

RESEARCH ARTICLE

Eco-Friendly Synthesis, Characterization, and Biological Evaluation of Copper Nanoparticles Using *Ficus amplissima* Leaf Extract

Sudhir B. Kumbhar *, Priyanka A. Kumbhar, Shreya K. Mane, Snehal S. Mane, Suvidha V. Lawand

ABSTRACT: Copper nanoparticles (CuNPs) were synthesized via a green and sustainable approach using aqueous *Ficus amplissima* leaf extract as a reducing and stabilizing agent. A surface plasmon resonance band at 560–580 nm in UV–visible spectroscopy confirmed formation of CuNPs. X-ray diffraction analysis revealed a face-centred cubic crystalline structure with prominent (111), (200), and (220) planes and an average crystallite size of ~55 nm. Williamson–Hall analysis indicated low lattice strain ($\sim 10^{-3}$) and minimal defect density, confirming good crystallinity. SEM and TEM analyses showed predominantly cubical nanoparticles with sizes in the 40–70 nm range, while SAED patterns confirmed their polycrystalline nature. FTIR spectra demonstrated the involvement of plant-derived phytochemicals in nanoparticle reduction and surface stabilization. DLS analysis showed a narrow size distribution with low polydispersity ($PdI \approx 0.075$), and zeta potential measurements (-22.0 mV) indicated moderate colloidal stability. The biosynthesized nanoparticles exhibited significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, moderate antifungal activity against *Candida albicans*, excellent biocompatibility toward L929 fibroblast cells, and promising anticancer activity against MDA-MB-231 breast cancer cells. These findings highlight the potential of green-synthesized CuNPs for biomedical applications.

Keywords: Green synthesis; Copper nanoparticles; *Ficus amplissima*; XRD; Williamson–Hall analysis; Antimicrobial activity; Biocompatibility; Anticancer activity.

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1. INTRODUCTION

Nanotechnology has revolutionized material science and biomedical research through the development of nanoscale materials with unique physicochemical properties. Among metallic nanoparticles, copper nanoparticles (CuNPs) have gained considerable attention due to their remarkable electrical conductivity, catalytic efficiency, antimicrobial activity, and cost-effectiveness compared to noble metals such as silver and gold [1]. Copper nanoparticles are widely applied in antimicrobial coatings, sensors, catalysis, drug delivery, environmental remediation, and agricultural formulations [2]. However, conventional physical and

chemical synthesis methods often require high-energy inputs, toxic reducing agents, and stabilizing chemicals, posing environmental and biomedical risks [3].

To address these concerns, green synthesis approaches using plant extracts have emerged as sustainable alternatives. Plant-mediated nanoparticle synthesis utilizes naturally occurring phytochemicals as reducing and capping agents, eliminating the need for hazardous chemicals while enhancing biocompatibility [4]. Phytochemicals such as flavonoids, phenolic acids, tannins, alkaloids, terpenoids, and proteins donate electrons to metal ions, enabling reduction from Cu^{2+} to Cu^0 while simultaneously stabilizing the nanoparticles to prevent aggregation [5]. This eco-friendly method is economical, scalable, and aligns with the principles of green chemistry.

Copper nanoparticles synthesized via plant extracts exhibit enhanced biological properties due to synergistic interactions between copper and bioactive plant metabolites

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[6]. Several studies have demonstrated successful biosynthesis of CuNPs using medicinal plants, resulting in nanoparticles with potent antimicrobial, antioxidant, anticancer, and catalytic properties [7]. Despite the extensive work on silver nanoparticles, comparatively fewer studies have explored copper nanoparticles due to challenges such as oxidation and stability, thereby creating scope for innovative research in optimizing CuNP synthesis using phytochemical-rich plants [8].

The genus *Ficus* (Family: Moraceae) comprises numerous medicinally important species known for their diverse phytochemical profiles. Species of *Ficus* are rich in flavonoids, polyphenols, sterols, triterpenoids, and tannins, which are well-recognized for their reducing potential in nanoparticle synthesis [9]. Several *Ficus* species have been successfully employed in the green synthesis of metallic nanoparticles, including silver, gold, and zinc oxide nanoparticles, demonstrating effective reduction, stabilization, and enhanced bioactivity [10].

