Research Article

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Antibacterial, Antifungal, Antioxidant, and Anticancer Activities of CuO Nanoparticles Green Synthesized Using *Solanum xanthocarpum*

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ABSTRACT

This study demonstrates a green and sustainable method for synthesizing copper oxide nanoparticles (CuO NPs) using *Solanum xanthocarpum* leaf extract as a natural reducing and stabilizing agent. Structural analysis by X-ray diffraction confirmed the formation of crystalline monoclinic CuO NPs. Fourier-transform infrared spectroscopy identified functional groups originating from phytochemicals, suggesting their role in nanoparticle capping and stabilization. UV–Visible spectroscopy revealed characteristic absorption in the visible range, while photoluminescence spectrum provided insights into the optical emission behavior of the CuO NPs. Field emission scanning electron microscopy showed aggregated nanoparticle morphology, and energy-dispersive X-ray spectroscopy along with elemental mapping confirmed the uniform distribution of copper and oxygen elements. The biosynthesized CuO NPs exhibited significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and antifungal activity against *Candida albicans* and *Aspergillus niger*. Antioxidant potential, evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, indicated strong free radical scavenging capacity. Furthermore, cytotoxicity assessment against A498 human kidney carcinoma cells revealed promising anticancer activity. These findings highlight the potential of *S. xanthocarpum*-mediated CuO NPs for diverse biomedical applications, including antimicrobial, antioxidant, and anticancer therapies.

Keywords: Green synthesis; CuO nanoparticles; *Solanum xanthocarpum;* Antibacterial activity; Antifungal activity; Antioxidant activity; Anticancer activity.

1. INTRODUCTION

Nanotechnology is a rapidly advancing and transformative field that has found widespread application across diverse industries, including chemical manufacturing, pharmaceuticals, mechanical engineering, and food processing. At its core, lies nanoscience, which focuses on the design, development, and manipulation of materials at the nanoscale, typically between 1 and 100 nanometers. At this scale, nanoparticles exhibit distinctive physical, chemical, and biological properties that differ significantly from their bulk counterparts, rendering them highly suitable for innovative applications in medicine, electronics, catalysis, and environmental remediation (Nair *et al.* 2022).

Among various nanomaterials, metal and metal oxide nanoparticles are particularly valued for their potent antimicrobial activity, attributed to their high surface area-to-volume ratio and unique surface reactivity (Sengul and Asmatulu, 2020). However, conventional nanoparticle synthesis methods often rely on hazardous chemical reducing and stabilizing agents, which pose significant environmental and biological risks, thereby limiting their practical utility in biomedicine and environmental sciences (Szczyglewska *et al.* 2023).

Copper oxide nanoparticles (CuO NPs), in particular, have gained increasing attention due to their favorable characteristics, including large surface area, chemical stability, and strong antimicrobial efficacy. These properties make CuO NPs as promising candidates for applications such as targeted drug delivery, biosensing, cancer therapeutics, and environmental detoxification (Grigore *et al.* 2016; Prakash *et al.* 2018; Radulescu *et al.* 2023; Nayal *et al.* 2024). A wide range of synthetic techniques, such as sol-gel, hydrothermal, solvothermal, co-precipitation, microwave-assisted synthesis, and thermal decomposition, have been employed to produce CuO NPs with controlled size and morphology. Despite their effectiveness, these methods typically require high temperatures, complex instrumentation, and toxic reagents, making them energy-intensive and environmentally hazardous (Uma *et al.* 2021).

In response to these limitations, green synthesis has emerged as a sustainable and eco-friendly alternative for nanoparticle production. This approach uses biological systems, such as plant extracts, microorganisms, enzymes, or biopolymers, as natural reducing and stabilizing agents, thereby eliminating the need for toxic chemicals and promoting environmental compatibility (Rafique *et al.* 2017). Plant-mediated synthesis, in particular, offers several advantages including cost-effectiveness, wide availability, simplicity, and the presence of diverse phytochemicals. Bioactive compounds such as flavonoids, alkaloids, terpenoids, phenolics, tannins, and saponins not only facilitate the reduction of metal ions but also act as capping agents that stabilize the nanoparticles and prevent agglomeration (Iravani, 2011).

Solanum xanthocarpum, a well-known medicinal plant in Ayurvedic medicine, has shown potential in the green synthesis of nanoparticles. Traditionally used for its anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and anticancer properties, *S. xanthocarpum* serves as a rich source of bioactive molecules (Amin *et al.* 2012; Bharathidasan *et al.* 2024). The phytochemicals present in its leaf extract aid in the reduction and stabilization of metal nanoparticles, while also enhancing their biological activities. Prior studies (Xu *et al.* 2022; Rahman *et al.* 2025) have demonstrated the successful synthesis of multifunctional silver and copper nanoparticles using *S. xanthocarpum* fruit extract, highlighting its potential for sustainable nanotechnology in antimicrobial and environmental applications.

The present study aims to develop an eco-friendly synthesis route for CuO NPs using *S. xanthocarpum* leaf extract. The synthesized nanoparticles are characterized for their structural and morphological features using advanced analytical techniques. Additionally, their biological efficacy is evaluated through antibacterial, antifungal, antioxidant, and anticancer assays. This research aligns with the principles of green chemistry and contributes to the development of cost-effective, biocompatible, and environmentally safe nanomaterials for potential biomedical applications.

