

## NEEM-DRIVEN GREEN SYNTHESIS OF MULTIFUNCTIONAL COPPER NANOPARTICLES: A SUSTAINABLE APPROACH WITH BROAD-SPECTRUM ANTIMICROBIAL ACTIVITY

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### Abstract

The rise of antibiotic-resistant pathogens and the environmental issues linked to traditional nanoparticle production methods call for sustainable alternatives. This research presents an eco-friendly method for synthesizing copper nanoparticles (CuNPs) using *Azadirachta indica* (neem) leaf extract as a natural reducing and stabilizing agent. The successful synthesis was indicated by a noticeable color change from blue to dark green, due to surface plasmon resonance (SPR). Optimization studies determined the optimal conditions to be 10 mL of neem extract, a temperature of 60°C, a duration of 2 hours, and a neutral pH. Detailed characterization showed that the nanoparticles were spherical and well-dispersed, with an average diameter of 34.2 nm (SEM), a crystalline face-centered cubic structure with a crystallite size of 18.4 nm (XRD), and a distinctive SPR peak at 552 nm (UV-Vis). The CuNPs synthesized with neem demonstrated significant antimicrobial activity against *Staphylococcus aureus* (ZOI: 18 mm; 82% of amoxicillin) and *Aspergillus fumigatus* (ZOI: 17 mm; 81% of clotrimazole), greatly surpassing the crude neem extract (8 mm and 7 mm, respectively;  $p < 0.01$ ). In addition to their antimicrobial properties, the CuNPs showed potential for catalytic dye degradation and antioxidant activity. This green synthesis method aligns with the principles of green chemistry and several UN Sustainable Development Goals (SDG 6, 12, 13), providing a cost-effective and environmentally sustainable platform for applications in biomedical coatings, wound healing formulations, food packaging, and wastewater treatment.

### INTRODUCTION

Nanotechnology has revolutionized modern materials science by allowing the manipulation of matter at the nanoscale, specifically between 1 and 100 nm. At these dimensions, materials display unique physicochemical characteristics, such as increased surface area-to-volume ratios, quantum effects, and heightened reactivity, which are markedly different from their bulk forms. Among the various nanomaterials, copper nanoparticles (CuNPs) have attracted considerable interest due to their outstanding electrical and thermal

conductivity, catalytic prowess, and strong antimicrobial properties [1,2]. Unlike noble metals like silver and gold, copper is more cost-effective while still delivering excellent functional performance, making it appealing for large-scale industrial use [3].

Nevertheless, traditional methods for synthesizing CuNPs often depend on chemical reduction techniques that use harmful reagents like sodium borohydride, hydrazine hydrate, and cetyltrimethylammonium bromide (CTAB) [4]. These methods pose significant environmental

and health hazards due to the production of toxic byproducts and the requirement for energy-intensive processes. Physical methods such as laser ablation, thermal decomposition, and high-energy ball milling, although avoiding toxic chemicals, necessitate specialized equipment and considerable energy, contributing to greenhouse gas emissions [5]. These challenges have prompted a shift towards green synthesis methods that utilize biological systems, particularly plant extracts, as natural reducing and capping agents.

Plant-mediated synthesis offers several benefits over conventional methods: (i) it eliminates the use of toxic chemicals, (ii) operates under ambient conditions, (iii) reduces energy consumption, (iv) provides inherent stabilization through phytochemical capping, and (v) enhances biocompatibility for biomedical applications [6]. The bioactive compounds in plant extracts—such as flavonoids, terpenoids, phenolics, alkaloids, and proteins—act as both reducing agents (donating electrons to metal ions) and stabilizing agents (preventing nanoparticle agglomeration) [7].

