (CuNPs) and silver (AgNPs) are well known for their unique physicochemical properties and potent antimicrobial activities. The nanoscale properties of these particles enable enhanced interaction with microbial cells, making them effective against a broad spectrum of bacterial and fungal pathogens [6,7] Recent advancements in green synthesis approaches, which utilize plant extracts as reducing and capping agents, have provided an eco-friendly pathway for nanoparticle production, aligning with the principles of green chemistry [8]. Plant-mediated synthesis of nanoparticles leverages the rich phytochemical content of plants, including alkaloids, flavonoids, and phenolic compounds, which play a dual role in reducing metal ions and stabilizing the resulting nanoparticles. Studies

have demonstrated the effectiveness of various medicinal plants in synthesizing nanoparticles with improved antimicrobial activity [9,10] For example, nanoparticles synthesized from *Azadirachta indica* have shown strong antibacterial effects against both Gram-positive and Gram-negative bacteria [11] Similarly, *Withania somnifera* and *Tinospora cordifolia* extracts have been utilized to produce nanoparticles with broad-spectrum antimicrobial and antioxidant properties [12,13] In line with these findings, the present study focuses on *Cissus quadrangularis*, a traditional medicinal plant renowned for its rich phytochemical profile and therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities [14]. Previous work has shown that green-synthesized nanoparticles from *Cissus quadrangularis* exhibit notable biological activities, making it an ideal candidate for nanoparticle synthesis [15,16] Furthermore, research comparing copper and silver nanoparticles has consistently highlighted the superior antimicrobial efficacy of CuNPs, attributed to their ability to generate reactive oxygen species (ROS) and disrupt microbial cell walls more effectively than AgNPs [17].

This study aims to synthesize and compare copper and silver nanoparticles using extracts of *Cissus quadrangularis* and evaluate their antimicrobial activity against a range of bacterial and fungal pathogens. By employing advanced characterization techniques such as UV-Vis spectroscopy, FTIR, and SEM, the research not only elucidates the structural properties of these nanoparticles but also establishes their potential for biomedical and industrial applications. Building on prior studies, this work contributes to the growing evidence supporting plant-mediated nanoparticle synthesis as a sustainable and effective approach in nanotechnology.

2. Material and Methods

2.1 Sample Collection

The present study was conducted at the Department of Botany, IIS (Deemed to be University) Jaipur, Rajasthan, during the academic year 2023-24. Fresh stems of Cissus quadrangularis L. were collected from Karpurchand Kulish Udhyan, Jaipur, Rajasthan, and subsequently identified at the herbarium of the Department of Botany, IIS (Deemed to be University), Jaipur.

2.2 Preparation of Plant Extract

To prepare the plant extract for the synthesis of silver nanoparticles, 10 grams of fresh stems of *Cissus quadrangularis* L. were weighed and crushed using a mortar and pestle to form a paste. For copper nanoparticle synthesis, 2 grams of dried stem powder were used. The dried stems were washed with distilled water, shade-dried, and ground into a fine powder. The prepared paste or powder was boiled with 100 mL of distilled water for 5 minutes to activate the metabolites. The solution was then filtered and strained to remove any solid residues. The resulting extract was stored at 4°C until further use.

3. Methods to Synthesize Nanoparticles

3.1 Synthesis of Silver Nanoparticles

To synthesise silver nanoparticles, 10 mL of fresh stem extract was gradually introduced to 90 mL of a 1 mM silver nitrate solution while continuously swirling with a magnetic stirrer for 50 minutes. The solution's hue gradually changed from white to red, indicating the formation of silver nanoparticles. The solution was allowed to rest for one hour and then centrifuged at 10,000 rpm to acquire the pellet. The pellet was thoroughly washed with ethanol to remove impurities, then oven-dried and stored for future analysis.

Figure 1: Schematic illustration of the green synthesis of silver nanoparticles (AgNPs) using *Cissus quadrangularis* stem extract. The process involves the reduction of Ag^+ ions by bioactive phytochemicals present in the extract

3.2 Synthesis of Copper Nanoparticle

In the conventional synthesis of copper nanoparticles, 10 ml of *C. vitiginea* leaf extract was combined with 90 ml of 10 mM copper sulphate solution and stirred continuously at room temperature. The visual investigation revealed a colour shift in the fluid, confirming the creation of nanoparticles. Centrifuge at 10,000 rpm, followed by washing with ethanol. Collect the pellet, oven-dry it, and store it for further analysis [18].

