

Phyto-synthesis of Copper Nanoparticles and Its Application in Medical Fabrics and Evaluation of Antibacterial Activity

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Abstract

Background: Copper's potent antimicrobial properties make it essential in medical fabrics, enhancing infection control and cleanliness. Phyto-synthesis, using plant-based materials for nanoparticle synthesis, offers an eco-friendly approach that harnesses the natural antimicrobial properties of plants. *Curcuma aromatica*, rich in bioactive compounds, shows potential for synthesizing antimicrobial copper nanoparticles (CuNPs).

Methods: CuNPs were synthesized using *Curcuma aromatica* extract and characterized using UV-Vis spectroscopy, Scanning Electron Microscope (SEM), Fourier transform infrared (FTIR) analysis, and X-ray diffraction (XRD). The antibacterial efficacy of the synthesized CuNPs was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the well diffusion method. The CuNPs embedded cotton fabrics were also evaluated for their antimicrobial activity.

Results: The UV-VIS Spectroscopy showed absorption peaks above 495 nm due to the plasmon resonance of CuNPs. The synthesized nanoparticles ranged in size from 54 to 69 nm which was confirmed by SEM images. FTIR analysis indicated the presence of various organic molecules associated with CuNPs. XRD analysis revealed the face-centered cubic crystal structure of the CuNPs. The CuNPs exhibited significant antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, as evidenced by the inhibition zones. The CuNPs embedded cotton fabrics demonstrated antimicrobial activity against the tested pathogens.

Conclusion: In this study, CuNPs were synthesized using *Curcuma aromatica* extract and exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The incorporation of CuNPs into cotton fabrics resulted in sustained antimicrobial activity, indicating their potential for antimicrobial textile development. These findings support the use of *Curcuma aromatica* as a suitable source for CuNP synthesis and emphasize the significance of CuNPs in the development of effective antimicrobial fabrics for medical applications.

INTRODUCTION

Textiles with antimicrobial property have garnered significant interest in various industries, including clothing, healthcare and defence owing to their potency in combating microbial contamination and improving hygiene standards (Jassal et al., 2021). Copper is essential in medical fabrics due to its potent antimicrobial properties, which inhibit the growth and dissemination of harmful bacteria, thereby enhancing infection control and standards of cleanliness. These textiles possess the ability to inhibit the growth and spread of microorganisms, making them invaluable in environments where infection control is paramount.

Conventional antimicrobial agents, such as synthetic chemicals and heavy metals, have been widely used in textile treatments. However, they often have limitations in terms of toxicity, environmental impact, and the emergence of antimicrobial resistance (Ahmad et al., 2020). These drawbacks have highlighted

the need for alternative and sustainable solutions that can provide effective antimicrobial properties without compromising safety and sustainability.

One such innovative approach is usage of nanoparticles as antimicrobial agents. Phyto-synthesis of nanoparticles offer dual advantage as the plant on their own possess antimicrobial properties (Das et al., 2020). Phyto-synthesis offers several advantages, including its eco-friendly nature, cost-effectiveness, and the potential to harness the natural antimicrobial properties of plants.

Copper nanoparticles, in particular, have shown promise as effective antibacterial agents (Vardhana et al., 2022). The unique properties of copper nanoparticles, such as their high surface-to-volume ratio and release of copper ions, contribute to their antimicrobial efficacy against a broad spectrum of microorganisms, including bacteria, fungi, and viruses (Fayaz et al., 2010). In textile applications, copper nanoparticles can be incorporated onto fabrics to provide durable and long-lasting antimicrobial activity. The copper based antimicrobial fabrics are widely used in controlling asthma, allergy caused by dust mites and in socks to control foot diseases like athlete's foot (New Cloth Market, 2013).

Curcuma aromatica, commonly known as wild turmeric or Vanaharidra, belongs to the ginger family (Zingiberaceae) and is rich in bioactive compounds such as curcuminoids, volatile oils, and phenolic compounds (Das et al., 2018). Curcuminoids, the major constituents of *Curcuma aromatica*, possess remarkable antioxidant, anti-inflammatory, and antimicrobial properties (Sethi et al., 2017). These compounds have been extensively studied for their therapeutic potential in various diseases and as natural antimicrobial agents.

The use of plant extracts in nanoparticle synthesis is gaining prominence due to their biocompatibility, non-toxicity, and sustainability (Das et al., 2020). Moreover, *Curcuma aromatica* extract offers advantages such as its abundance, cost-effectiveness, and eco-friendliness compared to synthetic chemicals used in conventional nanoparticle synthesis methods. Few reports demonstrated the efficacy of *Curcuma aromatica* extract in the synthesis of Nanoparticles (Shende et al., 2016).

The aim of this study is to synthesize copper nanoparticles (CuNPs) using *Curcuma aromatica* extract and evaluate their antimicrobial properties incorporated to cotton fabrics. The objectives include the synthesis and stabilization of CuNPs, evaluation of antibacterial property of synthesized CuNPs and incorporation of CuNPs onto cotton fabrics and assessment of the antibacterial activity of the treated cotton for developing antimicrobial textiles for its usage in healthcare industry.

MATERIAL AND METHODS

Collection of plant:

Dry rhizome of *Curcuma aromatica* (Family:Zingiberaceae) was procured from Super market in Chennai, Tamilnadu, India. The rhizome procured was crushed to powder with the help of mortar and pestle. The *Curcuma aromatica* rhizome powder was used for synthesizing CuNPs (Fig. 1).