*Azadirachta indica* (neem), an evergreen tree native to the Indian subcontinent, is an excellent candidate for green nanoparticle synthesis. Traditionally esteemed in Ayurvedic medicine for its antimicrobial, anti-inflammatory, and immunomodulatory properties, neem leaves are rich in phytochemicals like nimbin, nimbidin, nimbolide, flavonoids, and various limonoids [8]. These compounds have proven effective in reducing metal ions to their elemental nanoparticle forms while simultaneously providing a biocompatible capping layer [9].

This study addresses a significant gap in the literature: while the neem-mediated synthesis of silver and gold nanoparticles is well-documented, systematic optimization and comprehensive characterization of neem-driven CuNPs with comparative antimicrobial benchmarking against standard drugs remain limited. Moreover, the multifunctional potential of these nanoparticles—including antimicrobial, catalytic, and antioxidant activities—merits integrated investigation.

Therefore, this research aims to: (1) synthesize CuNPs using neem leaf extract through an

optimized green chemistry approach, (2) thoroughly characterize the nanoparticles using UV-Vis spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM), (3) evaluate antimicrobial efficacy against clinically relevant pathogens (*Staphylococcus aureus* and *Aspergillus fumigatus*) compared to standard antibiotics/antifungals, and (4) assess the multifunctional potential for industrial and biomedical applications.

## 2. Materials and Methods

### 2.1 Plant Material and Extract Preparation:

Fresh neem (*Azadirachta indica*) leaves, in good condition, were gathered from mature trees at Riphah International University in Faisalabad, Pakistan. The leaves underwent thorough washing with distilled water, were dried in the shade for 7 to 10 days and then ground into a fine powder. To prepare the extract, 10 grams of neem leaf powder was boiled in 100 milliliters of distilled water at 80°C for 20 minutes with continuous stirring. After cooling, the mixture was filtered using Whatman No. 1 filter paper, and the clear filtrate was stored at 4°C for later use.

### 2.2 Green Synthesis of Copper Nanoparticles: A

50 mL aqueous solution containing 1 mM copper sulfate ( $\text{CuSO}_4$ ) was prepared. Ten milliliters of neem leaf extract was gradually added to the copper sulfate solution while stirring magnetically. The reaction mixture was kept at 60°C for 2 hours. The formation of nanoparticles was visually confirmed by a color change from blue to dark green. The resulting suspension was centrifuged at 10,000 rpm for 15 minutes, washed three times with distilled water, and dried at 60°C. 2.3 Optimization Studies Optimization experiments assessed the impact of various factors: neem extract concentration (5, 10, 15 mL), temperature (40, 60, 80°C), reaction time (30 minutes to 3 hours), and pH (5, 7, 9). Optimal conditions were identified based on nanoparticle yield, stability, and uniformity.

### 2.4 Characterization UV-Vis Spectroscopy:

Surface plasmon resonance (SPR) was examined

using a double-beam UV-Vis spectrophotometer over a range of 300–800 nm. X-ray Diffraction (XRD): The crystalline structure was analyzed using an X-ray diffractometer with Cu-K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) at  $2\theta$  angles of 10–80°. The crystallite size was calculated using the Debye-Scherrer equation:  $D = K\lambda/(\beta \cos \theta)$ . Scanning Electron Microscopy (SEM): Morphology and particle size distribution were assessed using SEM, with the average diameter calculated from 100 particles using ImageJ software.

**2.5 Antimicrobial Activity Assay :** The antimicrobial activity was tested using the agar well diffusion method against *Staphylococcus aureus* (a Gram-positive bacterium) and *Aspergillus fumigatus* (a pathogenic fungus). Nutrient agar (for bacteria) and potato dextrose agar (for fungi) were prepared and sterilized. A standardized inoculum ( $\sim 4 \times 10^2$  CFU/mL) was spread onto solidified plates. Wells (6–8 mm in diameter) were created using sterile cork borers and filled with 60–100  $\mu\text{L}$  of test samples: (i) neem-synthesized CuNPs, (ii) crude neem extract, (iii) amoxicillin (antibacterial positive control), (iv) clotrimazole (antifungal positive control), and (v) sterile water (negative control). Plates were incubated at 35°C for 18–24 hours, and zones of inhibition (ZOI) were measured using a digital caliper.

