Research Article

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Giloy-mediated Copper Nanoparticles: Their Bioactive Components, Medicinal Properties, and Species-Specific Antibacterial Efficacy

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ABSTRACT

In this study, *Tinospora cordifolia* (Giloy), rich in bioactive compounds, such as berberine and giloin, was used to synthesize copper nanoparticles (CuNPs). The UV-Vis spectrum of the nanoparticles showed a prominent absorption band at 320 nm. The FTIR analysis confirmed stabilization by polyphenols and proteins, while X-ray diffraction (XRD) revealed 18 crystalline peaks. Scanning electron microscopy (SEM) showed agglomerated particles, with individual sizes below 100 nm. The antibacterial efficacy of the CuNPs was tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. A significant sensitivity at a minimum inhibitory concentration of 125 µg/mL was shown by *K. pneumoniae*, while other strains showed resistance. These findings suggest that Giloy-mediated CuNPs could serve as targeted antibacterial agents, especially for multidrug-resistant *K. pneumoniae*, offering eco-friendly and sustainable applications in infection control and biomedical fields.

Keywords: *Tinospora cordifolia*; Copper nanoparticles; Antibacterial activity; Bioactive compounds; Medicinal properties.

1. INTRODUCTION

The field of nanoparticle synthesis is increasingly shifting towards eco-friendly and sustainable methods utilizing bio-enzymes, microbial by-products, and plant extracts. These green synthesis techniques have gained significant attention due to their environmental compatibility, cost-effectiveness, and ability to produce nanoparticles with enhanced biocompatibility (Alsaiari *et al.* 2023; Bahrulolum *et al.* 2021). Among the plants explored, *Tinospora cordifolia* (commonly known as Giloy) has emerged as a particularly promising candidate for nanoparticle bio-fabrication, given its abundance of bioactive compounds, such as berberine and giloin. These compounds exhibit a wide array of medicinal properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities, making T. cordifolia an excellent choice for the biosynthesis of functional nanoparticles (Chowdhury, 2021). This study investigates the potential of *T. cordifolia* in synthesizing CuNPs and evaluates their antibacterial efficacy against key pathogenic strains.

Unlike conventional chemical methods that often involve toxic reagents and high energy input, green synthesis using plant extracts simplifies the process by utilizing the reducing and stabilizing capabilities of natural phytochemicals, minimizing the need for complex optimization or elaborate scale-up procedures (Kim and Song, 2009; Mohanpuria *et al.* 2008; Patil *et al.* 2012; Shankar *et al.* 2004; Tyagi *et al.* 2012). Additionally, *T. cordifolia* offers added value as a medicinal plant, known for its diverse biological functions such as regulating blood sugar levels, enhancing immunity, and offering therapeutic benefits for conditions like osteoporosis, osteoarthritis, and liver disorders (Arunachalam *et al.* 2022).

In traditional *Ayurvedic* medicine, *T. cordifolia* extract has been used for centuries to treat a range of ailments, including fever, jaundice, cancer, asthma, skin diseases, and urinary disorders. Its rich phytochemistry, including alkaloids, glycosides, steroids, and flavonoids, positions *T. cordifolia* as an ideal candidate for green nanoparticle synthesis. Prior studies have demonstrated the potent antibacterial, antioxidant, and anti-inflammatory effects of *T. cordifolia* extracts, suggesting their ability to enhance the stability and functionality of biosynthesized nanoparticles. These properties align with the growing demand for sustainable, plant-based therapeutic solutions, as well as the need for novel approaches to combat multidrug-resistant pathogens.