Preparation of Aqueous Extract of *Curcuma aromatica*:

Ten grams of *Curcuma aromatica* powder was dispensed in 100 ml sterile distilled water and boiled for 5 minutes for preparation of plant extract. The extract was then filtered using Whatman's No.1 filter paper and stored for further usage in CuNPs synthesis.

Synthesis and optimization of copper nanoparticles from the *Curcuma aromatica*:

Copper sulphate (CuSO_4) was used as the precursor for the synthesis of CuNPs. For the synthesis, 100ml of 1mM of copper sulphate solution was prepared and 10 mL of aqueous extract of *Curcuma aromatica* was added and mixed homogeneously on magnetic stirrer for 15 min so as to ensure the formation of CuNPs.

Characterization of copper nanoparticles:

The plasmon resonance of the synthesized nanomaterial is studied using UV–Vis (Shimadzu UV–Vis 2450) spectrophotometer with the wavelength ranging from 200–800 nm. The morphology of the synthesized CuNPs was studied using Scanning Electron Microscope (SEM) (CARL ZEISS, EVO 18), and X-ray Diffraction Spectroscopy (XRD) (PAN analytical: XPERT-PRO) and the associated chemicals of CuNPs using Fourier Transform Infra-Red (FTIR) (Shimadzu FTIR Prestige 21) spectrophotometer.

Antibacterial Efficacy of the biosynthesized CuNPS:

The antibacterial efficacy of green synthesised CuNPs was studied using well diffusion method against the bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The overnight bacterial cultures were uniformly swabbed over the surface of Nutrient Agar Plates and allowed to dry for 15 minutes. Well of 5mm diameter (4 numbers) was cut using corkborer. Each well was added with different concentrations of (10, 15, 20 and 25 μ l) CuNPs and the plates were incubated at 37°C for 24 h. After 24 h of incubation, the zone of inhibition was measured in millimetres. The tests were performed in triplicates.

Incorporation of CuNPs onto Cotton Fabrics and Evaluation of its Antibacterial activity

Cotton fabrics of size 2 × 2 cm were washed in sterile distilled water rinsed and autoclaved. The fabrics were then treated with green synthesized CuNPs for an hour and were allowed to dry at 50°C for 30 min. After drying, the cotton fabrics were analyzed using FE-SEM to ensure the incorporation of CuNPs onto the fibres. The NPs treated cotton fabrics were placed over the culture plates inoculated with respective bacteria. Untreated cotton fabrics were placed as control.

RESULTS

Synthesis of Copper Nanoparticles

After an addition of the extract of *Curcuma aromatica* to the specified concentration of Copper Sulphate (CuSO_4), change in color was observed within 10–15 minutes time. The blue colour solution changes to pale bluish green due to the Plasmon resonance, which confirms the formation of CuNPs (Fig. 2).

Characterization of Copper Nanoparticle

UV Adsorption Spectra

The UV – Vis spectra of synthesised CuNPs showed the absorption peaks above 554 nm (Fig. 3) which indicates that the reduction reaction occurred.

Scanning Electron Microscopy (SEM) Analysis

The SEM examination of the copper nanoparticles confirmed (Fig. 4) the synthesized nanoparticles ranged in size from 54.83 to 69.66 nm.

FTIR Spectral Analysis

The FTIR spectra was performed for the synthesized copper nanoparticle (Fig. 5). Peak at 3328 could be attributed to the stretching vibration of O-H bonds, indicating the presence of water molecules or hydroxyl groups. Peak at 2137 suggests the presence of carbon-carbon triple bonds ($\text{C} \equiv \text{C}$). Peak at 1636 corresponds to the stretching vibration of $\text{C} = \text{C}$ bonds, indicating the presence of carbon-carbon double bonds.

The X-Ray Diffraction (XRD) Analysis

The phase evaluation of the dried copper nanoparticles were analysed through X-ray Diffractometer with $\text{Cu-K}\alpha$ as a radiation source. The diffracted intensities 2θ angle recorded was at 28.5. The intense peaks at the values of 32.18, 34.87, 36.79, 38.53, 42.72 correspond to the characteristics of face centered cubic (fcc) structure (Fig. 6).

Scanning Electron Microscopy of Cotton Fibre Amended with CuNPs

Copper nanoparticles embedded in cotton fabric were subjected to SEM analysis, which revealed that the fabric contains a widespread distribution of copper nanoparticles (Fig. 7).

Anti -Bacterial Activity

CuNP's showed antibacterial activity against both gram positive and gram negative bacteria. Maximum zone of inhibition of about 2 cm was recorded against the gram negative bacteria *Escherichia coli* for the concentration of 25 μl and about 1.3 cm as the zone of inhibition against the gram positive bacteria, *Staphylococcus aureus* (Fig. 8).

Antibacterial Activity Of CuNPs Loaded Cotton Fabric

The antibacterial activity of CuNPs loaded cotton fabrics were evaluated against both gram negative and gram-positive bacteria. The *Escherichia coli* displayed sensitivity (0.8 cm) against the CuNPs amended cotton fabric followed by the *Staphylococcus aureus* (0.6 cm) when compared to Untreated cotton fabric (Fig. 9).