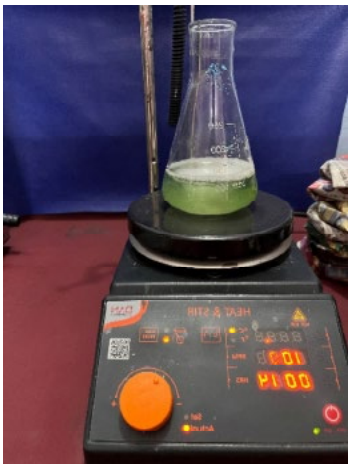

Figure 2: Schematic representation of the green synthesis of copper nanoparticles (CuNPs) from *Cissus quadrangularis* stem extract, showing the reduction of Cu^{2+} ions by plant metabolites

4. Antibacterial Activity

The Agar Well Diffusion method was employed for in vitro antibacterial assessment using standard microbial techniques. The various samples were diluted with 10% dimethyl sulfoxide (DMSO), and two concentrations (250 mg/L and 500 mg/L) of all chemicals were generated. Disinfected Petri dishes containing nutritional agar (NA) medium were utilised for the inoculation of test microorganisms; the inoculum was evenly distributed around the dish using a spreader and allowed to stand for 30 minutes. Wells with a diameter of 6 mm were created in the agar plates that had been seeded. A standard medication was similarly administered in a separate petri dish at equivalent concentrations. All varying concentrations of the samples and standard medication (30 µl) were dispensed into the designated wells of the seeded plates. The plates were incubated at 37ºC for 24 hours. The antibacterial spectrum of the test sample was assessed using the measurement of the inhibition zone (IZ) surrounding each prepared well. A comparison was conducted between the widths of the inhibition zones produced by the test sample and the commercial control antibiotic (streptomycin).

4.1 Minimum Inhibition Concentration (MIC)

The previous research employed the agar well diffusion method to determine the minimal inhibitory concentration. The organisms designated for testing were cultivated in broth at 37°C for a duration of 24 hours for bacterial growth. The MIC nutritional agar was poured into sterilised Petri plates and allowed to settle for 30 minutes under UV light. Upon solidification, the bacterial culture was distributed throughout the petri plate, and wells were created in the agar medium using a sterilized cork borer. Subsequently, silver and copper nanoparticles at varying concentrations (25, 50, 100, 150, 175, and 200 microgrammes per millilitre) were introduced into the wells. The inoculated plates were subsequently incubated at 37ºC for 24 hours, and the zones of inhibition were measured in millimetres. Three duplicates were created for inoculum concentrations of 25, 50, 100, 150, 175, and 200 micrograms/ml.

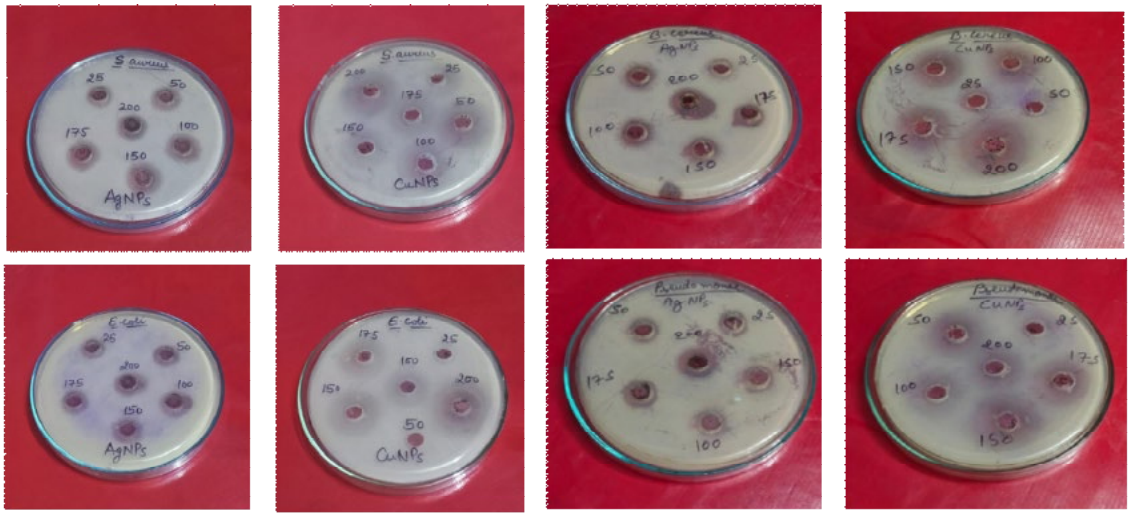

Figure 3: Minimum inhibitory concentration (MIC) assay results of silver and copper nanoparticles against bacterial strains **(***Staphylococcus aureus, Escherichia coli, Bacillus cereus,* and *Pseudomonas aeruginosa***)** at various concentrations (25, 50, 100, 150, 175, and 200 µg/mL)

4.2 Antifungal Activity

The Agar Well Diffusion method was employed as the standard microbiological methodology for *in vitro* antifungal assessment. The various samples were diluted with 10% dimethyl sulfoxide (DMSO), and two concentrations (250 mg/L and 500 mg/L) of all chemicals were generated. Disinfected petri plates with PDA medium were utilised for the inoculation of test microorganisms; the inoculum was evenly distributed around the dish using a spreader and allowed to stand for 30 minutes. Wells with a diameter of 6 mm were created in the seeded PDA plates. In a separate petri dish, the standard medication was administered at equivalent concentrations. All varying concentrations of the samples and standard medication (30 µl) were dispensed into the designated wells of the seeded plates. The plates were incubated at 37ºC for 72 hours. The antifungal spectrum of the test sample was assessed by measuring the inhibition zone (IZ) surrounding each prepared well. The sizes of the inhibition zones produced by the test sample and the commercial control antibiotic (ketoconazole) were compared.