Copper nanoparticles have gained attention in recent years for their potent antimicrobial properties, which are effective against a wide range of bacteria, fungi, and viruses. The unique properties of CuNPs, including their high surface area, conductivity, and catalytic activity, make them particularly valuable in biomedical applications, such as drug delivery, wound healing, and water treatment. However, the conventional chemical methods for synthesizing CuNPs often involve hazardous chemicals and energy-intensive processes. In this context, bio-fabrication using *T. cordifolia* not only offers a greener alternative but also enhances the therapeutic potential of the nanoparticles through synergistic effects with the plant's inherent bioactive compounds. Recent studies have shown that biogenic CuNPs synthesized using plant extracts exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacteria. For example, biosynthesized CuNPs have been tested against pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, with promising results in inhibiting bacterial growth (Aziz *et al.* 2024; Salah *et al.* 2021). This study seeks to build on this body of work by specifically exploring the antibacterial efficacy of *T. cordifolia*-mediated CuNPs, focusing on their species-specific responses to different bacterial strains. In particular, the study aims to evaluate the potential of these CuNPs as a sustainable solution for combating multidrug-resistant pathogens, especially *K. pneumoniae*, which is known for its role in hospital-acquired infections.

Given the increasing prevalence of antibiotic resistance and the need for new antimicrobial agents, the exploration of *T. cordifolia* as a source for bio-nanomaterials is both timely and critical (Jassim *et al.* 2016; Mittal *et al.* 2022; Saha and Ghosh, 2012). The results of this study will not only contribute to the field of green nanotechnology but also highlight the potential of traditional medicinal plants in the development of advanced biomedical applications.

2. EXPERIMENTAL SECTION

2.1 Materials and Methods

2.1.1 Chemicals and Bacterial Culture

Analytical grade copper sulphate pentahydrate and sodium hydroxide were used for the bio-fabrication of copper nanoparticles. The bacterial strains used in this study are cited in Table 1. Mature stems of *Tinospora cordifolia* were ethically harvested from natural habitats for the synthesis of CuNPs. To prepare the extract, 1 g of dried stem powder was soaked in 100 mL of sterile distilled water for 48 hours, filtered through muslin cloth, and used for nanoparticle synthesis.

Table 1: Pathogens used for the screening of antibacterial activity of bio-fabricated CuNPs.

S. No.	Acc. no of bacterial strains	Species
1	LC747145	*Escherichia coli (EC)*
2	LC747146	*Klebsiella pneumoniae (KP)*
3	LC747148	*Staphylococcus aureus (SA)*
4	LC747147	*Pseudomonas aeruginosa (PA)*

2.2 Bio-Fabrication Process

The bio-fabrication of CuNPs began with the preparation of a 1% aqueous extract from *Tinospora cordifolia* stem powder, which was mixed with 20 mM $CuSO_4 \cdot 5H_2O$ in a 1:1 ratio. The solution, kept in an amber-coloured container, was left undisturbed for 24 hours in darkness. A visible colour change indicated the formation of copper nanoparticles. The solution was then centrifuged at 15,000 RPM for 30 minutes, separating the pellet from the supernatant. The pellet was purified by treatment with ethanol to eliminate residual water and dried in an oven at 60°C for 72 hours. The dried material was stored in a 1.5-mL centrifuge tube, shielded from light until further analysis.

2.3 Characterization Tools and Techniques

Various characterization tools and techniques were used in this study, and a concise overview of these processes is presented in Table 2, addressing the specific needs of characterization.

1. Spectrophotometric Analysis: UV-Vis spectrophotometry was used to assess the optical properties of the CuNPs. The diluted suspension was measured across a wavelength range 200-800 nm to identify the characteristic surface plasmon resonance (SPR) peak, confirming nanoparticle formation and stability.

2. X-ray Diffraction (XRD): This analysis determined the crystalline structure of the CuNPs. The dried nanoparticles were placed on an XRD sample holder, and diffraction patterns were recorded with Cu-Kα radiation ($\lambda = 1.5406$ Å) over the 2θ range 20° to 80°. To identify crystalline phases, the diffraction peaks were compared to reference patterns.

3. Fourier Transform Infrared (FTIR) Analysis: The FTIR spectroscopy was used to identify functional groups responsible for the reduction and stabilization of CuNPs. The dried nanoparticles were mixed with KBr, pressed into pellets, and analyzed over the range 4000-400 cm^{-1}. The characteristic absorption peak was examined to determine interactions

between *Tinospora cordifolia* extract biomolecules and the nanoparticles.

4. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis: The morphology and surface structure of the CuNPs was observed by the SEM. A thin layer of gold-coated dried nanoparticles was examined at various magnifications. The EDX analysis provided elemental composition, confirming the presence of copper in the synthesized nanoparticles.

2.4 Preparation of Bacterial Inoculum

A loopful of each bacterial strain (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) was inoculated into 10 mL of nutrient broth and incubated overnight at 37°C. The bacterial cultures were standardized to an optical density of 0.5 at 600 nm, corresponding to approximately 1×10^8 CFU/ mL.

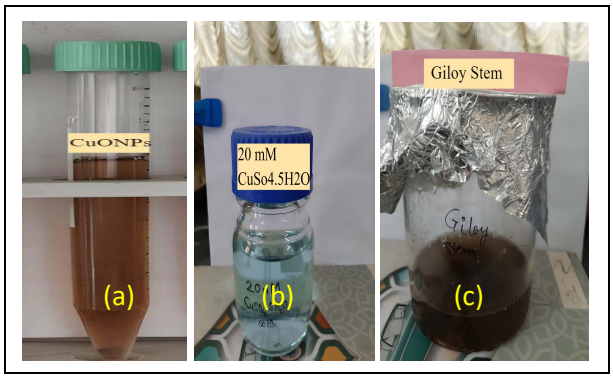

Fig. 1: (a) *Tinospora cordifolia* stem extract, (b) $CuSO_4 \cdot 5H_2O$ and (c) CuNPs.

2.5 Well Diffusion Assay

The antibacterial activity of the CuNPs was assessed using the well diffusion method. Sterile Mueller-Hinton agar plates were prepared, and 100 µL of the bacterial inoculum was evenly spread across the surface using a sterile cotton swab. Wells, each 6 mm in diameter, were created in the agar using a sterile cork borer.

2.6 Incubation and Measurement of Inhibition Zones

The plates were incubated at 37°C for 24 hours. After incubation, the antibacterial effect of the CuNPs was evaluated by measuring the diameter of the inhibition zones around the wells using a scale. The results, recorded in mm, indicated the effectiveness of the CuNPs against the bacterial strains.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of CuNPs was determined *via* the broth microdilution method. Serial dilutions of CuNPs, ranging from 1 to 100 µg/mL, were prepared in a 96-well microtiter plate. Each well was inoculated with 100 µL of bacterial suspension (approximately 1×10^6 CFU/mL) and incubated at 37°C for 24 hours. The MIC was defined as the lowest CuNPs concentration that visibly inhibited bacterial growth, indicated by the absence of turbidity in the wells.

3. RESULTS AND DISCUSSION

3.1 Synthesis and Optical Properties

The synthesis of CuNPs using *T. cordifolia* stem involved mixing its extract (Fig. 1a) with $CuSO_4 \cdot 5H_2O$ (Fig. 1b) in equal ratios. The solution mixture was left undisturbed for 24 hours in darkness. A blackish-brown colour appeared, indicating the formation of copper nanoparticles (Fig. 1c). The optical analysis confirmed successful formation of CuNPs, with a prominent absorption peak at 320 nm (Fig. 2). The pure *T. cordifolia* stem extract exhibited an absorption maximum at 300 nm, while the $CuSO_4 \cdot 5H_2O$ (2 mM) solution showed a peak at 290 nm. These results are comparable to similar studies by Gebremedhn et al. 2019, where CuNPs synthesized from *Catha edulis* leaf extract also exhibited a notable absorption peak.