2. MATERIALS AND METHODS

2.1 Extract Preparation

Fresh leaves of *Solanum xanthocarpum* were collected, thoroughly washed with tap water followed by distilled water to remove surface impurities, and subsequently shade-dried for 5–7 days. The dried leaves were then ground into a fine powder using a clean mortar and pestle. Approximately, 10 g of the powdered material was boiled in 200 mL of distilled water at a temperature of 60–70 °C for 30 minutes. After allowing the mixture to cool to room temperature, it was filtered using Whatman No. 1 filter paper to obtain a clear plant extract. This extract was stored at 28 °C and used as a natural reducing and stabilizing agent in the green synthesis of copper oxide nanoparticles.

2.2 Synthesis of CuO NPs Using *S. xanthocarpum* Leaf Extract

To synthesize CuO nanoparticles *via* a green route, 0.1 M copper nitrate trihydrate [$Cu(NO_3)_2 \cdot 3H_2O$] was dissolved in 100 mL of distilled water under continuous stirring to ensure complete dissolution. Subsequently, 100 mL of *Solanum xanthocarpum* leaf extract was added dropwise to the solution while maintaining constant stirring. A visible color change accompanied by the formation of a gel indicated the initiation of nanoparticle synthesis. The resulting gel was allowed to age, then dried in a hot air oven at 80 °C to remove moisture. The dried material was further calcined at 500 °C for 3 hours to obtain pure, crystalline CuO NPs. The synthesis process is depicted in Fig. 1.

Solanum xanthocarpum leaf extract is known to contain flavonoids, phenolic acids, alkaloids, tannins, and saponins. Among these, phenolics and flavonoids are most actively involved in the reduction and stabilization of CuO NPs. Phenolic –OH groups and flavonoid hydroxyl groups donate electrons to Cu^{2+} ions, facilitating their reduction to CuO nuclei during thermal treatment. Phenolic acids and flavonoids contain functional groups such as –OH, –COOH, –C=O, and –NH. These functional groups interact with the CuO surface through hydrogen bond, forming a protective organic shell that prevents excessive aggregation.

2.3 Antibacterial Activity

The antibacterial activity of the synthesized CuO NPs was evaluated using the agar well diffusion method. Nutrient agar plates were uniformly inoculated with bacterial cultures of *Staphylococcus aureus* and *Escherichia coli*. Sterile cork borers were used to punch wells into the agar medium, and different concentrations of the CuO nanoparticle suspension were carefully introduced into each well. The plates were then incubated at 37 °C for 24 hours. Following incubation, the antibacterial efficacy was assessed by measuring the diameter of the inhibition zones around each well, expressed in millimeters (Boyanova *et al.* 2005).

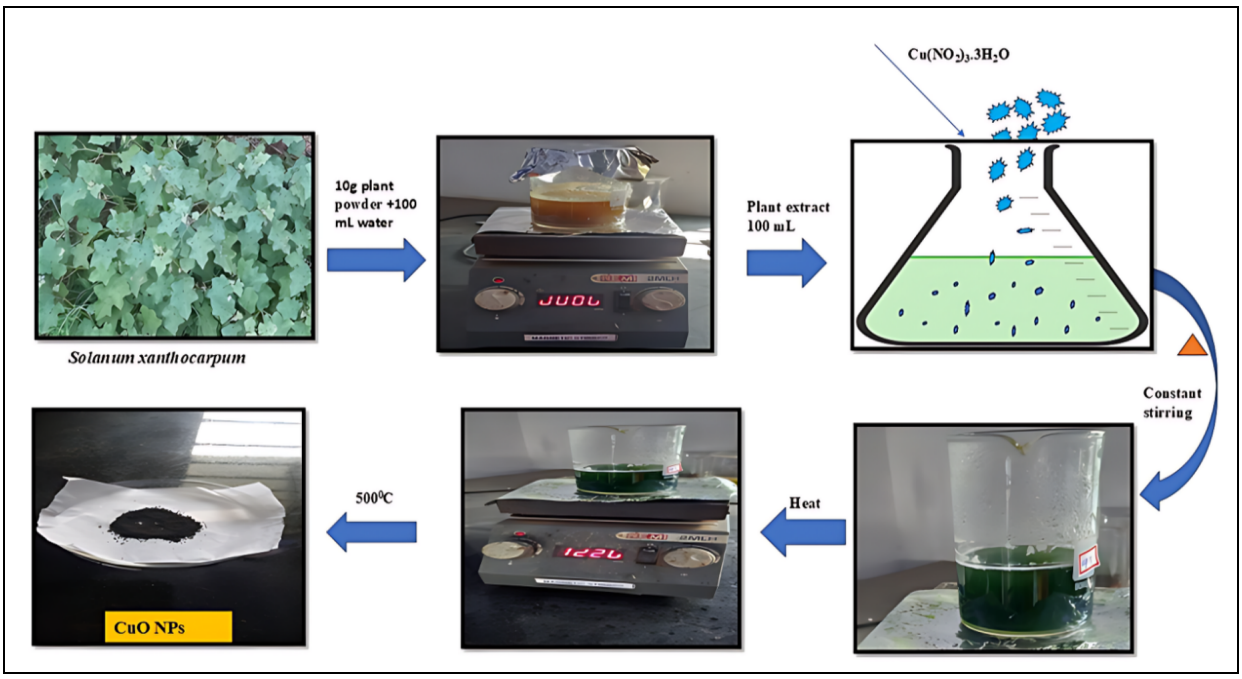


Fig. 1: Schematic representation of the green synthesis of CuO NPs using *Solanum xanthocarpum* leaf extract