DISCUSSION

The aim of this study was to synthesize copper nanoparticles (CuNPs) using *Curcuma aromatica* extract and evaluate their antimicrobial properties when incorporated into cotton fabrics. The results demonstrate the successful synthesis of CuNPs using the plant extract and their significant antimicrobial activity against tested pathogens. The use of plant-based materials for nanoparticle synthesis offers a sustainable and eco-friendly approach, highlighting the potential for developing environmentally friendly antimicrobial materials. Furthermore, the embedding of CuNPs onto cotton fabrics resulted in textiles with effective and durable antimicrobial activity, making them suitable for various applications, particularly in healthcare settings where infection control is crucial.

The UV-Vis spectroscopy analysis confirmed the formation of CuNPs, as indicated by the absorption peaks above 495nm. This is consistent with previous studies that reported similar absorption spectra for copper nanoparticles synthesized using plant extracts (Aguilar, et al., 2019). The change of native color, i.e. blue-to-pale bluish-green was observed during the reaction exhibiting plasmon resonance attributed to the reduction of copper ions.

Nanomaterial refers to a material that possesses nanoscale dimensions in at least one external dimension, such as length, width, or thickness. On the otherhand, a nanoparticle specifically refers to a particle with nanoscale dimensions in all three external dimensions (length, width, and thickness) (Nel et al., 2006). The scanning electron microscopic (SEM) image confirmed that the particles formed during the phytosynthesis method fell under the category of nanoparticles rather than nanomaterial. The size range of 54nm to 69nm, as recorded in the microscopic study, further supports their classification as nanoparticles.

The FTIR analysis of the synthesized CuNPs revealed the presence of various functional groups associated. These reactive oxidative species may also have an impact on inducing antibacterial property. The phytochemical compounds present in the extract of *Curcuma aromatica* like terpenoids, flavonoids, ketones, aldehydes, suggest their involvement in the stabilization and capping of the CuNPs (Jena et al., 2020).

The X-ray diffraction (XRD) analysis confirmed the face-centered cubic (fcc) crystal structure of the copper nanoparticles, as evidenced by the intense peaks at specific 2θ angle which is specific to CuNPs. The other associated peaks may be attributed to the phytocompounds of *Curcuma aromatica*. This is consistent with the characteristic XRD pattern of copper reported in previous studies (Sarkar et al., 2020). The presence of a single crystal structure indicates the formation of well-defined and crystalline CuNPs.

The antibacterial activity evaluation of the synthesized CuNPs against *Staphylococcus aureus* and *Escherichia coli* demonstrated significant inhibitory effects. The CuNPs exhibited significant zone of inhibition indicating potential antibacterial activity of the nanoparticles. These findings are consistent with previous studies that reported the antibacterial efficacy of copper nanoparticles against various pathogenic bacteria (Li et al., 2021; Raman et al., 2022). The release of copper ions from the nanoparticles are believed to contribute to their antimicrobial action by damaging the cell membranes and the nuclear material after penetration of cell wall. Copper used as an antimicrobial agent for decades has revealed a strong antibacterial activity and was able to decrease the microorganism concentration by 99.9% (N. Krithiga, A et al., 2013 and Ipsa Subhankari *et al.*, 2013).

The incorporation of CuNPs onto cotton fabrics resulted in antimicrobial textiles with effective bactericidal properties. The CuNP-loaded cotton fabrics exhibited larger zones of inhibition against both *Staphylococcus aureus* and *Escherichia coli* compared to untreated cotton fabrics. These results are in agreement with previous studies that investigated the antimicrobial activity of copper nanoparticles incorporated into textiles (Kumar et al., 2020; Sirelkhatim et al., 2015). The durable and long-lasting antimicrobial activity of CuNPs on cotton fabrics makes them suitable for various applications, particularly in healthcare settings where infection control is crucial (Li et al., 2021).

CONCLUSION

This study proves successful synthesis of copper nanoparticles using *Curcuma aromatica* extract and demonstrated their potent antimicrobial activity when incorporated into cotton fabrics. The results align with existing literature on the use of plant-based materials for nanoparticle synthesis and the antimicrobial efficacy of copper nanoparticles. The eco-friendly and sustainable nature of *Curcuma aromatica* extract, along with its abundance and cost-effectiveness, further highlight its potential as a valuable source for synthesizing antimicrobial nanoparticles. Future research can explore the optimization of CuNP loading onto textiles and evaluate their performance in real-world scenarios to assess their practical application in enhancing hygiene standards.

Declarations

Ethical Approval: Not Applicable

Competing Interests: The authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Dual Publication: The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another Publisher.

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Author Contributions Statement

1*- Conceptualization, design, and supervision of the study, Acquisition, analysis, and interpretation of data, Drafting and revising the manuscript, Final approval of the version to be submitted, Manuscript revision and editing. 2- Experimental work

Availability of data and materials: All of the material is owned by the authors and/or no permissions are required.

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Figures



Figure 1

Collection and Preparation of Sample.

(A) Dry *Curcuma Aromatica* rhizome: The image shows the rhizome of *Curcuma aromatica* in its dried form, which is used as the starting material for the experiment.

(B) Powdered rhizome: The image displays the powdered form of the *Curcuma aromatica* rhizome obtained after grinding and processing.



Figure 2

Preparation and Synthesis of Copper Nanoparticles.