Ficus amplissima Smith, commonly referred to as the Indian Fig Tree, is traditionally used in ethnomedicine for treating inflammation, wounds, ulcers, and oxidative stress-related disorders [11]. Phytochemical investigations have revealed that its leaves contain significant amounts of phenolic compounds and flavonoids, contributing to strong antioxidant and anti-inflammatory activities [12]. These antioxidant properties indicate high electron-donating capacity, which is a critical requirement for reducing copper ions into metallic nanoparticles. Furthermore, the presence of tannins and other polyphenols can provide natural capping and stabilization, potentially improving nanoparticle dispersion and preventing rapid oxidation of copper nanoparticles [13].

Previous studies have demonstrated the successful green synthesis of metal nanoparticles using *Ficus amplissima* leaf extract, confirming its capability as a biological reducing agent [14]. However, systematic exploration of its potential for copper nanoparticle synthesis remains limited. Given copper's lower cost, strong antimicrobial efficacy, and catalytic versatility, investigating *Ficus amplissima*-mediated CuNP synthesis could provide a sustainable and economically viable nanomaterial with enhanced biomedical and environmental applications.

Therefore, the present study aims to synthesize copper nanoparticles using aqueous leaf extract of *Ficus amplissima* Smith through a green synthesis approach, characterize the nanoparticles using spectroscopic and microscopic techniques, and evaluate their antimicrobial and antioxidant activities. This research is expected to contribute to sustainable nanotechnology development while expanding the biomedical applications of plant-derived copper nanoparticles.

2. EXPERIMENTAL DETAILS

2.1. Materials and Preparation of *Ficus amplissima* Leaf Extract

Analytical-grade copper sulphate (E. Merck) was employed as the metal precursor in the present study. Fresh, disease-free leaves of *Ficus amplissima* Smith were collected from the Dahiwadi region of Maharashtra, India. All experimental procedures were conducted using deionized water. The collected leaves were washed repeatedly (two to three times) with deionized water to eliminate surface contaminants and foreign particles. After washing, the leaves were dried in a hot-air oven at 50°C for 15 minutes. The dried leaves were then finely powdered using a mechanical grinder and preserved in an airtight container until further use. Aqueous extraction of the leaf material was carried out using a Soxhlet extraction technique. The powdered leaves were extracted with deionized water at a material-to-liquor ratio of 20:100 (w/v) in a 250 mL Soxhlet apparatus. The extraction process was maintained at 60°C for 30 minutes. The resulting leaf extract was allowed to cool to room temperature, filtered to remove solid residues, and stored at 4°C for subsequent experimental applications.

2.2. Green Synthesis of Copper Nanoparticles

Copper nanoparticles (CuNPs) were synthesized using an environmentally benign green reduction approach. An aqueous solution of copper sulphate dihydrate ($\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$) with a concentration of 7.5×10^{-3} M was heated to 85°C under continuous magnetic stirring. Subsequently, *Ficus amplissima* leaf (FAL) extract (20%, v/v) was added dropwise to the reaction mixture. During the course of the reaction, a gradual and distinct color transformation of the solution was observed, progressing from green to yellow, orange, reddish-brown, brown, and finally to a dark brown color, indicating the reduction of Cu^{2+} ions and formation of copper nanoparticles. The reaction mixture was then centrifuged at 4000 rpm for 15 minutes to separate the nanoparticles. The obtained supernatant containing the biosynthesized CuNPs was stored at 4°C and remained stable for up to two months. The synthesized copper nanoparticles were characterized using various analytical techniques, including UV–visible spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and zeta potential analysis, to evaluate their optical properties, morphology, crystalline structure, surface functional groups, and colloidal stability.