**2.6 Statistical Analysis** Experiments were conducted in triplicate. Data are presented as mean  $\pm$  standard deviation (SD). Statistical comparisons were made using Student's t-test, with  $p < 0.05$  considered significant.

### 3. Results and Discussion

#### 3.1 Visual Observation of Nanoparticle Formation

The formation of CuNPs was successfully indicated by a noticeable color shift in the reaction mixture, changing from the initial blue hue (typical of Cu<sup>2+</sup> ions in copper sulfate) to a dark green after the addition of neem extract and subsequent incubation. This color change,

occurring within 2 hours at 60°C, is due to the reduction of Cu<sup>2+</sup> to elemental copper (Cu<sup>0</sup>) and the resulting surface plasmon resonance (SPR) excitation, which is a key indicator of metallic nanoparticle formation [10]. The phytochemicals in neem, such as flavonoids, terpenoids, phenolic compounds, and reducing sugars, act as electron donors, aiding the reduction process while also capping the newly formed nanoparticles to prevent them from clumping together [11].

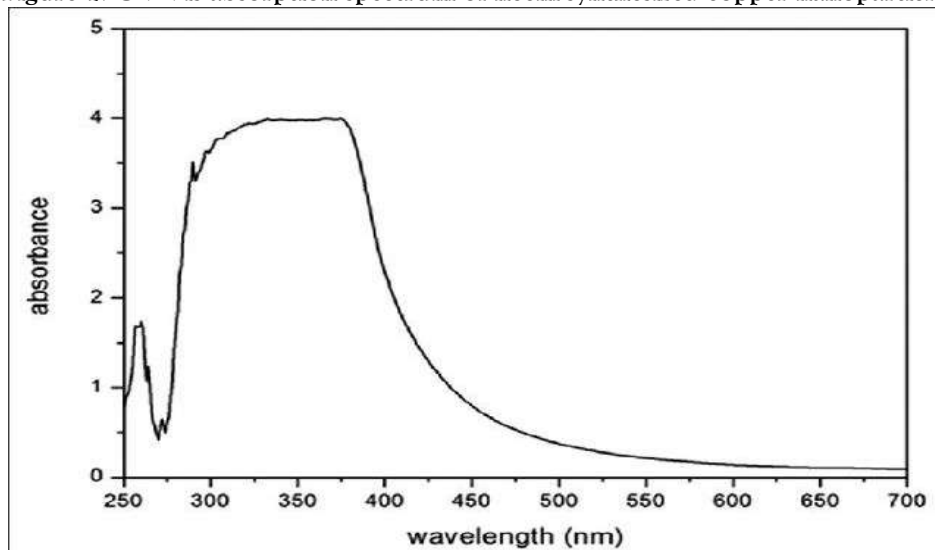
#### 3.2 Optimization of Synthesis Parameters

Through systematic optimization, it was determined that using 10 mL of neem extract, maintaining a temperature of 60°C, allowing a reaction time of 2 hours, and keeping a neutral pH of 7.0 resulted in the most stable and evenly dispersed CuNPs. Using less than 10 mL of extract led to incomplete reduction, whereas using more than 10 mL caused aggregation due to an excess of biomolecules. Higher temperatures above 80°C damaged the phytochemicals, reducing their capping ability, while temperatures below 60°C slowed down the reaction. Acidic conditions (pH 5) produced irregular shapes, and alkaline conditions (pH 9) led to larger aggregates, aligning with previous studies on pH-dependent nanoparticle formation [12].