(a) Aqueous Extract of the Sample: The image represents the aqueous extract obtained from the *Curcuma aromatica* rhizome, which serves as a key component in the synthesis of copper nanoparticles.

(b) *C. aromatica* Extract: The image showcases the extract derived from *Curcuma Aromatica*, highlighting its role in the synthesis process.

(c) Synthesized CuNP: The image exhibits the copper nanoparticles that have been successfully synthesized using the aqueous extract of *Curcuma Aromatica*.

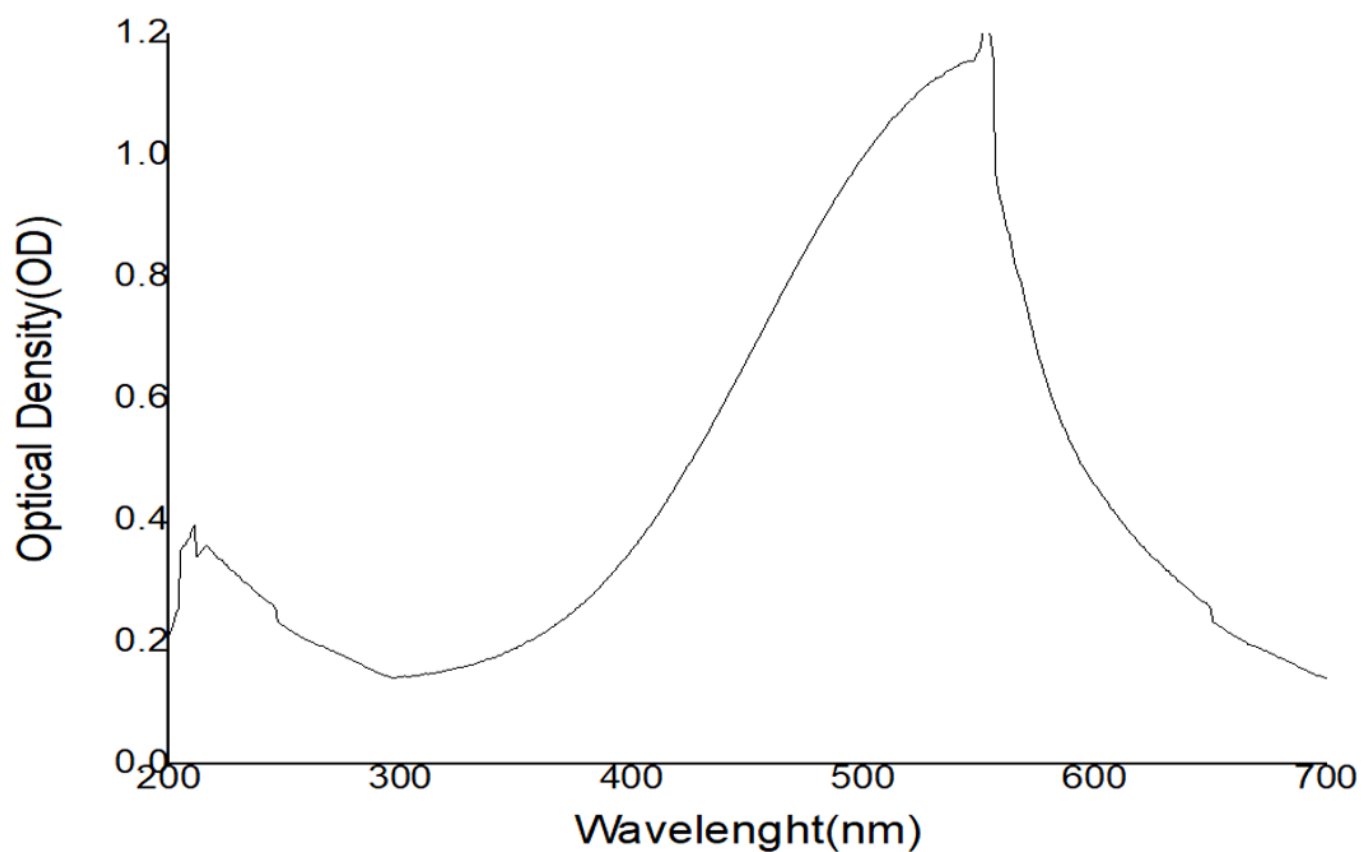


Figure 3

UV Absorption Spectra of Copper Nanoparticles: This figure displays the ultraviolet (UV) absorption spectra of the synthesized copper nanoparticles, providing information about their optical properties.