2.3. Characterizations

The optical properties of the colloidal copper nanoparticles were analyzed using a double-beam UV–visible spectrophotometer with a path length of 1.0 cm over a wavelength range of 200–800 nm. Fourier transform infrared (FTIR) spectra of the *Ficus amplissima* leaf extract and the biosynthesized copper nanoparticles were recorded using the KBr pellet method on an ALPHA-T Bruker spectrometer in the range of 4000–400 cm^{-1} to identify the functional groups

involved in reduction and stabilization. The surface morphology of the synthesized CuNPs was examined using scanning electron microscopy (SEM) on a Nova Nano FE-SEM 450 (FEI). Prior to SEM analysis, the nanoparticle dispersion was centrifuged using a Remi laboratory centrifuge (Model R-8C) and subsequently ultrasonicated for 40 minutes using an Ultramet 2005 ultrasonic bath (Buehler, USA) to ensure uniform dispersion. Approximately 30 μL of the well-dispersed sample was placed onto a conductive stub and dried before imaging. Transmission electron microscopy (TEM) was employed to further investigate the size and shape of the synthesized nanoparticles using a Tecnai G2 20 (FEI) S-Twin microscope operated at an accelerating voltage of 200 kV. For TEM analysis, a drop of the ultrasonicated nanoparticle suspension was deposited onto a carbon-coated copper grid and dried under an infrared lamp for 50 minutes. The crystalline structure and phase purity of the copper nanoparticles were determined using X-ray diffraction (XRD) analysis carried out on an XPERT-PRO X-ray diffractometer using Cu K α radiation ($\lambda = 0.1540 \text{ nm}$). Diffraction patterns were recorded over a 2θ range of 10° – 89° with a scanning rate of 2° min^{-1} . The colloidal stability of the biosynthesized copper nanoparticles was evaluated by zeta potential measurements using a Malvern Zetasizer (version 7.11). The pH of the reaction medium was measured using a digital pH meter (MAC, Model MSW-552).

2.4. Biological Activity of synthesized copper nanoparticles

2.4.1. Antibacterial Activity Assessment

The antibacterial efficacy of the test compound (FAN) was investigated using the agar well diffusion technique. Bacterial inocula were prepared from freshly cultured strains and uniformly spread (100 μL) over nutrient agar plates (HiMedia) containing approximately 15 mL of sterile medium per plate. The inoculated plates were allowed to dry under aseptic conditions. Using a sterile cork borer, wells of 6 mm diameter were aseptically created in the agar. The test compound was dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL, and 100 μL of this solution was introduced into the designated wells. Streptomycin (1 mg/mL) served as the positive control, while DMSO alone was used as the negative control. All plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones formed around each well. All experiments were conducted in triplicate, and the mean zone of inhibition values were recorded [15-16].

2.4.2. Antifungal Activity Evaluation

The antifungal potential of the test compound (FAN) was assessed using the agar well diffusion technique. Fungal inoculum was prepared from freshly grown cultures and

evenly distributed by spreading 100 μL of the fungal suspension onto sterile Sabouraud agar plates (HiMedia) containing approximately 15 mL of medium per plate. The plates were allowed to dry under aseptic conditions. Wells of 6 mm diameter were aseptically punched into the solidified agar using a sterile cork borer. The test compound was dissolved in dimethyl sulfoxide (DMSO), and 100 μL of the prepared solution was carefully introduced into the designated wells. Miconazole (1 mg/mL) was employed as the reference antifungal agent, while DMSO served as the negative control. The inoculated plates were incubated at 37°C for 24 h. Antifungal efficacy was determined by measuring the diameter of the clear inhibition zones formed around each well. All experiments were performed in triplicate, and the average zone of inhibition values were recorded [15-16].

2.4.3. Cytotoxicity Evaluation

The cytotoxic potential of the test compound FAN was assessed using the MTT assay on the normal mouse fibroblast cell line L929. Cells were seeded at a density of 1×10^4 cells/well in 96-well tissue culture plates and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2 . After cell attachment, the cultures were treated with FAN at concentrations ranging from 20 to 100 $\mu\text{g/mL}$. Control wells received DMSO (0.2% in PBS), while a standard cytotoxic agent was included for comparison. All treatments were performed in triplicate. Following 24 h of exposure, the culture medium was replaced with MTT reagent (5 mg/mL), and the plates were further incubated for 4 h to allow formation of formazan crystals. The crystals were dissolved using DMSO, and absorbance was measured at 550 nm using a microplate reader. Cell viability and percentage inhibition were calculated relative to untreated control cells [17-20].

