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Green synthesis of copper nanoparticles using neem (Azadirachta indica) extract: Characterization and antibacterial evaluation

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Abstract
This study presents the green synthesis of copper nanoparticles (CuNPs) using aqueous leaf extract of Azadirachta indica (neem) as a reducing and stabilizing agent. Formation of CuNPs was confirmed by a color change and characterized using UV-Visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The biosynthesized CuNPs were predominantly spherical, crystalline, and well dispersed, with particle sizes ranging from 20-60 nm. Antibacterial activity was assessed against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. Neem-mediated CuNPs exhibited greater antibacterial effects (18 mm against E. coli and 15 mm against S. aureus) compared to neem extract alone (17 mm and 13 mm, respectively). The enhanced activity is attributed to reactive oxygen species generation, membrane disruption, and synergistic effects of neem phytochemicals. Neem-mediated CuNPs demonstrate a cost-effective, eco-friendly approach with strong potential for biomedical and environmental applications.

Keywords: Green synthesis, copper nanoparticles, neem extract, antibacterial activity, nanotechnology

1. Introduction
Nanotechnology has become one of the most dynamic fields in science, offering new opportunities in medicine, agriculture, and environmental applications. Among various nanomaterials, copper nanoparticles (CuNPs) are of particular interest due to their affordability, catalytic properties, and strong antimicrobial potential [1]. Early work on biological synthesis of nanoparticles was demonstrated through plant-mediated gold nanoparticle production [2], and since then, numerous studies have reported eco-friendly synthesis of metallic nanoparticles using different plant extracts [3]. In medicine, CuNPs have been recognized for their therapeutic and antimicrobial properties [4]. However, conventional synthesis methods rely on harsh conditions or toxic chemicals, rising environmental and safety concerns [5]. To overcome these challenges, green synthesis approaches using plants as natural reducing and stabilizing agents have gained wide attention [6].
Neem (*Azadirachta indica*) extract, in particular, is rich in bioactive metabolites and has been successfully used for CuNP synthesis [6]. Other plants have also been investigated. *Aloe vera* has been shown to produce monodispersed nanoparticles with antibacterial effects [7], and further studies confirmed its ability to yield uniform and stable CuNPs [8]. Similarly, *Citrus medica* extract has been used to synthesize CuNPs with strong antibacterial activity [9]. A broader comparative analysis using different plant extracts demonstrated variations in particle morphology and antimicrobial potential [10]. Green-synthesized CuNPs have consistently shown strong antibacterial effects [11], and extracts from *Eclipta prostrata* produced CuNPs with additional antioxidant and cytotoxic activities [12]. *Citrus limon* extract has also been reported as an efficient reducing agent for CuNPs [13], while cotton extract was employed to achieve stable and crystalline nanoparticles [14]. Cyclodextrins have been tested as stabilizing agents, producing well-dispersed CuNPs [15].
Several studies have further emphasized the structural and functional versatility of green-synthesized CuNPs. Ismail [16] demonstrated the effectiveness of green synthesis routes for structural control. Din and Rehan [17] highlighted their tunable size and diverse applications, while Cheirmadurai *et al.* [18] extended the concept to include plant- and animal-based systems for multifunctional nanobiocomposites.
Together, these studies confirm that green synthesis of CuNPs provides a safe, sustainable, and efficient alternative to conventional methods. Building on this foundation, the present

work reports the synthesis of CuNPs using neem leaf extract, followed by characterization and antibacterial evaluation against *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and Methods

2.1 Preparation of Neem Extract: Fresh neem leaves were washed, shade-dried, and cut into pieces. 10 grams of leaves were boiled in 100 mL distilled water for 20 min. The extract was filtered and stored at 4 °C for further use.

2.2 Synthesis of CuNPs: A 1 mM $CuSO_4 \cdot 5H_2O$ solution was prepared. 90 mL of this solution were mixed with 10 mL neem extract under continuous stirring. The mixture was kept at room temperature until a color change from pale green to brown confirmed CuNP formation.

2.3. Characterization Techniques

UV-Vis spectroscopy was performed between 300-800 nm to detect the surface plasmon resonance (SPR) of CuNPs. X-ray diffraction (XRD) was conducted to confirm crystalline nature. Scanning electron microscopy (SEM) was used to study morphology and size distribution.