4.3 Minimum Inhibition Concentration (Mic)

The previously mentioned research employed the agar well diffusion method to determine the minimal inhibitory concentration. The organisms designated for testing were cultivated in potato dextrose media at 37°C for 72 hours for bacterial growth. The potato dextrose was prepared for inoculation, and the medium were poured into sterile Petri plates and allowed to settle for 30 minutes under UV light. Upon solidification, the bacterial culture was distributed throughout the petri plate, and wells were created in the PDA medium using a sterilised cork borer. Subsequently, silver and copper nanoparticles at varying concentrations (25, 50, 100, 150, 175, and 200 microgrammes per millilitre) were introduced into the wells. The inoculated plates were subsequently incubated at 37ºC for 72 hours, and the zones of inhibition were measured in millimetres. Three duplicates were created for inoculum concentrations of 25, 50, 100, 150, 175, and 200 micrograms/ml.

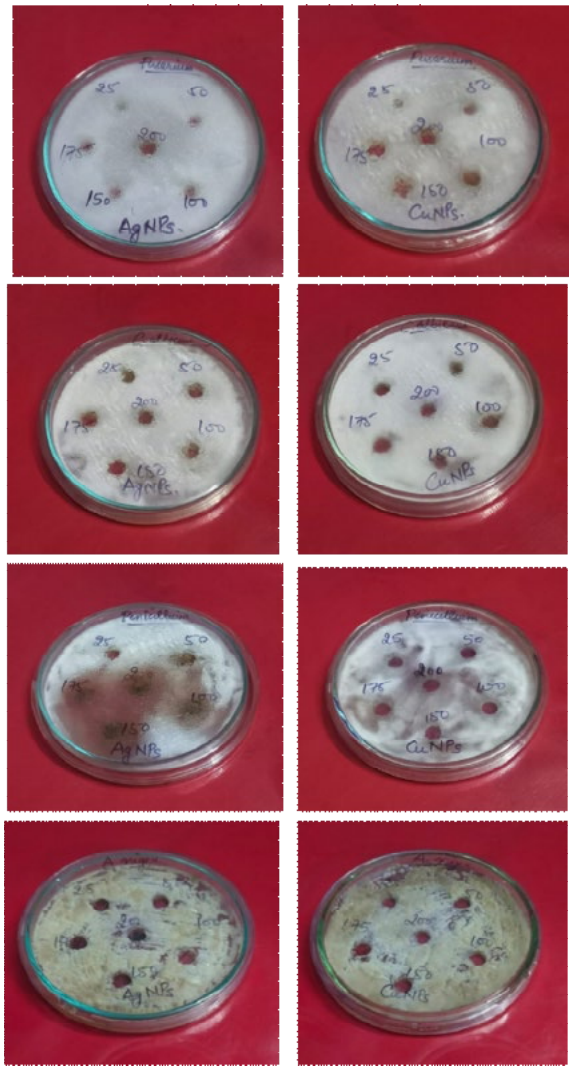

Figure 4: MIC assay results of silver and copper nanoparticles against fungal strains **(***Fusarium oxysporum, Aspergillus niger, Candida albicans,* and *Penicillium chrysogenum***)** at concentrations of 25, 50, 100, 150, 175, and 200 µg/mL

5. Result and Discussion

5.1 Characterization

5.1.1 Uv Visible Spectrophotometer

The synthesised copper nanoparticles were characterised using a Hitachi UV-2300 UV-Vis spectrophotometer, with continuous scanning conducted from 300 to 700 nm. Deionised water served as the blank. The reaction mixtures were analysed using UV–Visible spectroscopy to determine the maximum absorbance within the range of 300–700 nm. The results indicate that the creation of CuO nanoparticles was confirmed, with the maximum absorbance peak at 420 nm for silver nanoparticles and 470 nm for copper nanoparticles, where surface plasmon resonance was distinctly observed in the peaks. The surface plasmon resonance characteristics of metallic nanoparticles, influenced by their shape and size, can be ascertained using UV-visible absorption measurements. The growing size of nanoparticles (NPs) generally leads to a significant red shift in the wavelength absorption spectrum, whereas smaller NPs exhibit a blue shift. The distinctive peaks of silver and CuO nanoparticles were observed between 420-470 nm in the UV-Vis spectra, corresponding to the frequency of surface plasmon oscillations.9