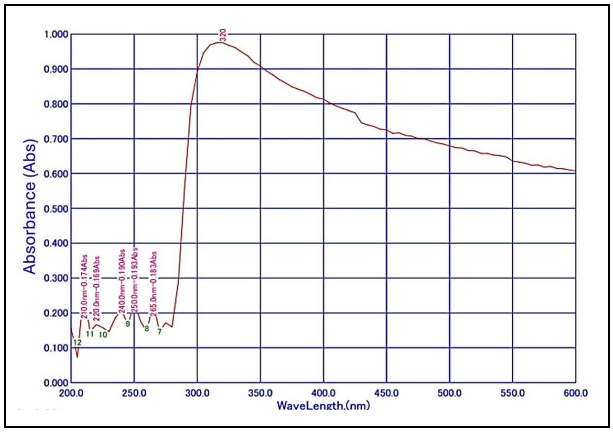

Fig. 2: UV- VIS Spectroscopic analysis of CuNPs after 24 hours reading

3.2 Crystalline Structure

X-ray diffraction analysis revealed the crystalline nature and phase composition of the CuNPs. The XRD pattern showed distinct diffraction peaks at 2θ values of 32.254°, 35.285°, 38.518°, 48.463°, 53.264°, 53.419°, 58.176°, 58.079°, 61.165°, 61.272°, 61.363°, 65.363°, 65.758°, 65.959°, 67.893°, 72.133°, 74.944°, and 75.104°, with intensities recorded as 307, 234, 275,

632, 213, 211, 270, 279, 338, 341, 351, 550, 630, 462, 172, 181, 192 and 231. These 18 peaks matched the standard reference pattern for monoclinic copper nanoparticles, confirming the formation of crystalline CuNPs (Fig. 3). The observed peaks align with previous findings (Kumar *et al.* 2015; Sarkar *et al.* 2020; Vigneshwaran *et al.* 2007).

3.3 Functional Groups

Fourier-transform infrared spectroscopy was used to identify functional groups and chemical bonds in the CuNPs. The FTIR spectrum using a Shimadzu IRTracer-100 device revealed several significant peaks (Fig. 4):

- **451.31 cm^{-1}:** Associated with the Cu-O stretching bond in CuNPs.
- **569 cm^{-1} and 1060 cm^{-1}:** Indicate metal-oxide bond transfer from tetrahedral to octahedral sites, supported by the shift from 451 cm^{-1}.
- **3664.75 cm^{-1}:** Represents the O-H group stretching vibration from plant phenolic compounds, suggesting the reduction of Cu^{2+} ions to CuNPs.
- **1708 cm^{-1} and 1543 cm^{-1}:** Indicate the presence of polyphenols or proteins that stabilize the CuNPs.

The FTIR analysis confirmed that the CuNPs are stabilized by biomolecules from *T. cordifolia*, including polyphenols and proteins, which play a crucial role in reducing Cu^{2+} ions and stabilizing the nanoparticles. Our findings are supported by other published works, where similar biomolecules have been shown to maintain the structural integrity of CuNPs (Rosa and Rubí, 2020; Dayana *et al.* 2022; Kombaiah *et al.* 2018; Luo *et al.* 2014; Manikandan *et al.* 2021; Mohanraj *et al.* 2014; Raeisi *et al.* 2021; Rengasamy *et al.* 2016).

3.4 Scanning Electron Microscopy

The SEM images at a 300 nm resolution revealed that the CuNPs were crystalline and exhibited a tendency to agglomerate, suggesting strong intermolecular interactions (Fig. 5). This agglomeration is likely due to the high surface energy of the nanoparticles, which promotes clustering. Individual particles measured less than 100 nm in at least one dimension, which is consistent with the findings of Das *et al.* 2020. These nanoscale dimensions provide a large surface area to volume ratio, which is essential for enhanced reactivity and makes these nanoparticles highly suitable for biological and medicinal applications. Additionally, the irregular shape of the nanoparticles may further contribute to their high surface energy, increasing the propensity for agglomeration. Similar observations have been reported in other studies (Amjad *et al.* 2021) where plant-mediated CuNPs exhibited comparable sizes, shapes, and tendencies to agglomerate.