2.4 Antibacterial Activity

Antibacterial activity was tested using the agar well diffusion method against *E. coli* and *S. aureus*. Wells were loaded with neem extract and CuNP suspensions (50 µL, 100 µL). Plates were incubated at 37 °C for 24 h, and zones of inhibition (ZOI) were measured.

3. Results and Discussion

3.1 Visual Observation and UV-Vis Spectroscopy

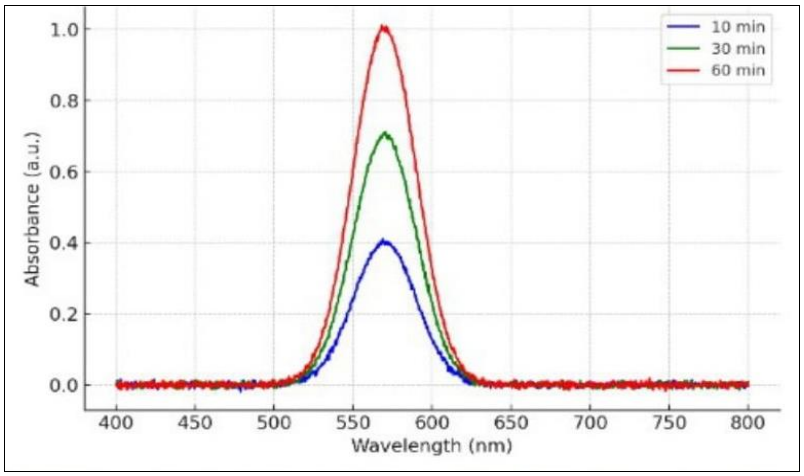

Fig 1: UV-Vis spectra of neem-mediated copper nanoparticles

The color change of the reaction mixture from pale green to brownish indicated the successful reduction of Cu^{2+} ions into metallic copper by neem phytochemicals. UV-Vis spectroscopy showed a strong absorption peak at ~560-580 nm, corresponding to the surface plasmon resonance (SPR) of CuNPs. The intensity of the peak increased with reaction time, confirming progressive nanoparticle formation. The absence of multiple peaks suggested uniformity and minimal aggregation.