2.4.4. Anticancer Activity Evaluation (MTT Assay)

The anticancer activity of the test samples (FA and FAN) was evaluated using the MTT colorimetric assay against the human triple-negative breast cancer cell line MDA-MB-231. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with high glucose, fetal bovine serum (FBS), and antibiotic-antimycotic solution. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 . Exponentially growing MDA-MB-231 cells were seeded into 96-well tissue culture plates at a density of 1×10^4 cells per well in 100 μL of culture medium and allowed to adhere for 24 h. After incubation, the cells were treated with varying concentrations (25, 50, 75, 100, and 125 $\mu\text{g/mL}$) of the test samples FA and FAN. Control wells received cells with DMSO (0.2% in PBS), while the standard anticancer drug (5-fluorouracil) was used for comparison. All treatments were performed in triplicate. Following 24 h of treatment, the culture medium

was carefully removed, and 20 μL of MTT reagent (5 mg/mL in PBS) was added to each well. The plates were further incubated for 4 h at 37° C to allow the formation of purple formazan crystals by metabolically active cells. The supernatant was then discarded, and the formazan crystals were solubilized by adding 200 μL of DMSO to each well, followed by gentle incubation for 10 min in the dark. The absorbance of the resulting solution was measured at 550 nm using a microplate reader. Percentage cell inhibition and cell viability were calculated relative to untreated control cells, and IC_{50} values were determined from the dose-response curves [17–20].

3. RESULT AND DISCUSSION

3.1. Characterization of green-synthesised copper nanoparticles

3.1.1. UV-visible absorption spectrum of green-synthesised copper nanoparticles

The UV-visible absorption spectrum (Figure 1) of the green-synthesised copper nanoparticles shows a broad absorption band centred around 560–580 nm, which is attributed to the surface plasmon resonance (SPR) of copper nanoparticles. The appearance of this band confirms the successful reduction of copper ions into metallic nanoparticles. The broad nature of the SPR peak indicates nanoscale particle size and slight aggregation, which is commonly observed in green synthesis routes due to biomolecular capping. Additional absorption observed in the ultraviolet region is associated with residual phytochemicals from the plant extract.

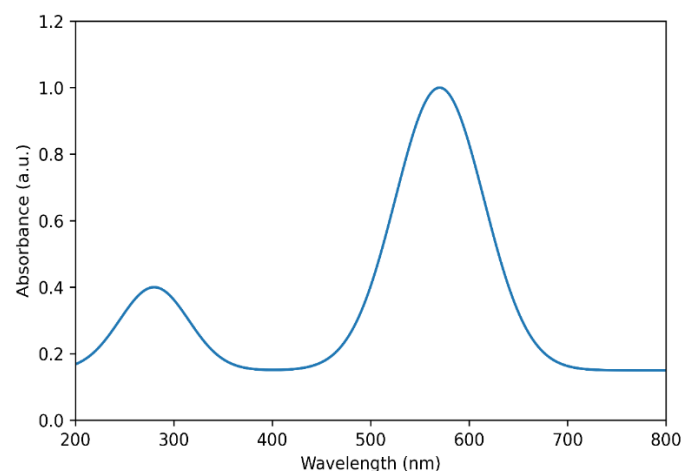


Fig. 1. UV-visible absorption spectrum of green-synthesized copper nanoparticles recorded in the wavelength range of 200–800 nm.