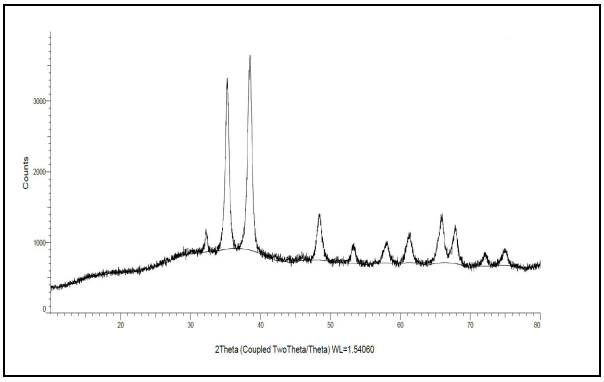

Fig. 3: XRD spectrum of copper nanoparticles prepared from *Tinospora cordifolia*

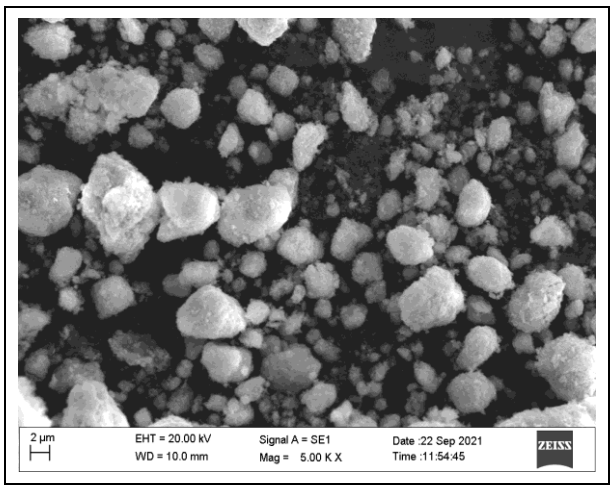

Fig. 5: SEM micrograph of CuNPs prepared from *Tinospora cordifolia*

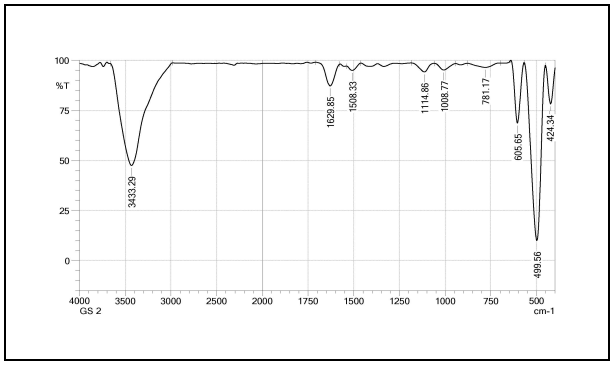

Fig. 4: FTIR spectrum of CuNPs prepared from *Tinospora cordifolia*

3.5 Energy-Dispersive X-ray Spectroscopy

The EDX analysis identified characteristic X-ray energies for copper, with prominent Kα and Lα lines

at 7.8 keV and 0.08 keV, respectively, along with a Kα1 peak for oxygen at 0.05 keV (Fig. 6). These peaks confirmed that the CuNPs are primarily composed of copper and oxygen, indicating high purity with minimal impurities. The presence of oxygen suggests that the surface of the copper nanoparticles may be partially oxidized, forming a thin oxide layer, which is common in plant-mediated synthesis. This oxide layer can enhance the stability of the nanoparticles, preventing further oxidation and preserving their antimicrobial properties. The high purity of the CuNPs, as confirmed by the absence of significant peaks for other elements, highlights the effectiveness of the biofabrication process in eliminating contaminants.