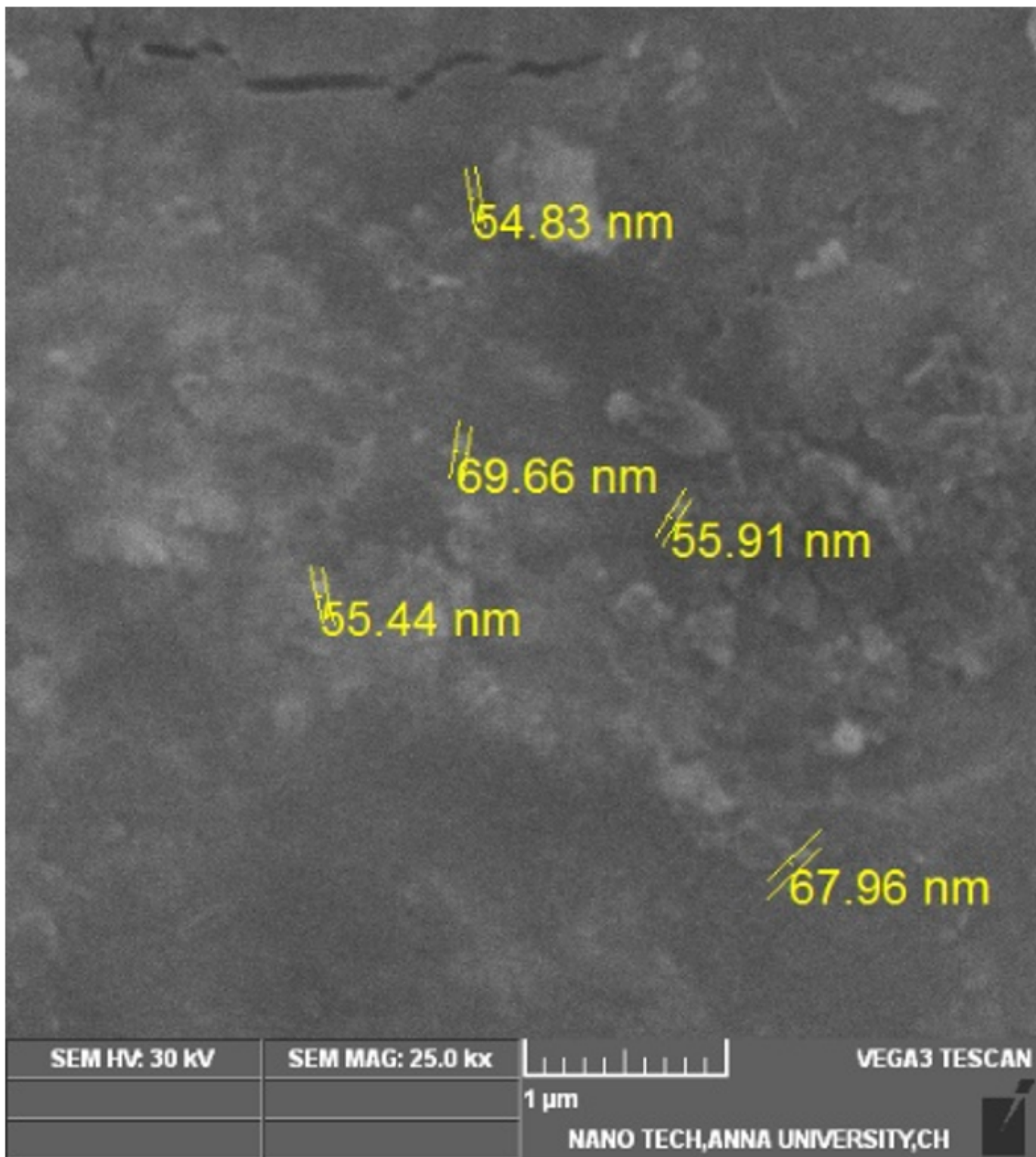


Figure 4

SEM Image of the Synthesized Copper Nanoparticles: The scanning electron microscope (SEM) image reveals the surface morphology and structure of the synthesized copper nanoparticles, allowing for a detailed examination at the nanoscale level.