2.4 Antifungal Activity

The antifungal activity of the synthesized CuO nanoparticles was assessed using the agar well diffusion method against *Candida albicans* and *Aspergillus niger*. Fungal cultures were evenly spread onto Potato Dextrose Agar (PDA) plates. Wells were then created in the agar using sterile cork borers and filled with varying concentrations of the CuO nanoparticle suspension. The plates were incubated at 28 °C for 48 hours. After incubation, the diameters of the inhibition zones were measured in millimeters and recorded to evaluate antifungal efficacy (Intra *et al.* 2019).

2.5 Antioxidant Activity

The antioxidant potential of the green-synthesized CuO NPs was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Various concentrations of the nanoparticle suspension (ranging from 1 to 10 µg/mL) were mixed with 1 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent photodegradation of the DPPH radicals. After incubation, the absorbance of each sample was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid (Vitamin C) was used as a standard reference antioxidant. A calibration curve was established using known concentrations of Vitamin C for comparative analysis.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad \ldots (1),$$

where A_0 is the absorbance of the control (DPPH solution without sample) and A_1 is the absorbance of the test sample or standard.

2.6 Anticancer Activity (MTT Assay)

The cytotoxic effects of the synthesized CuO NPs were assessed using the MTT assay on A498 human kidney carcinoma cell lines. Cells were seeded in 96-well plates and incubated for 24 hours to allow for proper attachment. Following this, various concentrations of the CuO nanoparticle suspension were added to the wells, and the plates were incubated for an additional 24 hours. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 4 hours at 37 °C to allow the formation of formazan crystals. The crystals were then solubilized using dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated to determine the cytotoxic and anticancer potential of the CuO NPs (Almutary and Sanderson, 2016).

3. RESULTS AND DISCUSSION

3.1 XRD Analysis

Fig. 2 displays the X-ray diffraction (XRD) pattern of CuO NPs synthesized *via* a green method using *Solanum xanthocarpum* leaf extract. The presence of sharp and well-defined diffraction peaks indicates the high crystallinity of the synthesized nanoparticles. Prominent peaks observed at 2θ values of 32.50°, 35.50°, 38.70°, 48.80°, 53.50°, 58.30°, and 61.60° correspond to

the (110), (–111), (111), (–202), (020), (202), and (113) crystal planes, respectively. These reflections are consistent with the monoclinic phase of CuO as indexed by the Joint Committee on Powder Diffraction Standards (JCPDS Card No. 89-5895), confirming the formation of phase-pure CuO nanoparticles. No additional peaks were detected, indicating the absence of impurities or secondary phases.

The average crystallite size (D) was estimated using the Debye–Scherrer equation:

$$D = \frac{0.9\lambda}{\beta\cos\theta} \quad \ldots (2),$$

where D is the crystallite size, λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) in radians, and θ is the Bragg diffraction angle. Based on the most intense diffraction peak at $2\theta \approx 35.50°$, the average crystallite size of the CuO NPs was calculated to be approximately 25 nm. These results confirm the successful synthesis of pure, crystalline CuO nanoparticles through an eco-friendly green synthesis approach (Sharma *et al.* 2015; Siddiqui *et al.* 2021; Surendhiran *et al.* 2021).

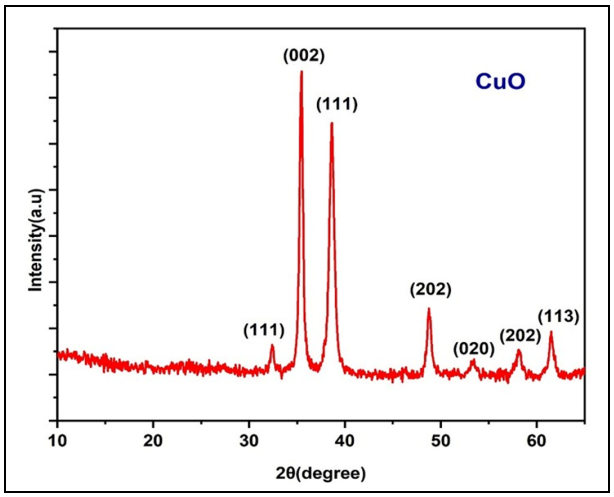


Fig. 2: XRD spectrum of CuO NPs

3.2 FT-IR Analysis

Fig. 3 presents the Fourier Transform Infrared (FT-IR) spectrum of CuO NPs synthesized *via* a green method using *Solanum xanthocarpum* leaf extract. The broad peak observed at 3386 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups from water molecules or phytochemicals present in the plant extract. The peak at 2921 cm^{-1} is attributed to the C–H stretching vibrations of aliphatic hydrocarbon chains.