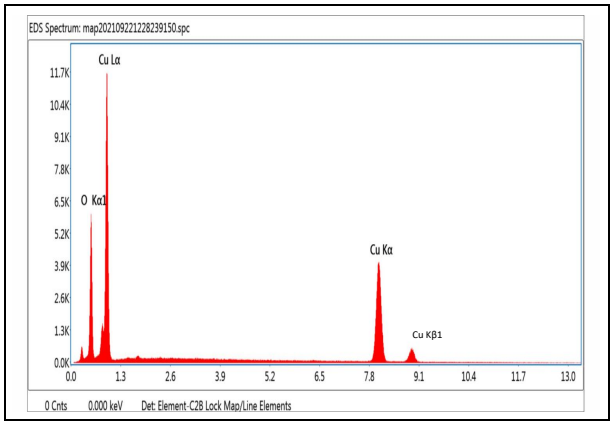

Fig. 6: EDX spectrum of *Tinospora cordifolia*-mediated CuNPs

3.6 Antibacterial Activity

The antibacterial efficacy of the CuNPs synthesized using *T. cordifolia* was evaluated against four clinically relevant bacterial strains: *E. coli*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa* at concentrations of 62.5, 125, 500, and 1000 µg/mL (Fig. 7). Among these, *K. pneumoniae* showed the highest sensitivity to the CuNPs, with significant growth inhibition observed at concentrations as low as 125 µg/mL and more pronounced effects at 500 and 1000 µg/mL. This species-specific response suggests a potent antibacterial effect of CuNPs against *K. pneumoniae*, which is known for its ability to cause severe infections, including pneumonia, bloodstream infections, and urinary tract infections. The other tested bacterial strains, *E. coli*, *S. aureus*, and *P. aeruginosa*, demonstrated resistance to the CuNPs across all tested concentrations (62.5, 125, 500, and 1000 µg/mL). These results indicate that the bio-fabricated CuNPs exhibit selective antibacterial activity, which may be attributed to differences in the cell wall structure or metabolic processes of the bacterial strains. For instance, *K. pneumoniae* has a complex polysaccharide capsule, which might render it more susceptible to oxidative stress and ion release from the CuNPs. In contrast, *S. aureus*, a Gram-positive bacterium, and *E. coli* and *P. aeruginosa*, both Gram-negative bacteria, may have more robust defense mechanisms against nanoparticle-induced stress, such as stronger antioxidant systems or efflux pumps that prevent nanoparticle accumulation (Avakh *et al.* 2023; Stephen *et al.* 2023).