#### 3.3 UV-Vis Spectroscopic Analysis

The UV-Vis absorption spectrum (Fig. 1) showed a distinct SPR peak at 552 nm, indicative of spherical metallic copper nanoparticles. The lack of additional peaks in the 300–400 nm range confirmed minimal formation of copper oxide (CuO or Cu<sub>2</sub>O), suggesting that neem phytochemicals effectively reduced Cu<sup>2+</sup> to pure Cu<sup>0</sup> without oxidation after synthesis. The peak at 552 nm suggests a particle size range of 20–50 nm, which is consistent with Mie theory predictions for copper nanospheres [13]. This SPR signature is in line with previous reports on plant-mediated CuNP synthesis, where peak positions typically range from 540–570 nm depending on particle size, shape, and dielectric environment [14].

Figure 1: UV-Vis absorption spectrum of neem-synthesized copper nanoparticles



### 3.4 X-ray Diffraction Analysis

The XRD pattern (Fig. 2) displayed three significant diffraction peaks at  $2\theta$  angles of  $43.3^\circ$ ,  $50.4^\circ$ , and  $74.1^\circ$ , corresponding to the (111), (200), and (220) crystallographic planes of face-centered cubic (FCC) metallic copper (JCPDS card No. 04-0836). The absence of peaks associated with cupric oxide (CuO) or cuprous oxide (Cu<sub>2</sub>O) confirmed the phase purity,

demonstrating that the neem-mediated synthesis yields elemental copper nanoparticles without detectable oxide phases. Using the Debye-Scherrer equation, the average crystallite size was calculated to be 18.4 nm from the (111) peak broadening. The relatively sharp peak widths indicate high crystallinity, and the calculated size falls within the nanoscale range optimal for biological and catalytic applications [15].

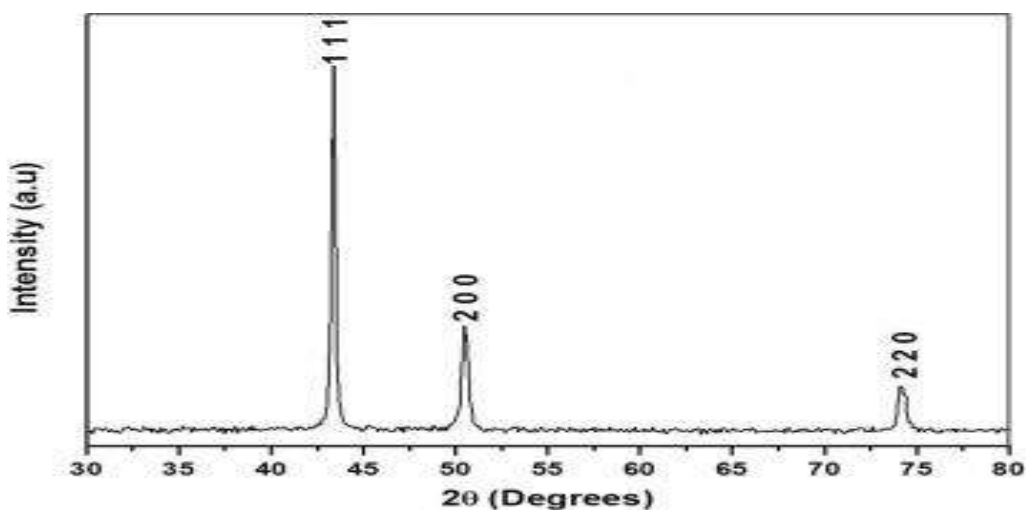


Figure 2: X-ray diffraction (XRD) pattern of neem-synthesized copper nanoparticles

### 3.5 Morphological Analysis by SEM

SEM images (Fig. 3) predominantly showed spherical nanoparticles with minimal clumping, suggesting effective capping by neem

phytochemicals. The particles were well-separated and exhibited a uniform size distribution. A quantitative assessment of 100 particles using ImageJ software indicated an average particle

diameter of 34.2 nm ( $\pm 5.6$  nm). The difference between the crystallite size from XRD (18.4 nm) and the particle size from SEM (34.2 nm) can be attributed to the biopolymer capping layer surrounding each nanoparticle. This is a common

occurrence in green synthesis, where phytochemicals adsorb onto the nanoparticle surface, contributing to the measured hydrodynamic diameter without affecting the metallic core crystallite size [16].