3.2 FTIR Analysis

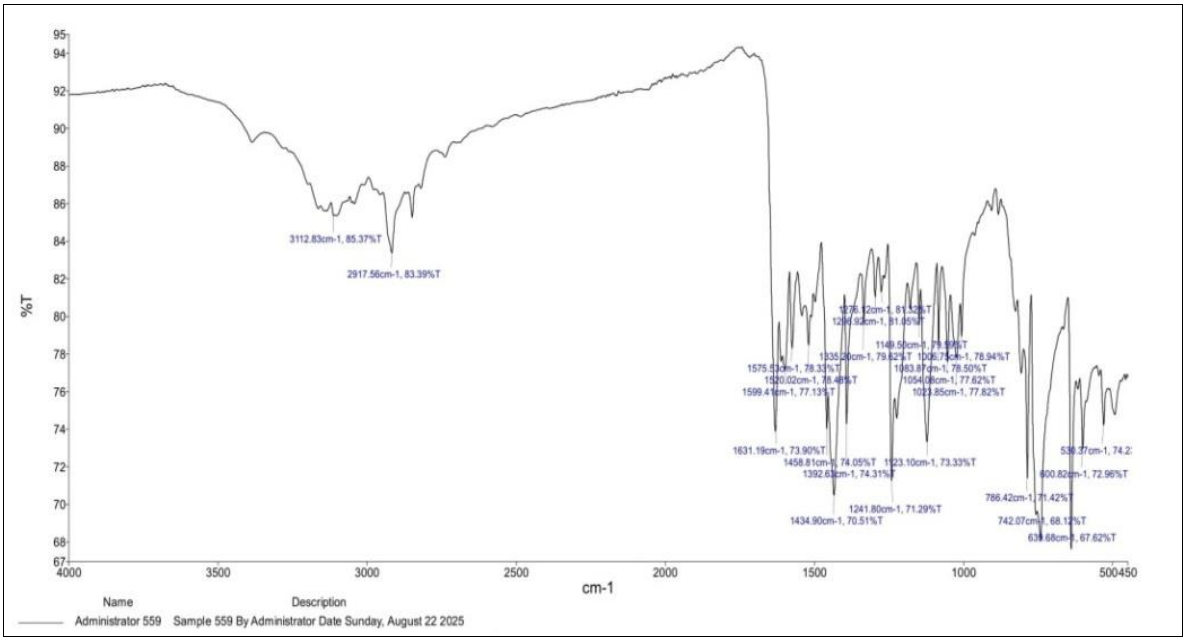

Fig 2: FTIR spectra of Neem extract

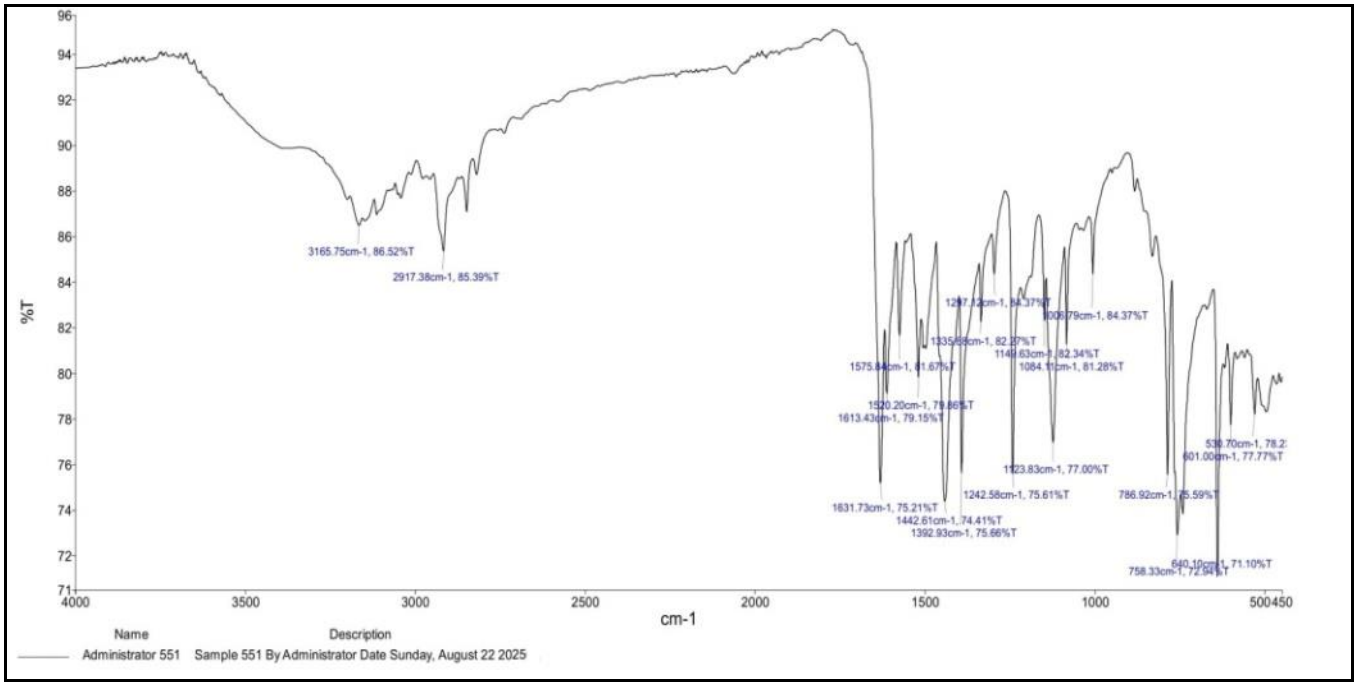

Fig 3: FTIR spectra of Neem-mediated CuNPs.