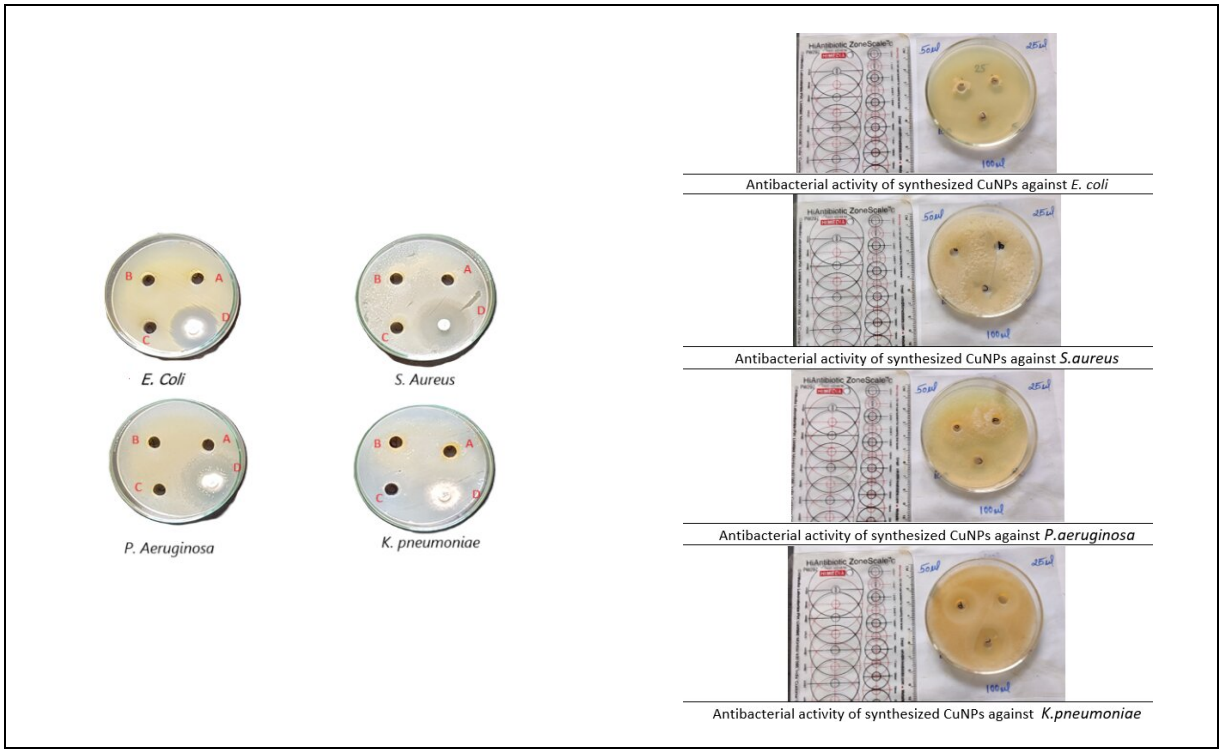

Fig. 7: Species-specific antibacterial efficacy of pathogenic bacteria against CuNPs

Similar observations have been reported in studies where CuNPs synthesized using plant extracts exhibited varying degrees of antibacterial activity depending on the bacterial species. For example, CuNPs derived from *Ocimum americanum* (Manikandan *et al.* 2021; Manikandan *et al.* 2017) and *Sida acuta* (Sathiyavimal *et al.* 2018) were found to be particularly effective against Gram-negative bacteria, while Gram-positive bacteria showed higher levels of resistance.

This selective antibacterial activity highlights the importance of exploring plant-mediated nanoparticle synthesis as a means of developing targeted therapies against specific pathogens.

3.7 Species-Specific Antibacterial Efficacy

The emergence of multidrug-resistant bacterial strains has prompted an urgent need for novel antibacterial agents that are both effective and sustainable. Among the various solutions explored, nanoparticle-based therapies have gained considerable attention due to their unique properties and potential for selectively targeting pathogens (Avakh *et al.* 2023; Stephen *et al.* 2023). In this regard, CuNPs synthesized using *T. cordifolia* represent a promising approach for antibacterial treatments. With its rich composition of bioactive compounds such as, berberine alkaloids and giloin, it provides an eco-friendly platform for CuNP bio-fabrication, resulting in nanoparticles with demonstrated species-specific antibacterial efficacy. Recent research has highlighted the selective antibacterial activity of *T. cordifolia*-mediated CuNPs, particularly against *K. pneumoniae* (a highly resistant pathogen implicated in numerous hospital-acquired infections). In a study by (Kumar *et al.* 2024),

The selective inhibition of *K. pneumoniae* is likely due to the interaction between the bioactive compounds present in *T. cordifolia* and the bacterial cell membrane, which may lead to enhanced membrane disruption and increased oxidative stress in *K. pneumoniae* compared to other species. Copper ions released from the CuNPs are known to generate reactive oxygen species, which can damage essential bacterial components such as DNA, proteins, and lipids, thereby preventing bacterial proliferation (Manjula *et al.* 2022). The eco-friendly synthesis of CuNPs using *T. cordifolia* not only enhances their therapeutic properties but also addresses the growing demand for sustainable medical solutions (Das *et al.* 2020). The species-specific efficacy of Giloy-mediated CuNPs marks a significant advancement in the development of targeted antibacterial therapies and presents a promising alternative to conventional antibiotics in combating antibiotic-resistant pathogens.

4. CONCLUSION

This study highlights the successful bio-fabrication of copper nanoparticles using *Tinospora cordifolia* (Giloy) and their notable antibacterial efficacy against *Klebsiella pneumoniae*. Synthesized through an eco-friendly method, these CuNPs showed significant antibacterial activity at 125 µg/mL, while other tested strains remained resistant. The bioactive compounds in *T. cordifolia*, such as berberine alkaloids and giloin, might have enhanced the antimicrobial properties of the nanoparticles. This targeted inhibition, combined with a sustainable synthesis approach, makes *T. cordifolia*-mediated CuNPs a promising alternative for treating multidrug-resistant pathogens. Future research should explore the mechanisms of action and potential clinical and environmental applications.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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