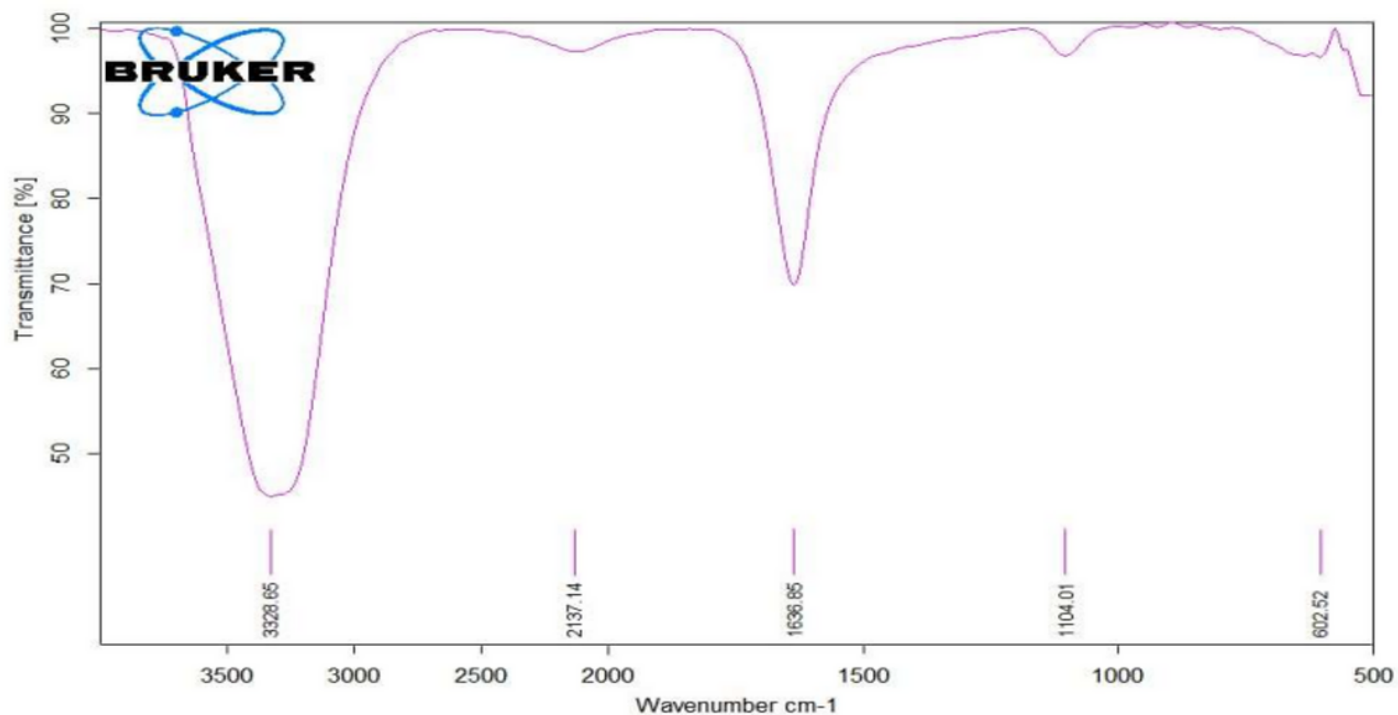


Figure 5

FTIR Spectra of Synthesized Copper Nanoparticles: The figure presents the Fourier-transform infrared (FTIR) spectra of the synthesized copper nanoparticles, which provides information about the chemical bonds and functional groups present in the nanoparticles.

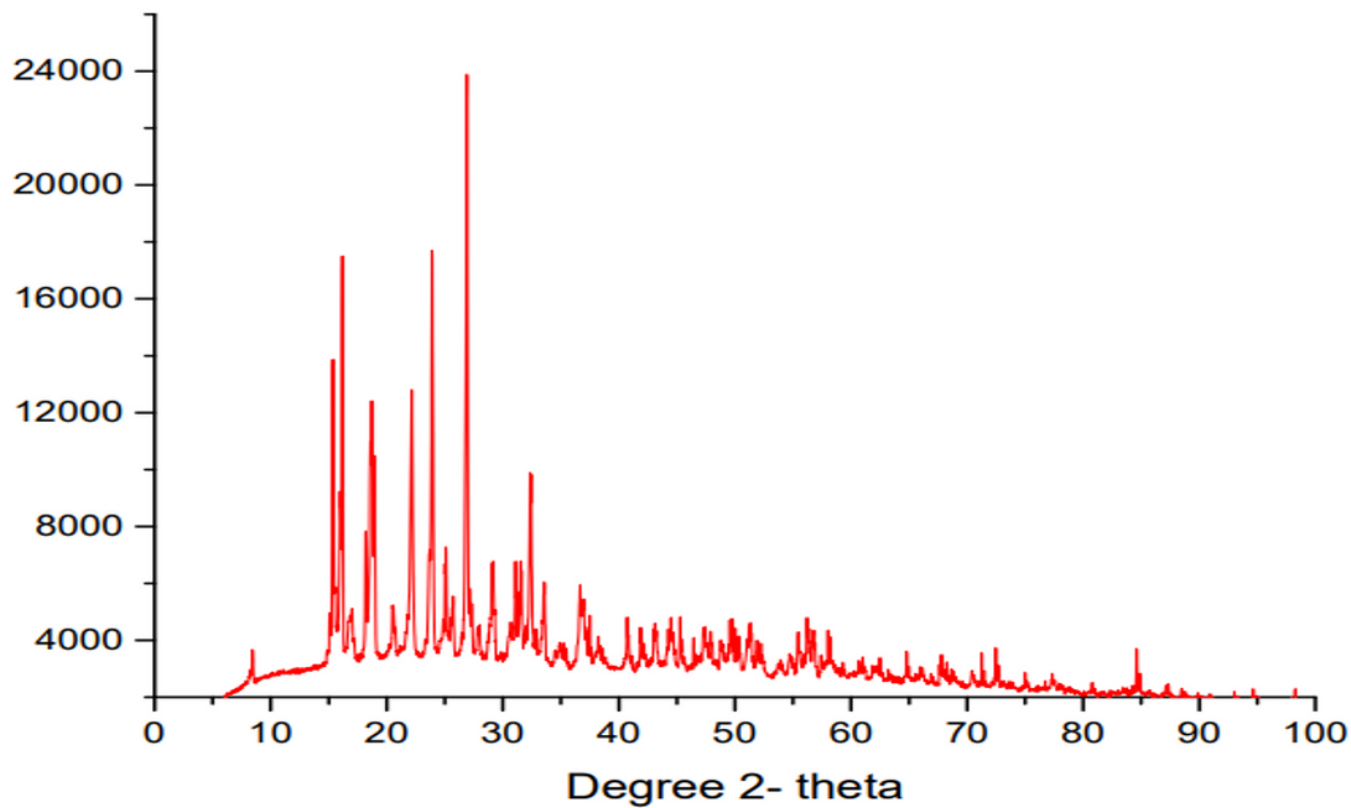


Figure 6

XRD Spectral Analysis of Copper Nanoparticles: This figure illustrates the X-ray diffraction (XRD) spectra of the copper nanoparticles, enabling the identification and characterization of their crystal structure and phase composition.