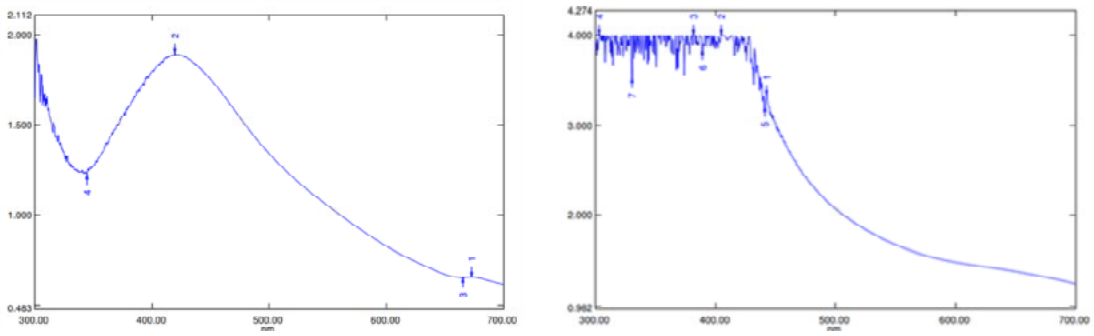

Figure 5: UV-Vis spectra of synthesized silver nanoparticles (AgNPs) (a) and copper nanoparticles (CuNPs) (b), showing surface plasmon resonance peaks at 420 nm and 470 nm, respectively

5.2 Fourier Transform Infrared Spectroscopy

FTIR analysis identifies the functional groups of the silver and copper nanoparticles synthesised utilising stem extracts of C. quadrangularis, as illustrated in the figure. The silver nanoparticles exhibit distinct peaks at 2921 cm, indicating C-H and C-H2 stretching of the aliphatic group in alkanes. The weak bands at 1601 cm correspond to C=C unsaturated molecules. The band at 1362 cm corresponds to the CH aliphatic bending group, while a moderate peak at 1040 cm is attributed to the alkyl aryl ether group. Conversely, FTIR examination of copper nanoparticles reveals a significant peak at 3211, indicating the NH2 stretching bonds of the amino acid group. A little peak at 2886 cm indicates the stretching vibrations of CH and CH2 groups, whereas a comparable peak at 2819 cm is attributed to the stretching of C=C conjugates and --C≡C stretches. The peak at 1648 cm indicates the presence of a -C=O amide bond, while the minor peak at 1407 cm signifies the stretching of C=O in inorganic carbonate. A pronounced peak at 1099 cm indicates the presence of C-O-C polysaccharide bonds. These groups are accountable for the reduction of silver and copper nanoparticles and the production of nanoparticles of silver and copper.

5.3 Scanning Electron Microscopy

Scanning Electron Microscopy elucidated the surface shape and dimensions of nanoparticles. The SEM analysis revealed an average size of 100 nm. The two synthesised nanoparticles of silver and copper are hereby shown. The SEM image of silver nanoparticles indicates that the synthesised particles range from 30 to 74 nm in size, exhibiting round and oval shapes, but the size of the copper nanoparticles is unspecified.

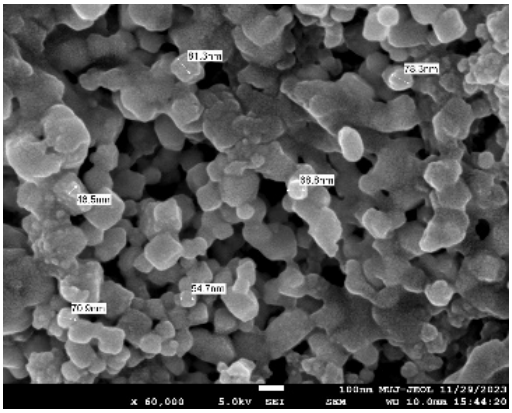

Figure 6: Scanning electron microscopy (SEM) images of silver nanoparticles (AgNPs), showing their morphology and size distribution (30–74 nm)

6. Antimicrobial Activity

This study demonstrates the microbiological resistance of synthesized silver and copper nanoparticles against P. aeruginosa, B. cereus, S. aureus, and E. coli at two concentrations of 250 and 500 microgrammes per litre.

S.no	Organism	Standard	Silver NP'S				Cu NP'S			
			250		500		250		500	
			AI	IZ	AI	IZ	A I	IZ	AI	IZ
1	*P.aeroginosa*	32	0.5	16+_0.25	0.485	17+_0.35	0.343	11+_1.02	0.571	20+_1.05
2	*B.cereus*	35	0.428	15+-0.52	0.486	18+_0.69	0.742	28+-1.55	0.756	26+_1.33
3	*S.aureus*	35	0.428	15+-0.85	0.555	20+_0.78	0.542	19+-1.03	0.777	28+_0.22
4	*E.coli*	32	0.468	15+-0.62	0.5	17+_0.95	0.281	9+_0.66	0.735	25+_1.05

Table 1: Antibacterial activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against Gram-positive and Gram-negative bacteria (*Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli*) at concentrations of 250 mg/L and 500 mg/L, compared to the standard antibiotic (Streptomycin)