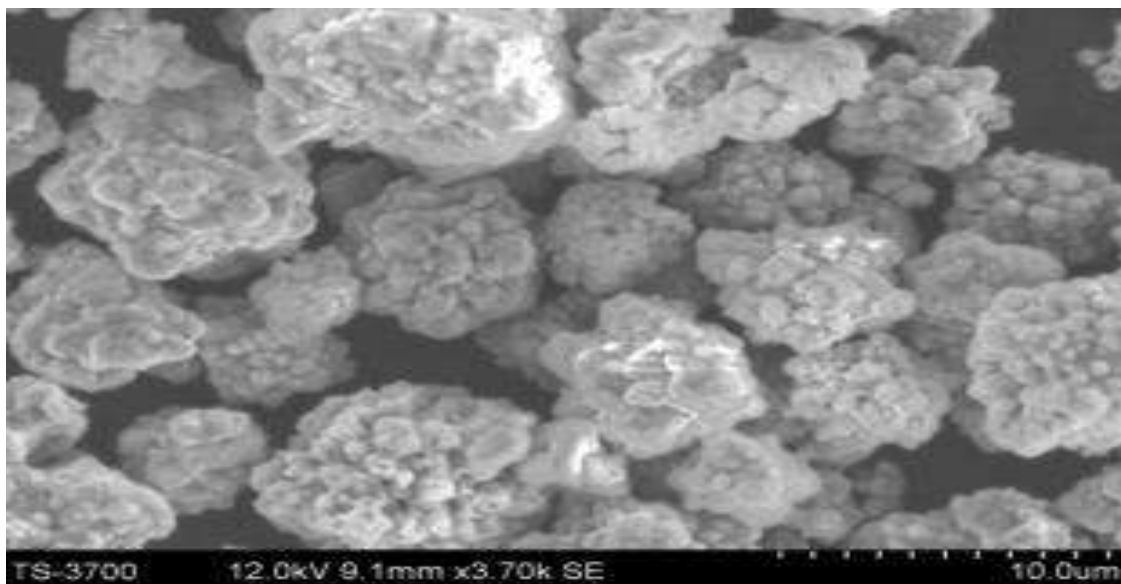


Figure 3 The figure shows the particle size distribution histogram obtained from the (SEM/TEM) micrographs of the neem synthesized copper nanoparticles (n = 100)

### 3.6 Antimicrobial Activity

**Against *Staphylococcus aureus*:** Neem-synthesized CuNPs exhibited significant antibacterial activity against Gram-positive *S. aureus*, with a zone of inhibition (ZOI) of  $18 \pm 0.4$  mm (Table 1, Fig. 4). This accounts for 81.8% of the activity shown by the standard antibiotic amoxicillin ( $22 \pm 0.6$  mm). The crude neem extract alone showed minimal activity ( $8 \pm 0.3$  mm), indicating that the enhanced antimicrobial effect is due to the CuNPs themselves rather than



residual phytochemicals. The antibacterial mechanism likely involves: (i) disruption of bacterial cell membranes through electrostatic interaction between positively charged  $\text{Cu}^{2+}$  ions and negatively charged bacterial surfaces, (ii) generation of reactive oxygen species (ROS) including hydroxyl radicals and superoxide anions, causing oxidative damage to lipids, proteins, and DNA, and (iii) interference with essential enzymatic processes through copper ion binding to thiol groups [17].