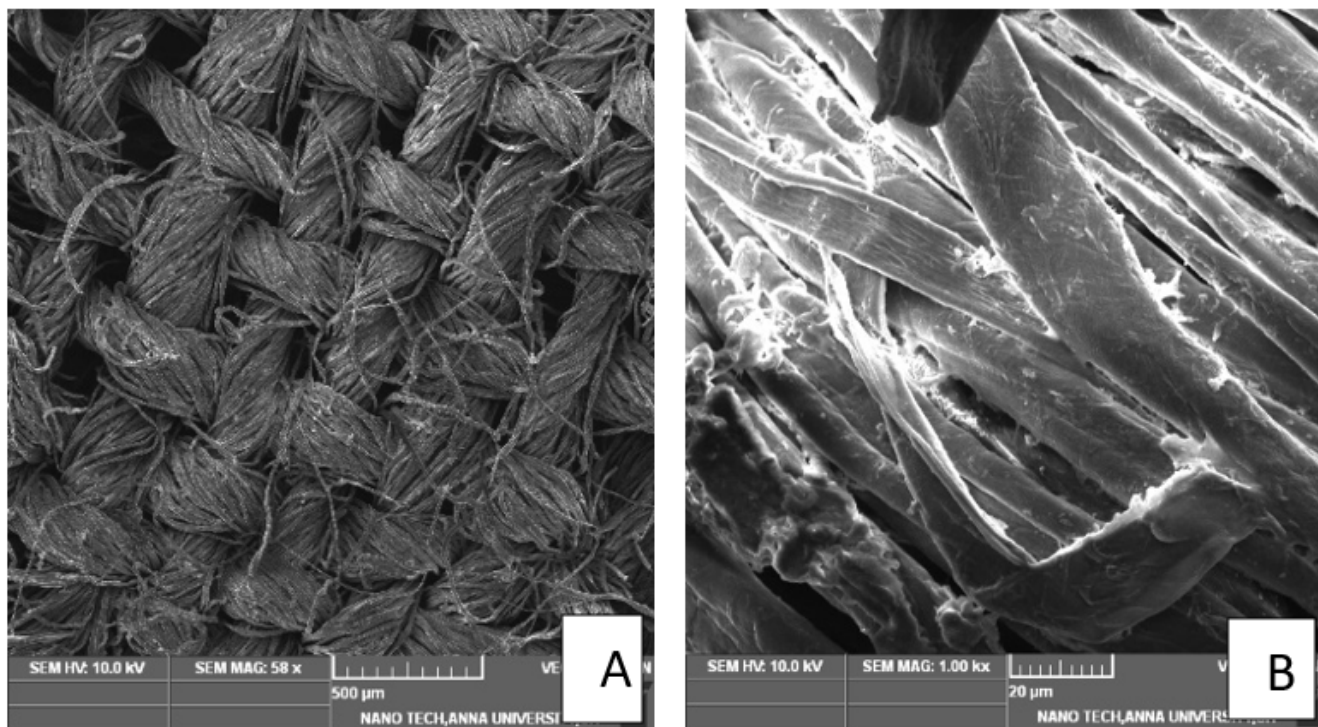


Figure 7

SEM Image of Copper Nanoparticles Embedded Cotton Fabric.

(A) Control: The SEM image shows the surface of cotton fabric without any copper nanoparticles embedded.

(B) CuNP Deposition on the Surface of the Fabric: The SEM image demonstrates the presence of copper nanoparticles deposited on the surface of the cotton fabric, indicating successful embedding.

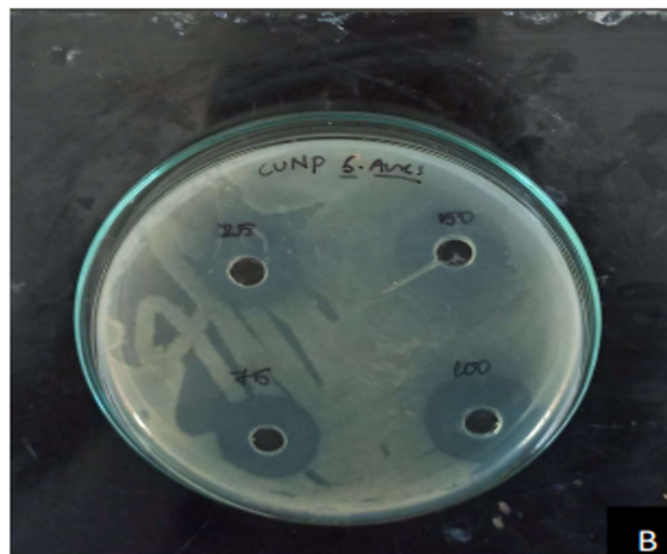


Figure 8

Anti-bacterial Activity of CuNPs.

(A) *Escherichia coli*: The figure exhibits the antibacterial activity of copper nanoparticles against *Escherichia coli* bacteria.

(B) *Staphylococcus aureus*: The figure demonstrates the antibacterial activity of copper nanoparticles against *Staphylococcus aureus* bacteria.