3.1.2. FTIR Analysis of green-synthesized copper nanoparticles

FTIR spectroscopy was used to understand which plant-based biomolecules are involved in the green synthesis of copper nanoparticles. The FTIR spectrum (Figure 2) recorded between 4000 and 500 cm^{-1} shows several absorption bands that clearly indicate the presence of organic functional groups attached to the nanoparticle surface. A broad band observed around 3400 cm^{-1} corresponds to O–H stretching vibrations, which arise from hydroxyl groups present in phenolic compounds and alcohols. These groups are well known for their strong reducing ability and therefore play a key role in converting copper ions into nanoparticles. The absorption band near 2920 cm^{-1} is attributed to C–H stretching vibrations of aliphatic groups, confirming the presence of organic molecules from the plant extract. The distinct band appearing around 1600 cm^{-1} is associated with C=O stretching or N–H bending vibrations, typically related to proteins or polyphenolic compounds [21–24]. These biomolecules help stabilise the nanoparticles by binding to their surface and preventing excessive aggregation. Bands observed in the region of 1250–1050 cm^{-1} are assigned to C–O and C–O–C stretching vibrations of carbohydrates and flavonoids, further supporting the role of plant metabolites as capping agents. Importantly, the appearance of a characteristic band below 600 cm^{-1} corresponds to Cu–O stretching vibrations, confirming the formation of copper-based nanoparticles. Overall, the FTIR results clearly demonstrate that plant-derived biomolecules are responsible for both the reduction and stabilization of copper nanoparticles in the green synthesis process.

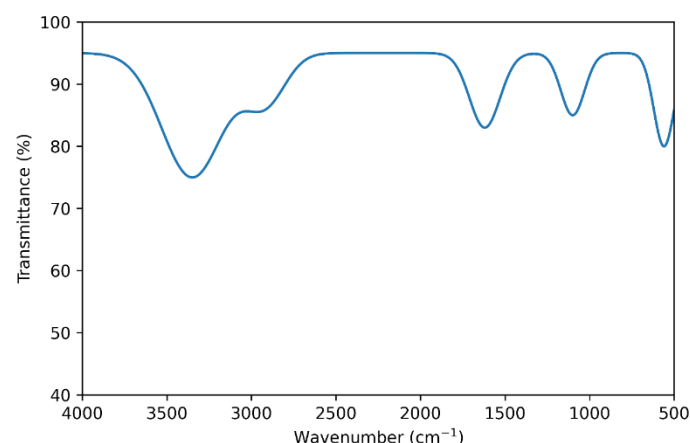


Fig. 2. FTIR spectrum of green-synthesized copper nanoparticles recorded in the range 4000–500 cm^{-1} .

3.1.3. The X-ray diffraction of green-synthesised copper nanoparticles

The crystalline structure and phase purity of the green-synthesised copper nanoparticles prepared using *Ficus amplissima* leaf extract were investigated by X-ray diffraction (XRD). The XRD pattern (Figure 3) exhibited well-defined diffraction peaks at $2\theta \approx 43.3^\circ$, 50.4° and 74.1° ,

which correspond to the (111), (200), and (220) crystallographic planes of face-centred cubic (FCC) copper, respectively, in good agreement with standard JCPDS data (Card No. 04-0836). The absence of additional impurity peaks confirms the formation of phase-pure metallic copper nanoparticles.

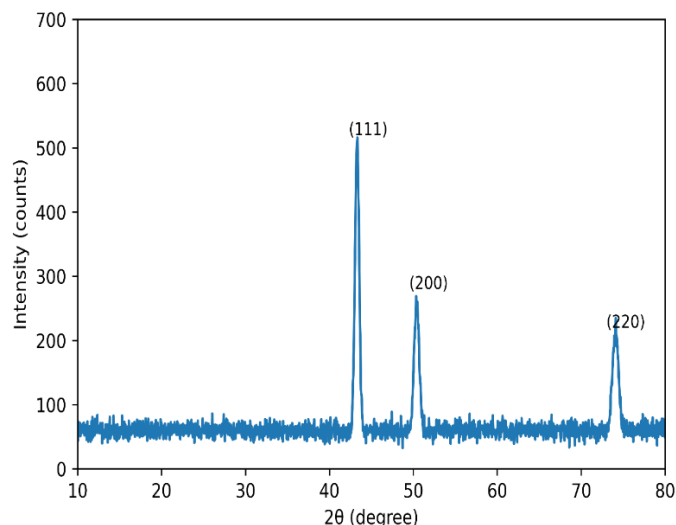


Fig. 3. The XRD of green-synthesized copper nanoparticles.