The peaks at 1642 cm^{-1} and 1384 cm^{-1} are assigned to C=O stretching and bending vibrations, respectively, which are likely associated with carboxylate or amide groups from plant metabolites involved in the reduction and capping processes. Additional peaks at 1352 cm^{-1} and 1096 cm^{-1} correspond to C–N stretching vibrations of amines and C–O stretching vibrations of alcohols or esters, respectively, further indicating the presence of bioorganic compounds.

Importantly, strong peaks at 600 cm^{-1} and 545 cm^{-1} are characteristic of Cu–O stretching vibrations, confirming the successful formation of CuO nanoparticles. These findings demonstrate that phytochemicals present in the leaf extract not only act as reducing agents but also serve as stabilizing (capping) agents by attaching to the nanoparticle surface, thereby preventing agglomeration and enhancing dispersion stability.

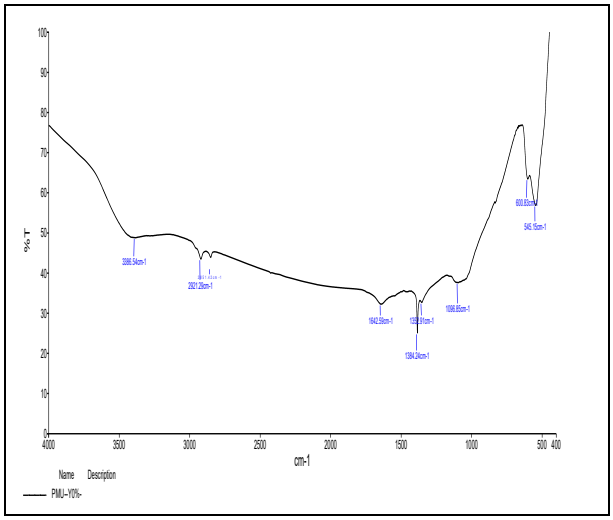


Fig. 3: FT-IR spectrum of CuO NPs

The FT-IR results validate the plant-mediated green synthesis approach and highlight the crucial role of biofunctional groups in both the synthesis and stabilization of CuO NPs.

3.3 UV-visible Analysis

Fig. 4 presents the UV-Visible absorption spectrum of CuO nanoparticles synthesized *via* a green synthesis approach using *Solanum xanthocarpum* leaf extract. A distinct band is observed at 364 nm, which is characteristic of CuO nanoparticles and arises from electronic transitions between the valence and conduction bands. The appearance of this peak confirms the successful formation of nanoscale CuO NPs.

The narrow and well-defined absorption feature reflects good crystallinity and uniform dispersion of the nanoparticles. Overall, the UV-Vis analysis confirms the successful synthesis of semiconducting CuO nanomaterials through green synthesis method (Sharma *et al.* 2015).

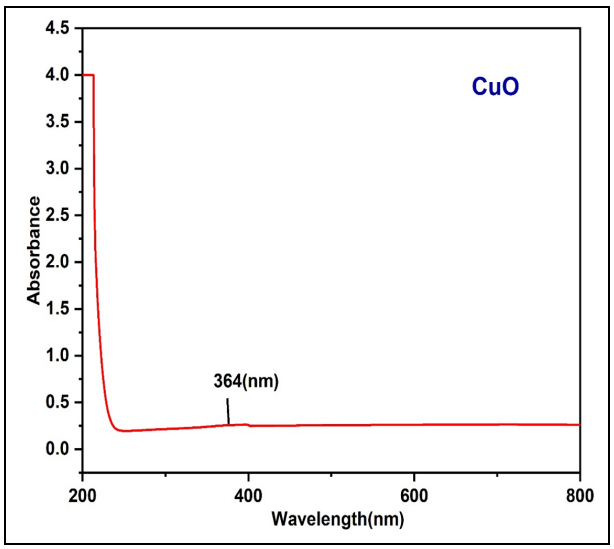


Fig.4: UV-visible spectrum of CuO NPs

3.4 PL Analysis

Fig. 5 illustrates the photoluminescence (PL) spectrum of CuO nanoparticles synthesized *via* a green method using *Solanum xanthocarpum* leaf extract. A prominent emission peak is observed at 805 nm in the near-infrared (NIR) region, which is primarily attributed to intrinsic surface defects and particle size, such as oxygen vacancies, within the CuO lattice. These defect-related emissions are influenced by the green synthesis route, where natural phytochemicals act as reducing and stabilizing agents, thereby impacting the defect chemistry and optical behavior of the nanoparticles. Plant-derived phytochemicals influence oxygen vacancy formation and surface defects by acting as mild reducing agents, altering local oxygen stoichiometry, controlling nucleation and growth rates. The green synthesis routes introduce surface defect states, leading to enhanced defect-related PL emissions in CuO nanoparticles (Nagore *et al.* 2001). Photoluminescence emission linked to defect states and UV–Vis absorption at 364 nm clearly support semiconducting behaviour of CuO nanoparticles.