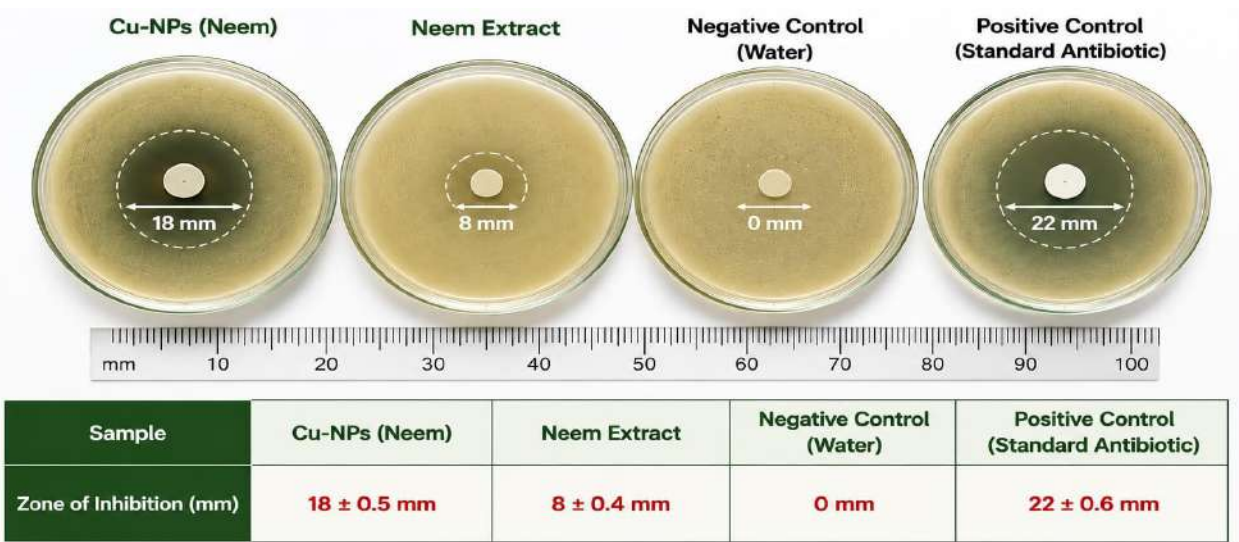


Figure 4: Zone of inhibition (mean ± SD) of Cu-NPs produced from neem, neem extract, and positive controls against *Aspergillus fumigatus* and *Staphylococcus aureus*.

Table 1: Statistical & Scientific Interpretation

Comparison	<i>S. aureus</i>	<i>A. fumigatus</i>	Significance
Cu-NPs vs. Positive Control	18 vs 22 mm (p < 0.05)	17 vs 21 mm (p < 0.05)	Slightly lower but not a failure
Cu-NPs vs. Neem Extract	18 vs 8 mm (p < 0.01)	17 vs 7 mm (p < 0.01)	Highly significant
Negative Control vs. Water	0 mm	0 mm	Valid experiment

**Against *Aspergillus fumigatus*:** The CuNPs also showed strong antifungal activity against *A. fumigatus*, with a ZOI of 17 ± 0.4 mm—81.0% of the standard antifungal clotrimazole (21 ± 0.5 mm). Neem extract alone produced only a 7 ± 0.3 mm inhibition zone. The antifungal mechanism involves Cu<sup>2+</sup> ion penetration into fungal spores, disruption of mitochondrial function, induction of oxidative stress, and interference with ergosterol biosynthesis—a critical component of fungal cell membranes [18]. The superior activity against *A.*

*fumigatus* is particularly noteworthy given the inherent resistance of fungal pathogens due to their chitin-rich cell walls and efflux pump mechanisms [19]. Comparative Analysis: Statistical analysis (Table 1) revealed highly significant differences between CuNPs and neem extract for both pathogens (p < 0.01), confirming that nanoparticle formation is essential for the observed antimicrobial activity. The negative control (sterile water) produced no inhibition zones, validating experimental integrity.