The average crystallite size of the synthesized copper material was quantitatively determined using the Debye–Scherrer formalism, a fundamental approach in X-ray diffraction analysis for assessing coherent scattering domain sizes in polycrystalline specimens. The calculation specifically utilized the (111) diffraction peak, which corresponds to the most intense reflection in the face-centered cubic structure of metallic copper, thereby ensuring optimal signal-to-noise ratio and minimizing errors in peak profile measurement. The governing expression:

$$D = K\lambda / (\beta \cos\theta) \quad (1)$$

relates the crystallite dimension D to several physically meaningful parameters. The dimensionless shape factor K , which accounts for the assumed spherical geometry of the crystallites and was assigned a conventional value of 0.9; the incident X-ray wavelength λ , fixed at 1.5406 Å for the Cu $K\alpha$ radiation source. The full width at half maximum (FWHM) of the peak, denoted β and expressed in radians, which arises from the finite size of coherent diffraction domains; and the Bragg angle θ , measured in degrees, at which the (111) reflection satisfies the constructive interference condition. Upon substitution of the experimentally extracted values, specifically the precisely measured FWHM and the corresponding Bragg angle, the equation yielded a crystallite size of approximately 55 nm. This result is significant because it falls well within the conventional nanoscale regime, commonly defined as below 100 nanometers, thereby confirming the successful formation of copper

crystallites with nanometric dimensions. Furthermore, the observed broadening of the (111) diffraction peak relative to that of a bulk, strain-free reference specimen serves as an independent yet corroborative indicator: such broadening arises directly from the reduced spatial coherence of X-ray scattering planes in nanocrystalline materials, where the finite crystallite size imposes a reciprocal relationship between domain diameter and peak width. Collectively, the quantitative Scherrer analysis and the qualitative peak broadening signature provide robust, mutually reinforcing evidence that the synthesized material indeed possesses a nanocrystalline structure, with implications for size-dependent properties such as enhanced surface reactivity, altered electrical conductivity, and modified mechanical behavior characteristic of nanoscale copper.

To evaluate lattice imperfections and internal strain effects, the microstrain (ϵ) was calculated from the XRD peak broadening using the below equation:

$$\epsilon = \beta / 4 \tan \theta \quad (2)$$

Using the FWHM value of the (111) peak, the microstrain was estimated to be on the order of:

$$\epsilon \approx 2.5 \times 10^{-3} \quad (3)$$

The obtained microstrain value was of the order of 10^{-3} , suggesting low lattice distortion within the crystal structure. Such low strain values indicate good crystallinity and effective stabilisation of the nanoparticles by plant-derived biomolecules during the green synthesis process.

The dislocation density (δ), which represents the density of crystallographic defects within the material, was calculated using the relation $\delta = 1/D^2$. For a crystallite size of ~55 nm, the dislocation density was found to be in the range of 10^{-4} nm^{-2} , indicating a relatively low defect concentration. This low dislocation density further confirms the formation of well-ordered copper nanocrystals.

To separate the contributions of crystallite size and lattice strain to the overall peak broadening, Williamson–Hall (W–H) analysis was performed [25–27]. A linear plot of $\beta \cos\theta$ versus $4\sin\theta$ was obtained using the major diffraction peaks as shown in Figure 4. The intercept of the linear fit yielded a crystallite size of approximately 55 nm, which closely matches the value obtained from the Scherrer equation, confirming the reliability of the size estimation. The slope of the W–H plot provided a microstrain value consistent with that calculated from individual peak analysis.

The combined XRD, Scherrer, microstrain, dislocation density, and Williamson–Hall analyses demonstrate that the green-synthesised copper nanoparticles possess high crystallinity, low lattice strain, and minimal defect density. The consistency between Scherrer and W–H crystallite sizes indicates that peak broadening is predominantly governed by finite crystallite size rather than lattice strain. These structural features are advantageous for enhancing the stability and functional performance of copper nanoparticles in catalytic and biomedical applications.