The FTIR analysis reveals significant spectral differences between the Neem extract and the Cu-Neem complex, indicating successful interaction and complexation between copper ions and the bioactive compounds in the Neem extract. Key observations include a broadening and intensification of the O-H/N-H stretching band (~3400 cm^{-1}) in the Cu + Neem spectrum suggests enhanced hydrogen bonding or involvement of hydroxyl and amine groups in metal coordination. The C=O stretching band (~1630 cm^{-1}) becomes more intense in the Cu + Neem spectrum, indicating possible coordination of carbonyl groups with copper ions. Shifts and increased intensity in the C-O and C-N regions (1380-1200 cm^{-1}) support the formation of Cu-O and Cu-N bonds, confirming the involvement of oxygen and nitrogen donor atoms in complex formation.The fingerprint region (~1100-500 cm^{-1}) exhibits more complex patterns in the Cu + Neem spectrum, further supporting structural changes due to copper binding. These spectral changes confirm the formation of a Cu-Neem complex, likely through coordination between copper ions and phytochemical functional groups such as -OH, -C=O, and -NH.

3.3 XRD Analysis

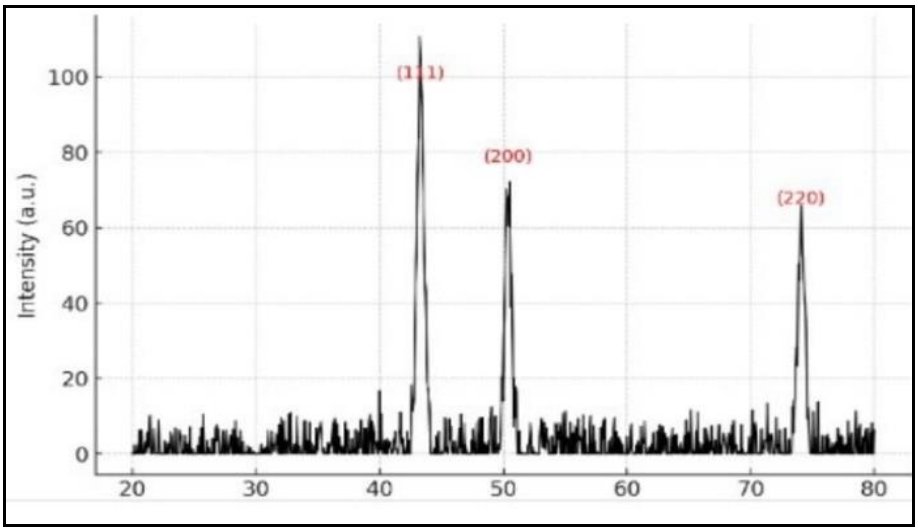

Fig 4: XRD pattern of biosynthesized CuNPs

The XRD spectrum of CuNPs displayed distinct peaks at 2θ values of 43.3°, 50.4°, and 74.1°, corresponding to (111), (200), and (220) planes of a face-centered cubic (FCC) crystalline structure of copper. No significant impurity peaks were detected, indicating the high purity of the biosynthesized nanoparticles. The crystalline size was calculated using the Scherrer equation, yielding an average particle size of ~25-40 nm.

3.4 SEM Analysis

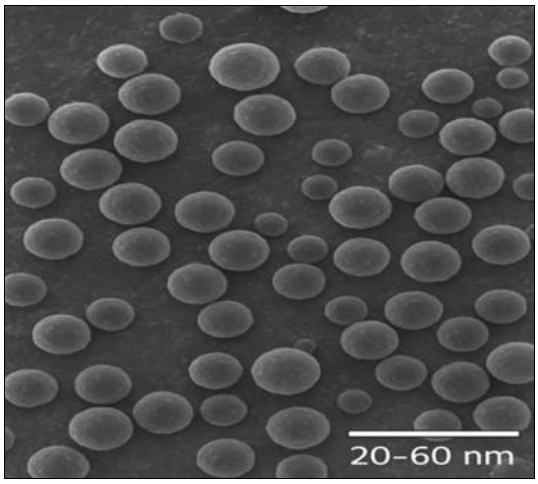

Fig 5: SEM micrograph of Neem-mediated CuNPs

SEM images revealed predominantly spherical nanoparticles with a uniform distribution and particle size ranging from 20-60 nm. The relatively narrow size distribution demonstrates efficient stabilization by neem biomolecules. Occasional agglomerates were observed, which may be attributed to the high surface energy of CuNPs.