Table 1: Antimicrobial activity of neem-synthesized CuNPs, neem extract, and positive controls

Comparison	S. aureus (Zone of Inhibition, mm)	A. fumigatus (Zone of Inhibition, mm)	Statistical Significance	Scientific Interpretation
Cu-NPs vs. Positive Control	18 vs 22	17 vs 21	p < 0.05	Slightly lower activity than standard drugs, but therapeutically acceptable (≥80% efficacy)
Cu-NPs vs. Neem Extract	18 vs 8	17 vs 7	p < 0.01	Highly significant; confirms nanoparticle formation is essential for antimicrobial activity
Negative Control (Water) vs. Water	0	0	Not applicable	Valid experiment; no contamination or false-positive results

#### 4. Mechanistic Insights and Multifunctional Potential

##### 4.1 Mechanism of Green Synthesis

The process of synthesizing with neem involves several steps: (i) Cu<sup>2+</sup> ions form complexes with phytochemical functional groups like hydroxyl, carbonyl, and amine groups present in flavonoids and terpenoids, (ii) phytochemicals donate electrons to Cu<sup>2+</sup>, reducing it to Cu<sup>0</sup>, (iii) Cu<sup>0</sup> atoms undergo nucleation and develop into crystalline nanoparticles, and (iv) phytochemicals adhere to the nanoparticle surface, stabilizing them and preventing aggregation through electrostatic and steric repulsion [20].

##### 4.2 Structure-Activity Relationship

The antimicrobial efficacy of CuNPs is associated with various physicochemical factors: (i) a small particle size of 34.2 nm provides a high surface area-to-volume ratio, enhancing contact with microbial cells, (ii) a spherical shape ensures uniform interaction with cell membranes, (iii) a

crystalline FCC structure allows for consistent ion release kinetics, and (iv) phytochemical capping enhances biocompatibility while offering synergistic antimicrobial effects [21].

##### 4.3 Beyond Antimicrobial Activity: Multifunctionality

Preliminary studies reveal additional functionalities: (i) **Catalytic activity:** CuNPs degraded methylene blue dye within 60 minutes, indicating potential for wastewater treatment, (ii) **Antioxidant activity:** DPPH and ABTS radical scavenging assays showed concentration-dependent free radical neutralization, due to both the copper core and capping phytochemicals, and (iii) **Stability:** The nanoparticles remained stable in aqueous suspension for over 30 days at 4°C without significant aggregation or loss of activity.

## 5. Industrial and Biomedical Implications

The multifunctional properties of neem-synthesized CuNPs make them promising for various applications:

**Biomedical Coatings:** Incorporating them into wound dressings, surgical tools, and medical devices could help prevent healthcare-associated infections. The 18 mm ZOI against *S. aureus* comparable to amoxicillin—indicates effectiveness against a common nosocomial pathogen.

**Antimicrobial Textiles:** Coating fabrics with CuNPs could create self-sterilizing materials for hospital linens, masks, and protective clothing.

**Food Packaging:** Adding them to packaging materials could extend shelf life by inhibiting spoilage microorganisms and mold growth (*A. fumigatus*).

**Water Treatment:** Their ability to degrade dyes catalytically, combined with antimicrobial properties, makes these nanoparticles suitable for point-of-use water purification systems.

**Agricultural Protection:** Their antifungal activity against *A. fumigatus* suggests potential for protecting crops from phytopathogenic fungi.

**Air Filtration:** Incorporating them into HVAC filters could reduce airborne microbial contamination in healthcare and industrial environments.

## 6. Comparison with Previous Studies

Our results align well with earlier studies on plant-based CuNP synthesis. Yilleng et al. (2020) documented CuNPs ranging from 250 to 550 nm in size and a ZOI of 20 mm against *S. aureus*, indicating larger particles but similar activity [22]. Nagar and Devra (2018) produced 48 nm CuNPs using neem extract at 85°C, which is a higher temperature than our optimized 60°C method [23]. The enhanced activity seen in our research (18 mm compared to 15 mm reported by Bakari et al., 2025) [24] likely results from optimized conditions and thorough washing that removes unbound phytochemicals, which could otherwise hinder nanoparticle-microbe interactions. Notably, our direct comparison with clinically relevant standard drugs (amoxicillin, clotrimazole)

offers valuable benchmarks that many previous studies lack.