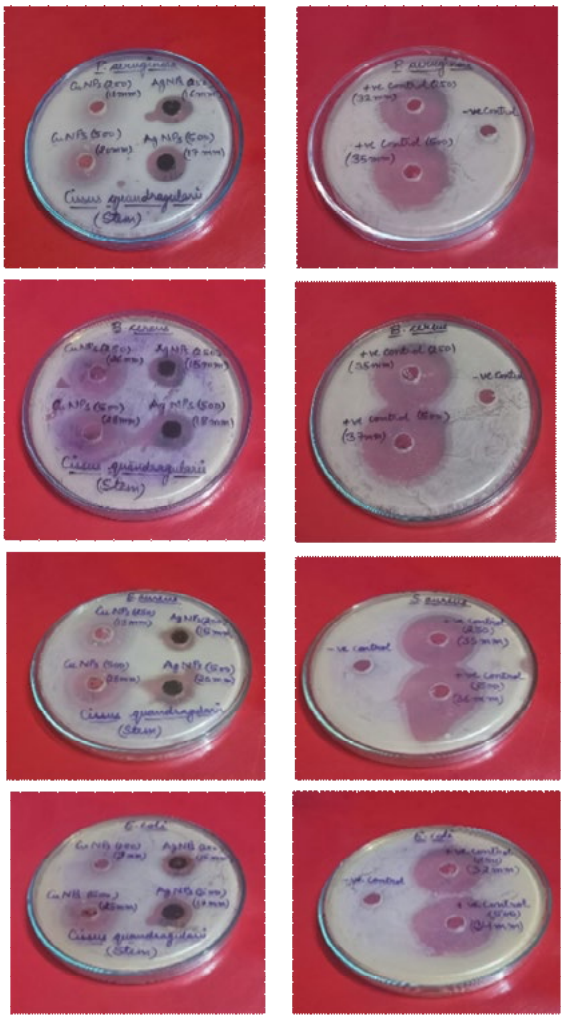

Figure 6: Antibacterial activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, and Escherichia coli. Inhibition zones (IZ) were measured at concentrations of 250 mg/L and 500 mg

7. Antifungal Activity

Antifungal activity was assessed against four fungal strains: *Penicillium chrysogenum, Fusarium oxysporum, Aspergillus niger,* and *Candida albicans*, using silver and copper nanoparticles synthesised from the stem of *Cissus quadrangularis* L.

Sr.no.	Organism	Standard	Silver NP'S				Copper NP'S			
			250		500		250		500	
			AI	IZ	AI	IZ	AI	IZ	AI	IZ
1	*Penicillium chrysogenum*	15	1.0	15+_1.02	0.89	17+_0.95	0.86	13+_0.84	0.84	16+_0.45
2	*Fusarium oxysporum*	17	1.11	19+-.065	1.05	21+_1.052	0.88	15+_0.78	0.95	19+_0.95
3	*A.niger*	15	1.2	18+_0.99	1.11	20+_0.82	0.933	14+_.046	1	18+_0.84
4	*C.albicans*	*20*	0.85	17+_1.01	0.68	20+_0.94	0.8	16+_0.94	0.72	21+_1.11

Table 2: Antifungal activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against fungal strains (*Penicillium chrysogenum*, *Fusarium oxysporum*, *Aspergillus niger*, and *Candida albicans*) at concentrations of 250 mg/L and 500 mg/L, compared to the standard antifungal drug (Ketoconazole)

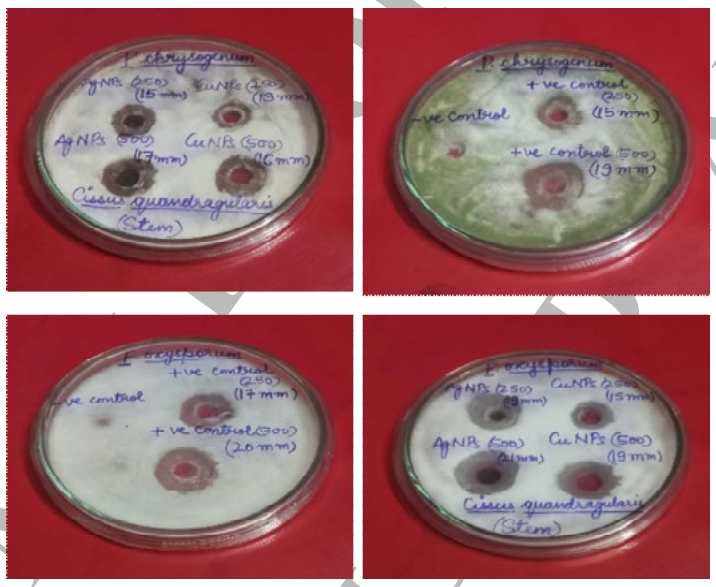

Figure 7: Antifungal activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against Fusarium oxysporum and Penicillium chrysogenum. Inhibition zones (IZ) were measured at concentrations of 250 mg/L and 500 mg/L