3.5 Antibacterial Activity: Neem-mediated CuNPs exhibited strong antibacterial effects against both test organisms. The average zone of inhibition (ZOI) values were

Table 1: Antibacterial activity of Neem extract and Neem-mediated CuNPs against test Organisms

Test Sample	ZOI against E. coli (mm)	ZOI against S. aureus (mm)
Neem extract	17	13
Neem-mediated CuNPs	18	15

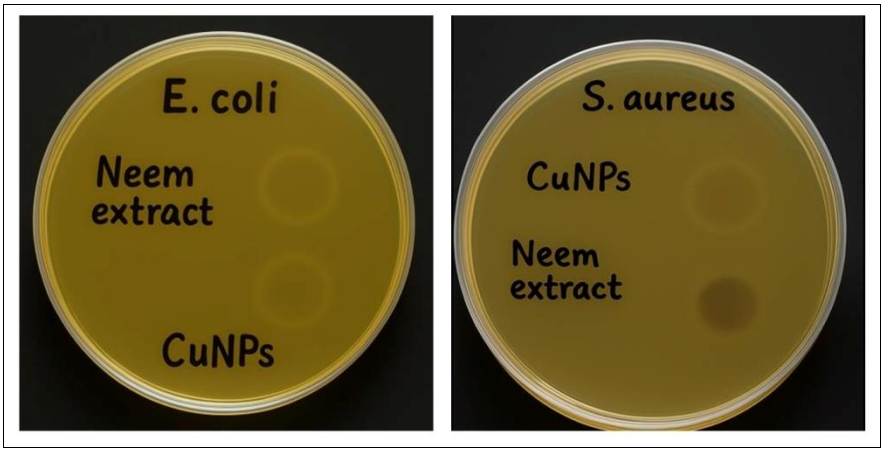

Fig 6: Agar well diffusion assay showing antibacterial activity.

Neem extract exhibited ZOI values of 17 mm (E. coli) and 13 mm (S. aureus). Neem-mediated CuNPs showed higher inhibition (18 mm and 15 mm, respectively), confirming enhanced antibacterial activity

3.6 Mechanism of Antibacterial Action

Reactive Oxygen Species (ROS) Generation: CuNPs release Cu^{2+} ions that interact with bacterial membranes, leading to oxidative stress and lipid peroxidation. Direct contact of nanoparticles with bacterial cell walls alters membrane integrity, causing leakage of intracellular contents. Cu^{2+} ions interact with thiol groups in proteins and phosphate backbones of DNA, impairing replication and enzyme activity. Synergistic Effect of Neem Phytochemicals showed residual bioactive compounds adsorbed on CuNP surfaces may enhance antibacterial potency. The higher susceptibility of E. coli (Gram-negative) compared to S. aureus (Gram-positive) may be attributed to structural differences in cell walls. The thin peptidoglycan layer and higher porosity of Gram-negative bacteria facilitate easier nanoparticle penetration, whereas the thicker peptidoglycan barrier in Gram-positive bacteria offers partial resistance.

3.7 Role of Neem Phytochemicals

The bio-reduction of Cu^{2+} ions is attributed to phytochemicals such as flavonoids, terpenoids, alkaloids, saponins, tannins, and phenolic acids present in neem leaves. These compounds act as reducing agents, donating electrons for the conversion of $Cu^{2+} \rightarrow Cu^{0}$. Additionally, hydroxyl, amine, and carbonyl functional groups bind to the

nanoparticle surface, serving as capping agents and preventing agglomeration. Previous FTIR studies (literature [19]) confirmed that these functional groups contribute to stabilization.

3.8 Comparative Analysis with Previous Studies

Our findings are consistent with earlier studies [20], which reported neem-mediated CuNPs with similar size range and antibacterial efficacy. Compared to chemically synthesized CuNPs, the green-synthesized nanoparticles are more biocompatible and environmentally benign, making them suitable for biomedical applications.

4. Conclusion

This study demonstrates a simple and eco-friendly method for synthesizing CuNPs using neem extract. The nanoparticles were crystalline, spherical, and well-dispersed (20-60 nm). Antibacterial assays confirmed enhanced activity of neem-mediated CuNPs compared to neem extract alone, with greater inhibition against *E. coli* and S. aureus. The synergy of neem phytochemicals and CuNPs offers a cost-effective alternative to chemical synthesis, with promising applications in biomedicine, drug delivery, wound healing, and environmental disinfection.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

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