The enhanced PL intensity reflects improved surface activity and crystal quality of the CuO NPs. The emission at 805 nm highlights the material's potential for optoelectronic and sensing applications, particularly in NIR-responsive technologies. Furthermore, the use of an environmentally friendly synthesis process underscores the dual advantage of achieving high-performance optical properties while adhering to sustainable and non-toxic production methods

3.5 FESEM, EDAX and Mapping Analysis

Fig. 6 presents the field emission scanning electron microscopy (FESEM) images of green-synthesized CuO nanoparticles at various magnifications. In image (a), taken at 10,000× magnification, the particles exhibit irregular morphology with noticeable agglomeration, a common feature in biologically synthesized nanoparticles due to the presence of organic stabilizing agents. Image (b), at 20,000× magnification, reveals a more compact arrangement and finer dispersion of the nanoparticles. At higher magnification (100,000×) in image (c), the particles appear well-defined and uniformly distributed, confirming their nanoscale dimensions and relatively high degree of crystallinity.

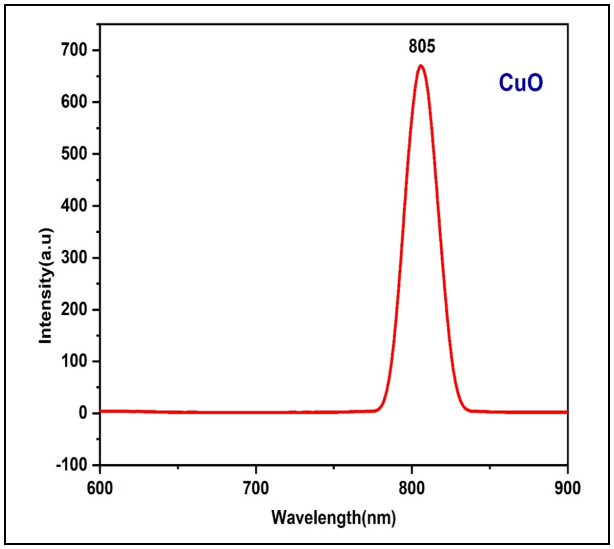


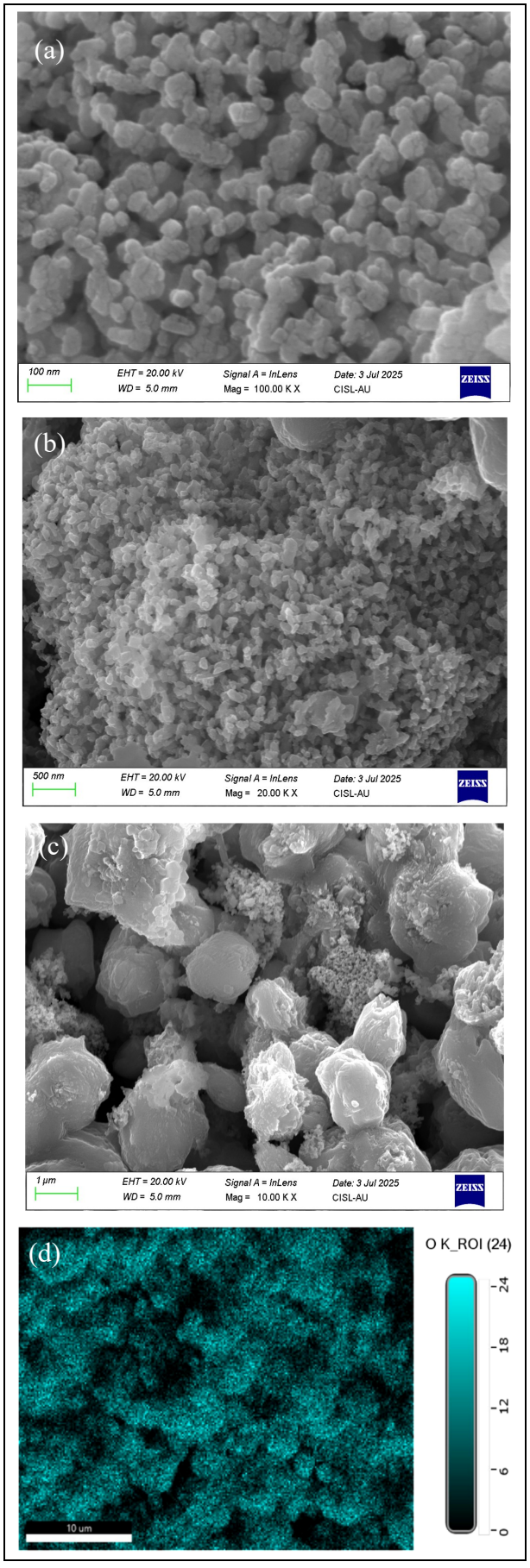
Fig. 5: PL spectrum of CuO NPs

Elemental mapping shown in images (d) and (e) confirms the homogeneous distribution of copper (Cu) and oxygen (O) throughout the sample, indicating the formation of well-dispersed CuO nanoparticles. Further confirmation is provided by the energy-dispersive X-ray spectroscopy (EDAX) analysis displayed in image (f), which identifies copper and oxygen as the major elemental constituents. Quantitatively, the EDAX spectrum shows that copper is present at 77.36 wt% and 46.25 at%, while oxygen accounts for 22.64 wt% and 53.75 at%. These results collectively validate the successful formation and elemental purity of CuO nanoparticles synthesized *via* a green, plant-mediated approach (Saif *et al.* 2016).