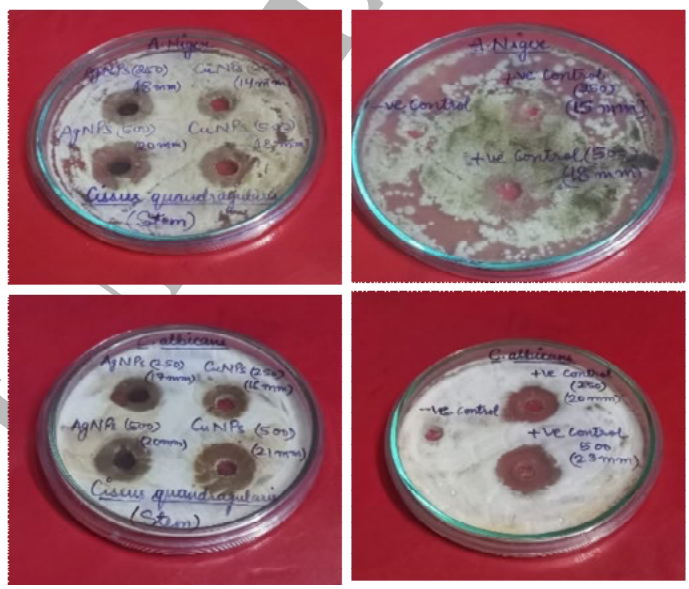

Figure 8: Antifungal activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against *Candida albicans* and *Aspergillus niger*. Inhibition zones (IZ) were assessed at concentrations of 250 mg/L and 500 mg/L

8. Discussion

This study successfully synthesized copper (CuNPs) and silver nanoparticles (AgNPs) using stem extracts of *Cissus quadrangularis*, demonstrating their efficacy as antimicrobial agents against a broad range of bacterial and fungal pathogens. The green synthesis approach, utilizing phytochemical constituents as reducing and capping agents, offers a sustainable alternative to conventional chemical methods. This aligns with previous research highlighting the advantages of plant-mediated nanoparticle synthesis, including reduced toxicity, cost-effectiveness, and environmental compatibility [19,20]. The antimicrobial results revealed that CuNPs exhibited superior activity compared to AgNPs. At a concentration of 500 µg/mL, CuNPs produced inhibition zones of 28 mm for Bacillus cereus and 25 mm for Escherichia coli, while AgNPs achieved zones of 18 mm and 17 mm, respectively [21,22]. This enhanced efficacy of CuNPs can be attributed to their higher reactivity and the generation of reactive oxygen species (ROS), which induce oxidative stress and damage microbial cell membranes [23]. Similar trends were observed in antifungal assays, where CuNPs demonstrated significant inhibition, with zones of 21 mm for *Candida albicans* and 19 mm for *Fusarium oxysporum* [24]. These findings are consistent with prior studies, which reported that CuNPs synthesized using other medicinal plants, such as *Azadirachta indica* and *Withania somnifera*, displayed potent antimicrobial properties due to their ability to disrupt microbial structures and interfere with essential cellular processes [25].

The characterization results further validate the successful synthesis and stability of the nanoparticles. UV-Vis spectroscopy confirmed the formation of CuNPs and AgNPs, with absorbance peaks at 470 nm and 420 nm, respectively, indicative of surface plasmon resonance (SPR) [26]. The observed size range, 30–74 nm for AgNPs and approximately 100 nm for CuNPs, aligns with previous studies emphasizing that smaller nanoparticles exhibit increased surface area and higher antimicrobial activity [27]. FTIR analysis identified functional groups such as C-H, C=C, and NH_2, which are likely involved in the reduction and stabilization processes, further supporting the role of phytochemicals in nanoparticle synthesis [28].

The superior antimicrobial performance of CuNPs over AgNPs is particularly significant in the context of multidrug-resistant (MDR) pathogens. Copper ions released from CuNPs can penetrate bacterial membranes, bind to proteins and DNA, and induce cellular damage through ROS generation [29]. This dual mechanism of action makes CuNPs highly effective against both Gram-positive and Gram-negative bacteria, as well as fungal strains [30]. In contrast, while AgNPs also exhibit strong antimicrobial properties, their mode of action is primarily through the disruption of bacterial cell membranes and inhibition of cellular respiration, which may be less effective against certain resistant strains [31]. In comparison with previous works, the current study highlights the enhanced efficacy of CuNPs synthesized using *Cissus quadrangularis*. For instance, studies involving *Tinospora cordifolia* and *Withania somnifera* have reported similar findings, where CuNPs exhibited greater antimicrobial activity than AgNPs, suggesting a broader spectrum of efficacy and a lower likelihood of resistance development [32,33]. The plant's rich phytochemical profile, including flavonoids, phenolics, and alkaloids, likely contributes to the reduction and capping processes, enhancing the stability and bioactivity of the synthesized nanoparticles [34].