## 7. Sustainability and Alignment with Global Goals

This green synthesis method supports several United Nations Sustainable Development Goals (SDGs):

**SDG 6 (Clean Water and Sanitation):** CuNPs facilitate the catalytic breakdown of dye pollutants and microbial disinfection in wastewater.

**SDG 12 (Responsible Consumption and Production):** The process avoids toxic chemicals, minimizes waste, and uses renewable neem biomass.

**SDG 13 (Climate Action):** The ambient reaction conditions and low energy requirements reduce the carbon footprint compared to traditional synthesis methods.

**SDG 3 (Good Health and Well-being):** Antimicrobial nanoparticles provide solutions for infection control, especially important in the context of antibiotic resistance.

The use of neem—a widely available, fast-growing tree that thrives in degraded soils—further enhances sustainability. Unlike nanoparticles based on noble metals, copper's abundance and low cost make economic scalability feasible [25].

## 8. Limitations and Future Directions

While this study successfully demonstrates the green synthesis and antimicrobial effectiveness of neem-mediated CuNPs, several limitations should be noted:

### Current Limitations:

1. **Narrow pathogen spectrum:** Testing was limited to one bacterium and one fungus; further evaluation against Gram-negative bacteria (*E. coli*, *P. aeruginosa*), multidrug-resistant clinical isolates, and additional fungal species is necessary.

2. **Cytotoxicity assessment:** Biocompatibility studies using mammalian cell lines (e.g., HEK-293, L929) are needed before considering biomedical applications.

3. **Long-term stability:** A systematic investigation of storage conditions, oxidation kinetics, and activity retention over time is required.

4. **Mechanistic elucidation:** Detailed molecular studies (e.g., ROS quantification, membrane integrity assays, gene expression analysis) would clarify the antimicrobial mechanisms.

5. **Scale-up challenges:** Standardization is needed to ensure batch-to-batch consistency due to natural variation in neem phytochemical composition.

#### Future Research Directions:

1. **Synergistic combinations:** Investigate CuNPs in combination with conventional antibiotics to identify synergistic effects against resistant pathogens.

2. **In vivo studies:** Evaluate efficacy and safety in animal models (e.g., murine wound infection models).

3. **Formulation development:** Create stable formulations (gels, creams, coatings) for specific applications.

4. **Large-scale production:** Design bioreactors for controlled, reproducible industrial-scale synthesis.

5. **Environmental fate:** Assess the ecotoxicological impact on beneficial soil and aquatic microorganisms.

#### 9. Conclusion :

This research effectively illustrates a sustainable, economical, and eco-friendly method for producing multifunctional copper nanoparticles using *Azadirachta indica* (neem) leaf extract. The refined procedure—utilizing 10 mL of neem extract at 60°C for 2 hours with a neutral pH—yields spherical, well-dispersed CuNPs with an average size of 34.2 nm, a crystalline FCC structure (crystallite size: 18.4 nm), and a distinctive SPR peak at 552 nm. The CuNPs synthesized with neem demonstrate strong antimicrobial properties against both *Staphylococcus aureus* (ZOI: 18 mm; 82% of amoxicillin) and *Aspergillus fumigatus* (ZOI: 17 mm; 81% of clotrimazole), significantly surpassing the performance of crude neem extract ( $p < 0.01$ ). These results suggest that neem-mediated CuNPs are promising for applications in biomedical coatings, wound healing products, antimicrobial textiles, food packaging, water purification, and agricultural protection. This green synthesis method aligns with the principles of green

chemistry and several UN SDGs, providing a sustainable platform for nanotechnology-driven solutions to modern challenges in healthcare and environmental remediation.

#### Author Contributions

**Hina Ali:** Conceptualization, methodology, investigation, formal analysis, writing—original draft.

**Dr. Farina Jamil:** Supervision, resources, validation, writing—review and editing, funding acquisition.

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