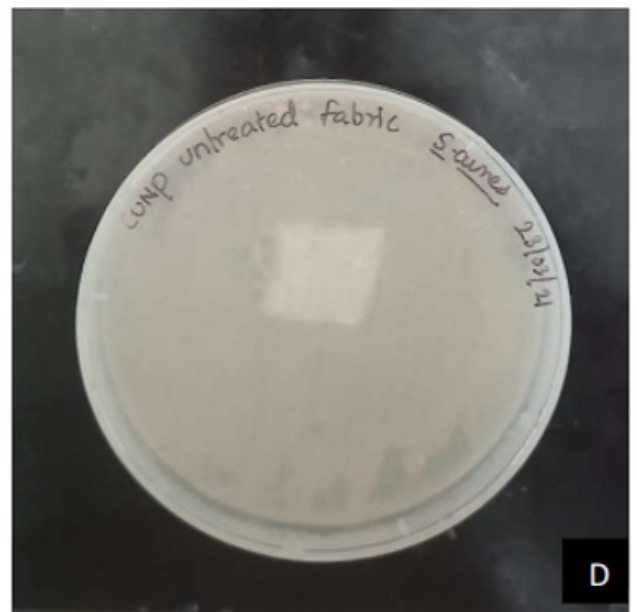
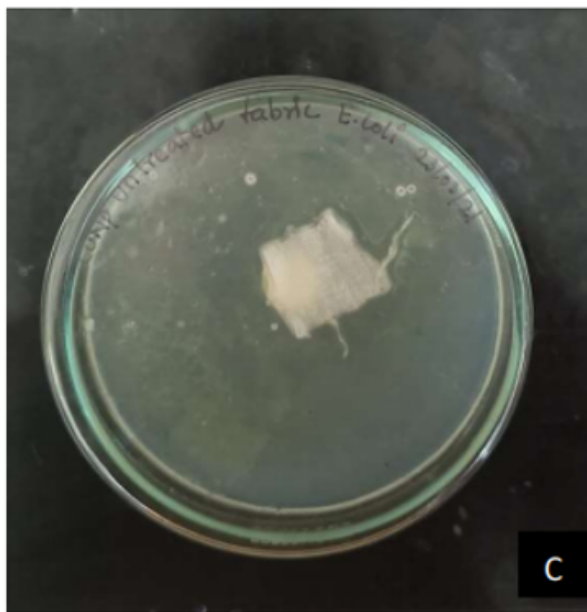
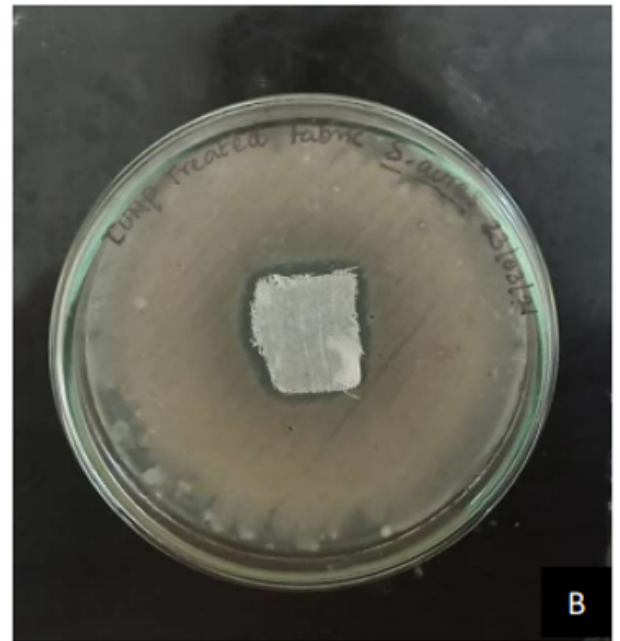
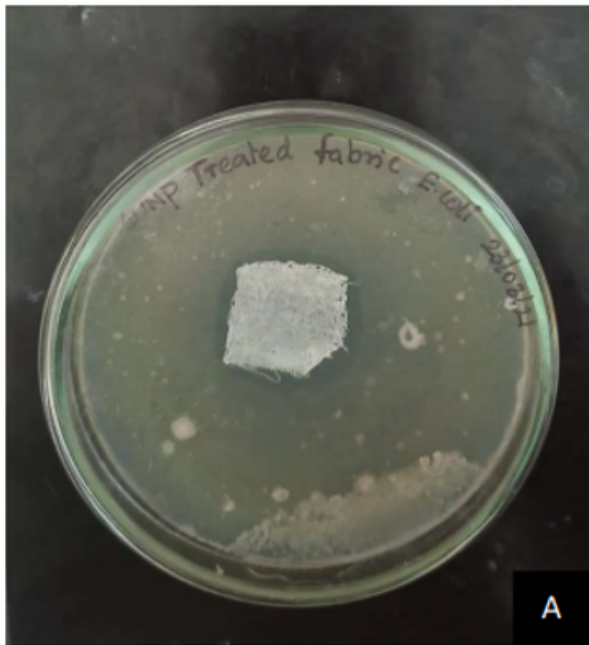


Figure 9

a: Antibacterial Activity of CuNP-Embedded Fabric.

(A) *E. coli*: The figure displays the antibacterial activity of cotton fabric embedded with copper nanoparticles against *Escherichia coli* bacteria.

(B) *S. aureus*: The figure demonstrates the antibacterial activity of cotton fabric embedded with copper nanoparticles against *Staphylococcus aureus* bacteria.

b: Antibacterial Activity of CuNP Untreated Fabric.

(C) *E.coli*: The figure illustrates the antibacterial activity of untreated cotton fabric without copper nanoparticles against Escherichia coli bacteria.

(D) *S. aureus*: The figure showcases the antibacterial activity of untreated cotton fabric without copper nanoparticles against Staphylococcus aureus bacteria.