9. Conclusion and Future Prospective

The research effectively illustrated the green synthesis of copper (CuNPs) and silver nanoparticles (AgNPs) utilising extracts from Cissus quadrangularis, underscoring the plant's viability as a biogenic source for sustainable nanoparticle fabrication. The synthesised nanoparticles had significant antibacterial activity against various Gram-positive and Gram-negative bacteria, as well as fungal species, with CuNPs exhibiting more efficacy than AgNPs. Advanced characterization techniques, including UV-Vis spectroscopy, FTIR, and SEM, validated the production, stability, and nanoscale shape of the particles. The results highlight the promise of CuNPs as a sustainable substitute for traditional antibacterial medicines, especially in combating multidrug-resistant infections [35].

Subsequent investigations ought to concentrate on amplifying the green synthesis procedure to assess its commercial viability and ecological implications. Furthermore, examining the processes that govern the antibacterial efficacy of these nanoparticles, particularly their interactions with microbial cells and the formation of reactive oxygen species (ROS), might yield more profound insights. The integration of CuNPs in biomedical applications, including wound healing and medication delivery systems, as well as their prospective utilisation in agricultural and industrial contexts, warrants additional investigation. This research advances green nanotechnology and underscores the significance of plant-derived technologies in the sustainable and novel synthesis of nanoparticles.

Reference

1. Agarwal, H., Menon, S., Kumar, S. V., & Rajeshkumar, S. (2018). Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. *Chemico-biological interactions, 286*, 60-70.
2. Rajeshkumar, S., & Bharath, L. V. (2017). Mechanism of plant-mediated synthesis of silver nanoparticles–a review on biomolecules involved, characterisation and antibacterial activity. *Chemico-biological interactions, 273*, 219-227.
3. Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, 1227-1249.
4. Ahmed, Q., Gupta, N., Kumar, A., & Nimesh, S. (2017). Antibacterial efficacy of silver nanoparticles synthesized employing Terminalia arjuna bark extract. *Artificial cells, nanomedicine, and biotechnology, 45*(6), 1192-1200.
5. El-Sayyad, G. S., Elfadil, D., Mosleh, M. A., Hasanien, Y. A., Mostafa, A., Abdelkader, R. S., ... & El-Batal, A. I. (2024). Eco-friendly strategies for biological synthesis of green nanoparticles with promising applications. BioNanoScience,