4. BIOLOGICAL APPLICATIONS

4.1 Antibacterial Activity

Fig. 7 illustrates the antibacterial activity of CuO nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. In Fig. 7(a), agar well diffusion plates display wells loaded with varying concentrations of CuO NPs (25, 50, 75, and 100 µL), while the central well marked "C" serves as the control, containing no nanoparticles. Clear zones of inhibition surrounding the wells indicate the suppression of bacterial growth due to the presence of CuO NPs.



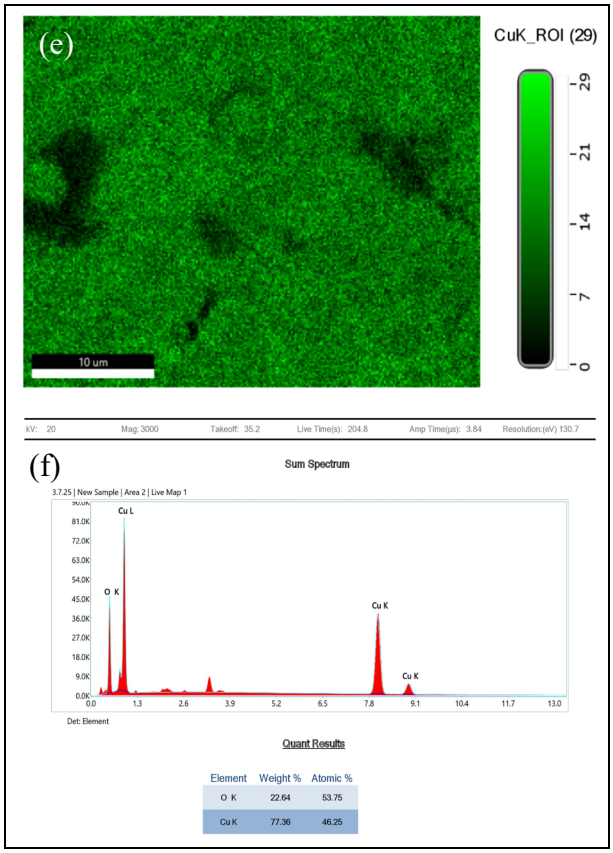


Fig. 6: (a-c) FESEM images, (d and e) mapping and (f) EDAX spectrum of CuO NPs

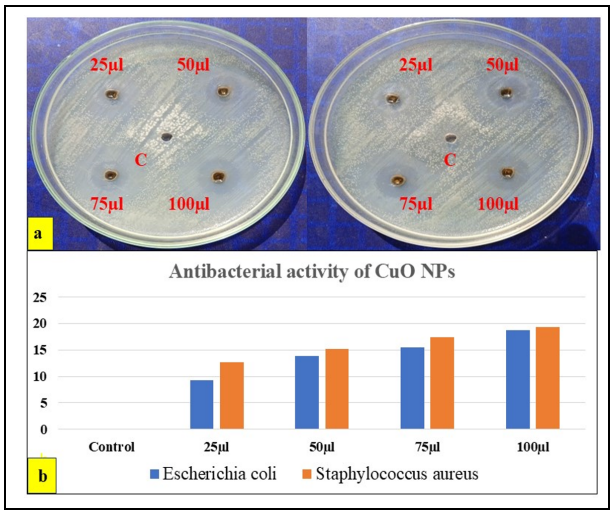


Fig. 7: (a) Antibacterial effect demonstrated on agar plates and (b) Impact of green-synthesized CuO NPs against *S. aureus* and *E. coli* (quantitative analysis)

Fig. 7(b) presents a bar graph quantifying the diameters of the inhibition zones in millimeters. The results reveal a clear concentration-dependent antibacterial response. In the case of *E. coli*, inhibition zones increased from 9.3 mm at 25 µL to 18.7 mm at 100 µL. Similarly, for *S. aureus*, the zones expanded from 12.6 mm to 19.3 mm across the same concentration

range. These findings confirm that CuO NPs possess strong antibacterial activity against both Gram-negative and Gram-positive bacteria, with *S. aureus* displaying slightly higher sensitivity at all tested concentrations. This concentration-dependent inhibition suggests that CuO NPs could serve as effective antimicrobial agents, particularly in applications requiring precise dose control (Khairy *et al.* 2024).

4.2 Antifungal Activity

Fig. 8 (a) displays agar plates showing zones of inhibition formed by varying concentrations (25, 50, 75, and 100 µL) of CuO NPs, with the central well labeled "C" serving as the control (no nanoparticles). Clear inhibition zones around the wells confirm the antifungal activity of the CuO NPs. Fig. 8 (b) presents a bar graph comparing the inhibition zone diameters for both fungal strains. For *Aspergillus niger*, the zone of inhibition increased from approximately 8 mm at 25 µL to 17 mm at 100 µL. In the case of *Candida albicans*, no inhibition was observed at 25 µL; however, the zone size increased markedly with higher concentrations, reaching approximately 20 mm at 100 µL. These results demonstrate a strong, concentration-dependent antifungal effect of the CuO NPs, with *C. albicans* showing slightly higher sensitivity than *A. niger* at higher concentrations.

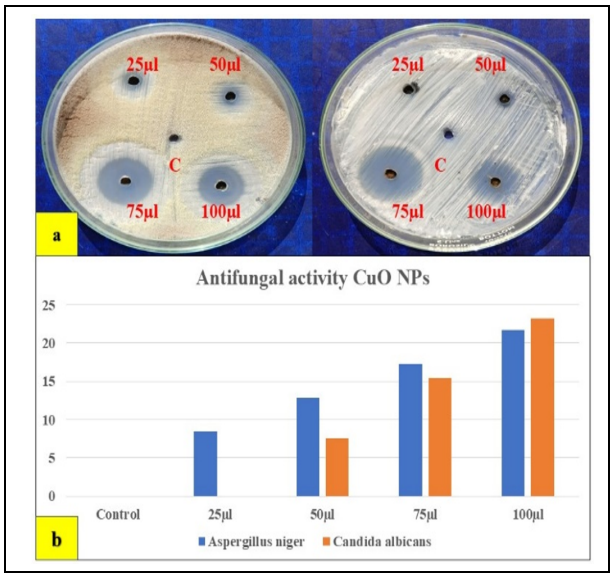


Fig. 8: (a) Antifungal activity demonstrated using agar plate assay and (b) inhibition zone measurements of green-synthesized CuO NPs against *C. albicans* and *A. niger*

The findings highlight the potential of green-synthesized CuO NPs as effective antifungal agents.

4.3 Antioxidant Activity

Fig. 9 illustrates the antioxidant activity of green-synthesized CuO NPs in comparison with standard Vitamin C across various concentrations (1–10 µg). The percentage of inhibition increased with concentration for both samples, indicating a dose-dependent antioxidant response. At 1 µg, CuO NPs exhibited 16.63% inhibition, slightly lower than Vitamin C at 18.36%. At the highest tested concentration of 10 µg, CuO NPs achieved 52.28% inhibition, closely approaching that of Vitamin C (53.15%). This comparable activity suggests that CuO NPs possess substantial antioxidant potential. The observed activity may be attributed to surface defects and reactive sites inherent in the nanostructures. Furthermore, the green synthesis route likely enhances antioxidant performance due to the presence of bioactive phytochemical capping agents.

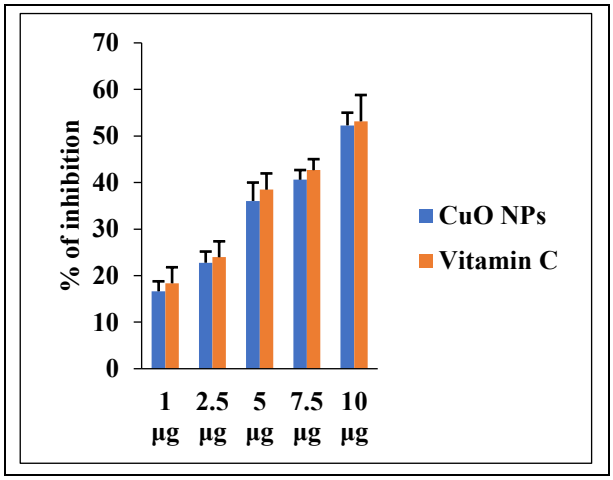


Fig. 9: Antioxidant activity of green-synthesized CuO NPs

These results highlight the promising antioxidant capabilities of CuO NPs for potential biomedical applications (Palani *et al.* 2024).

4.4 Anticancer Activity

Fig. 10 illustrates the anticancer activity of green-synthesized CuO nanoparticles on A498 human kidney carcinoma cells. The microscopic images depict morphological changes in A498 cells following treatment with increasing concentrations of CuO NPs. The control group (image a) shows healthy, densely packed cells with normal morphology. Upon treatment with 5 µg/mL of CuO NPs (image b), a reduction in cell density and early signs of cytotoxicity become evident. At 7.5 µg/mL (image c), pronounced morphological changes, such as cell shrinkage, rounding, and detachment from the surface, are observed, indicating significant cellular damage and apoptotic features.

The corresponding bar graph in Fig. 10(d) presents a quantitative analysis of cell viability across varying concentrations of CuO NPs. A clear concentration-dependent decline in viability is observed, decreasing from 100% in the untreated control to approximately 26% at the highest tested concentration

(10 µg/mL). Error bars representing standard deviation indicate high reproducibility and consistency of the experimental data