1-43.
6. Nisar, P., Ali, N., Rahman, L., Ali, M., & Shinwari, Z. K. (2019). Antimicrobial activities of biologically synthesized metal nanoparticles: an insight into the mechanism of action. *JBIC Journal of Biological Inorganic Chemistry, 24*, 929-941.
7. Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., ... & Souto, E. B. (2020). Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials, 10*(2), 292.
8. Pirsaheb, M., Gholami, T., Seifi, H., Dawi, E. A., Said, E. A., Hamoody, A. H. M., ... & Salavati-Niasari, M. (2024). Green synthesis of nanomaterials by using plant extracts as reducing and capping agents. *Environmental Science and Pollution Research, 31*(17), 24768-24787.
9. Dheyab, M. A., Oladzadabbasabadi, N., Aziz, A. A., Khaniabadi, P. M., Al-ouqaili, M. T., Jameel, M. S., ... & Ghasemlou, M. (2024). Recent advances of plant-mediated metal nanoparticles: Synthesis, properties, and emerging applications for wastewater treatment. *Journal of Environmental Chemical Engineering*, 112345.
10. Shafey, A. M. E. (2020). Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. *Green Processing and Synthesis, 9*(1), 304-339.
11. Wylie, M. R., & Merrell, D. S. (2022). The antimicrobial potential of the neem tree Azadirachta indica. *Frontiers in pharmacology, 13*, 891535.
12. Stan, D., Enciu, A. M., Mateescu, A. L., Ion, A. C., Brezeanu, A. C., Stan, D., & Tanase, C. (2021). Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. *Frontiers in pharmacology, 12,* 723233.
13. Pal, R., & Choudhury, B. (2024). A Comprehensive Systematic Review on the Immunomodulatory Properties and Therapeutic Potential of Withania somnifera, Tinospora cordifolia, and Cinnamomum zeylanicum: Molecular Mechanisms and. *Recent Advances In Pharmaceutical & Medical Sciences*, 13.
14. Bafna, P. S., Patil, P. H., Maru, S. K., & Mutha, R. E. (2021). Cissus quadrangularis L: A comprehensive multidisciplinary review. *Journal of Ethnopharmacology, 279*, 114355.
15. Nocedo-Mena, D., & Kharissova, O. V. (2024). Nanoparticles derived from the Cissus genus and their antibacterial potential. *Environmental Nanotechnology, Monitoring & Management*, 100967.
16. Wahab, S., Salman, A., Khan, Z., Khan, S., Krishnaraj, C., & Yun, S. I. (2023). Metallic nanoparticles: a promising arsenal against antimicrobial resistance—unraveling mechanisms and enhancing medication efficacy. *International journal of molecular sciences, 24*(19), 14897.
17. Rajeshkumar, S., Menon, S., Ponnanikajamideen, M., Ali, D., & Arunachalam, K. (2021). Anti-inflammatory and Antimicrobial Potential of Cissus quadrangularis-Assisted Copper Oxide Nanoparticles. *Journal of Nanomaterials, 2021*(1), 5742981.
18. Kashyap, V. K., Peasah-Darkwah, G., Dhasmana, A., Jaggi, M., Yallapu, M. M., & Chauhan, S. C. (2022). Withania somnifera: progress towards a pharmaceutical agent for immunomodulation and cancer therapeutics. *Pharmaceutics, 14*(3), 611.
19. Hussain, I., Singh, N. B., Singh, A., Singh, H., & Singh, S. C. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnology letters, 38*, 545-560.
20. Dubey, S., Virmani, T., Yadav, S. K., Sharma, A., Kumar, G., & Alhalmi, A. (2024). Breaking Barriers in Eco-Friendly Synthesis of Plant-Mediated Metal/Metal Oxide/Bimetallic Nanoparticles: Antibacterial, Anticancer, Mechanism Elucidation, and Versatile Utilizations. *Journal of Nanomaterials, 2024*(1), 9914079.
21. Alavi, M., & Moradi, M. (2022). Different antibacterial and photocatalyst functions for herbal and bacterial synthesized silver and copper/copper oxide nanoparticles/nanocomposites: A review. *Inorganic Chemistry Communications, 142*, 109590.
22. Mammari, N., Lamouroux, E., Boudier, A., & Duval, R. E. (2022). Current knowledge on the oxidative-stress-mediated antimicrobial properties of metal-based nanoparticles. *Microorganisms, 10*(2), 437.
23. Parveen, J., Sultana, T., Kazmi, A., Malik, K., Ullah, A., Ali, A., ... & Rehman, S. U. (2023). Phytosynthesized Nanoparticles as Novel Antifungal Agent for Sustainable Agriculture: A Mechanistic Approach, Current Advances, and Future Directions. *Journal of Nanotechnology, 2023*(1), 8011189.
24. Sarkar, S., Singh, R. P., & Bhattacharya, G. (2021). Exploring the role of Azadirachta indica (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *3 Biotech, 11*(4), 178.
25. El-Sayyad, G. S., Elfadil, D., Mosleh, M. A., Hasanien, Y. A., Mostafa, A., Abdelkader, R. S., ... & El-Batal, A. I. (2024). Eco-friendly strategies for biological synthesis of green nanoparticles with promising applications. *BioNanoScience, 14*(3), 3617-3659.
26. Shah, K. W., & Lu, Y. (2018). Morphology, large scale synthesis and building applications of copper nanomaterials. *Construction and Building Materials, 180*, 544-578.
27. Aminzai, M. T., Yildirim, M., & Yabalak, E. (2024). Metallic nanoparticles unveiled: Synthesis, characterization, and their environmental, medicinal, and agricultural applications. *Talanta*, 126790.
28. Ishak, N. M., Kamarudin, S. K., & Timmiati, S. N. (2019). Green synthesis of metal and metal oxide nanoparticles via plant extracts: an overview. *Materials Research Express, 6*(11), 112004.
29. Wahab, S., Salman, A., Khan, Z., Khan, S., Krishnaraj, C., & Yun, S. I. (2023). Metallic nanoparticles: a promising arsenal against antimicrobial resistance—unraveling mechanisms and enhancing medication efficacy. *International journal of molecular sciences, 24*(19), 14897.
30. Badoni, A., & Prakash, J. (2024). Noble metal nanoparticles and graphene oxide based hybrid nanostructures for antibacterial applications: recent advances, synergistic antibacterial activities, and mechanistic approaches. *Micro and Nano Engineering*, 100239.

31. Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in microbiology, 7*, 1831.
32. Puri, A., Mohite, P., Maitra, S., Subramaniyan, V., Kumarasamy, V., Uti, D. E., ... & Atangwho, I. J. (2024). From nature to nanotechnology: The interplay of traditional medicine, green chemistry, and biogenic metallic phytonanoparticles in modern healthcare innovation and sustainability. Biomedicine & Pharmacotherapy, 170, 116083.
33. Tortella, G., Rubilar, O., Fincheira, P., Pieretti, J. C., Duran, P., Lourenço, I. M., & Seabra, A. B. (2021). Bactericidal and virucidal activities of biogenic metal-based nanoparticles: Advances and perspectives. *Antibiotics, 10*(7), 783.
34. Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M., ... & Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied microbiology and biotechnology, 102*, 6799-6814.
35. Chidambara Murthy, K. N., Vanitha, A., Mahadeva Swamy, M., & Ravishankar, G. A. (2003). Antioxidant and antimicrobial activity of Cissus quadrangularis L. *Journal of medicinal food, 6*(2), 99-105.