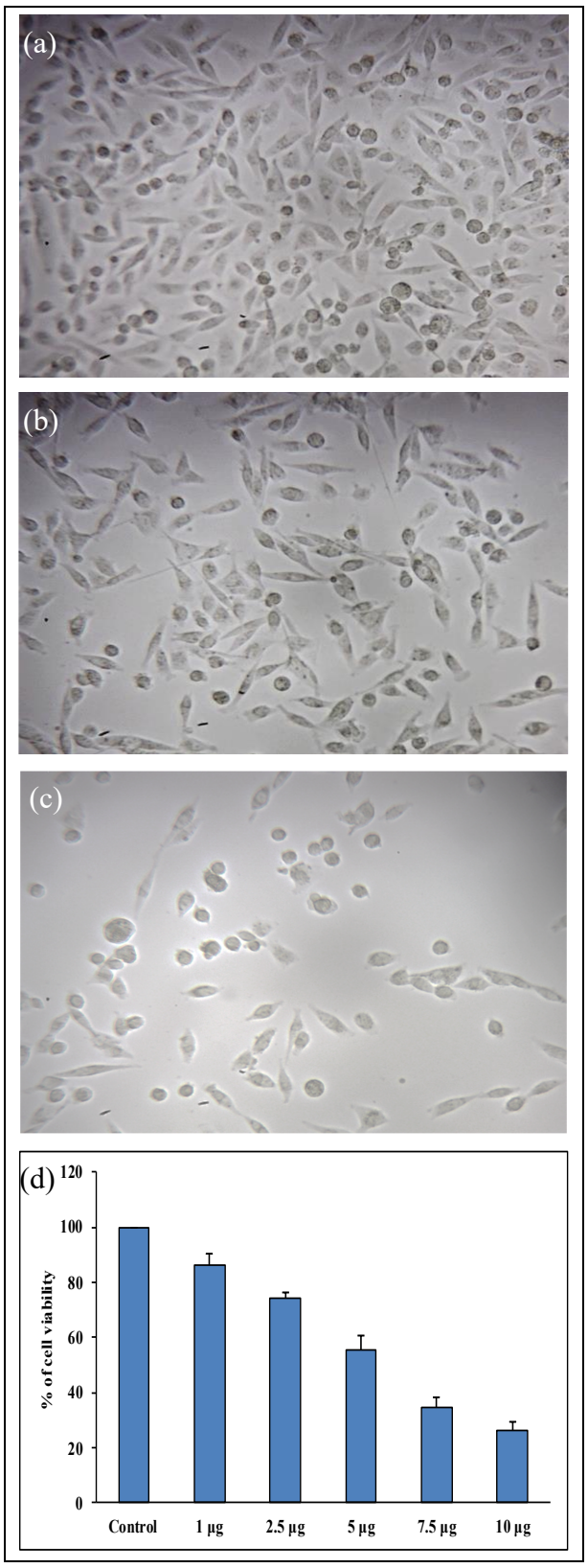


Fig. 10: Anticancer potential of green-synthesized CuO NPs

These findings confirm the potent cytotoxic effect of CuO NPs against A498 kidney cancer cells and support their potential as effective anticancer agents. The proposed mechanism of CuO NP-induced cell death, potentially involving oxidative stress and apoptosis, is schematically illustrated in Fig. 11. CuO surfaces catalyze ROS and may release Cu^{2+} that participates in Fenton-like reactions; ROS drives lipid peroxidation, protein oxidation, and DNA damage. Intracellular copper contributes to oxidative stress, while nanoparticle surfaces amplify redox cycling.

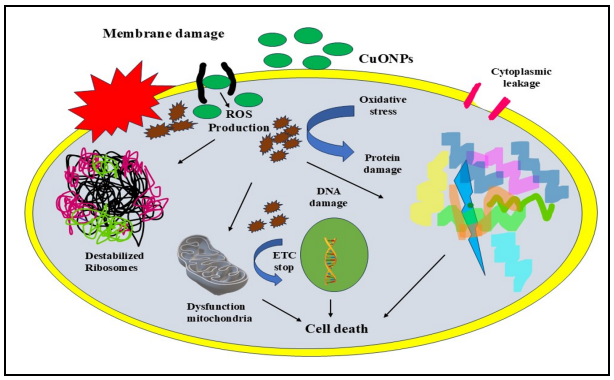


Fig. 11: Proposed mechanism of cell death in A498 cell lines induced by green-synthesized CuO NPs

5. CONCLUSION

In this study, CuO NPs were successfully synthesized *via* an eco-friendly and sustainable green synthesis route using *Solanum xanthocarpum* leaf extract. The structural characterization using XRD confirmed the formation of highly crystalline monoclinic-phase CuO NPs. Fourier-transform infrared spectroscopy revealed the presence of phytochemical functional groups, highlighting their role in the reduction and stabilization of the nanoparticles.

The optical properties, examined through UV-Visible and PL spectroscopy, exhibited characteristic absorption and emission features consistent with semiconducting CuO nanomaterials. Field emission scanning electron microscopy revealed agglomerated yet nanoscale morphology, while EDAX and elemental mapping confirmed a uniform distribution of copper and oxygen elements, validating the purity and composition of the synthesized nanoparticles.

The green synthesized CuO NPs demonstrated notable bioactivities. They showed strong antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*, and antifungal efficacy against *Candida albicans* and *Aspergillus niger*, in a concentration-dependent manner. Additionally, the nanoparticles exhibited significant antioxidant activity in the DPPH free radical scavenging assay. Importantly, CuO NPs also exerted potent cytotoxic effects against A498 human kidney

carcinoma cells, highlighting their potential anticancer properties.

Overall, the results highlight the multifunctional potential of *S. xanthocarpum*-mediated CuO nanoparticles, supporting their prospective application in biomedical fields, particularly as antimicrobial, antioxidant, and anticancer agents.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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