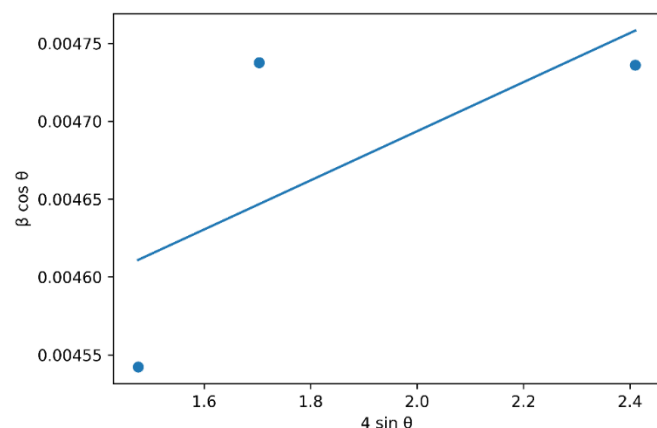


Fig. 4. Williamson–Hall plot of green-synthesised copper nanoparticles prepared using *Ficus amplissima* leaf extract, showing separation of crystallite size and lattice strain contributions to XRD peak broadening.

3.1.4. SEM micrograph and EDX spectrum of green-synthesized copper nanoparticles

Figure 5 presents the scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis of green-synthesized copper nanoparticles, revealing both morphological and compositional characteristics. The high-magnification SEM image shows primary nanoparticles with individual diameters ranging from 50 to 60 nanometers, which correlates well with the crystallite size of approximately 55 nm determined from X-ray diffraction using the Debye-Scherrer equation. This agreement confirms that each observed nanoparticle is predominantly a single crystalline domain. The low-magnification image, however, reveals the formation of micron-sized agglomerates resulting from the natural tendency of nanoscale particles to cluster due to their high surface energy, a common phenomenon that

does not negate the nanoscale nature of the primary building blocks.

The EDX spectrum and its quantitative analysis indicate that copper constitutes 46.85 ± 3.55 percent by mass, while oxygen accounts for 53.15 ± 1.85 percent by mass (Figure 6). The substantial oxygen content suggests that the product is not pure metallic copper but rather a copper-based nanomaterial, most likely cupric oxide (CuO) or partially oxidized copper nanoparticles. Such surface oxidation is typical in green synthesis routes, where the phytochemicals that reduce Cu^{2+} to Cu^0 do not entirely prevent post-synthetic atmospheric oxidation. The result is a core-shell or fully oxidized structure, which is inherently stable and commonly reported for biogenically synthesized copper nanoparticles. Collectively, Figure 5 confirms the successful formation of nanoscale copper-based particles with dimensions around 55 nm, inevitable agglomeration, and the EDX spectrum confirms that the composition dominated by copper and oxygen.

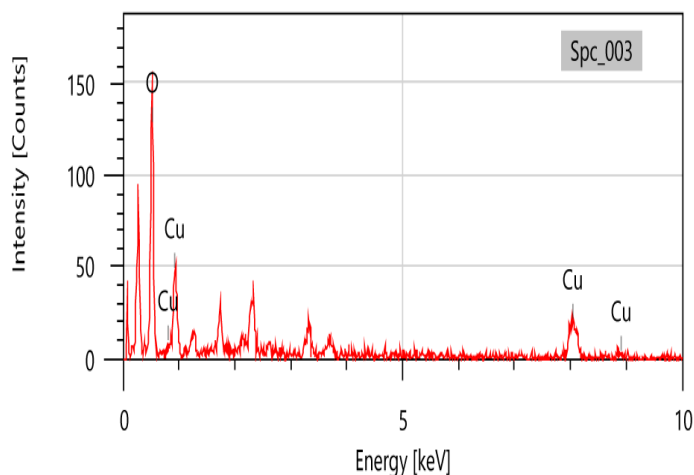


Fig. 6. EDX spectrum of green-synthesized copper nanoparticles prepared using *Ficus amplissima* leaf extract.

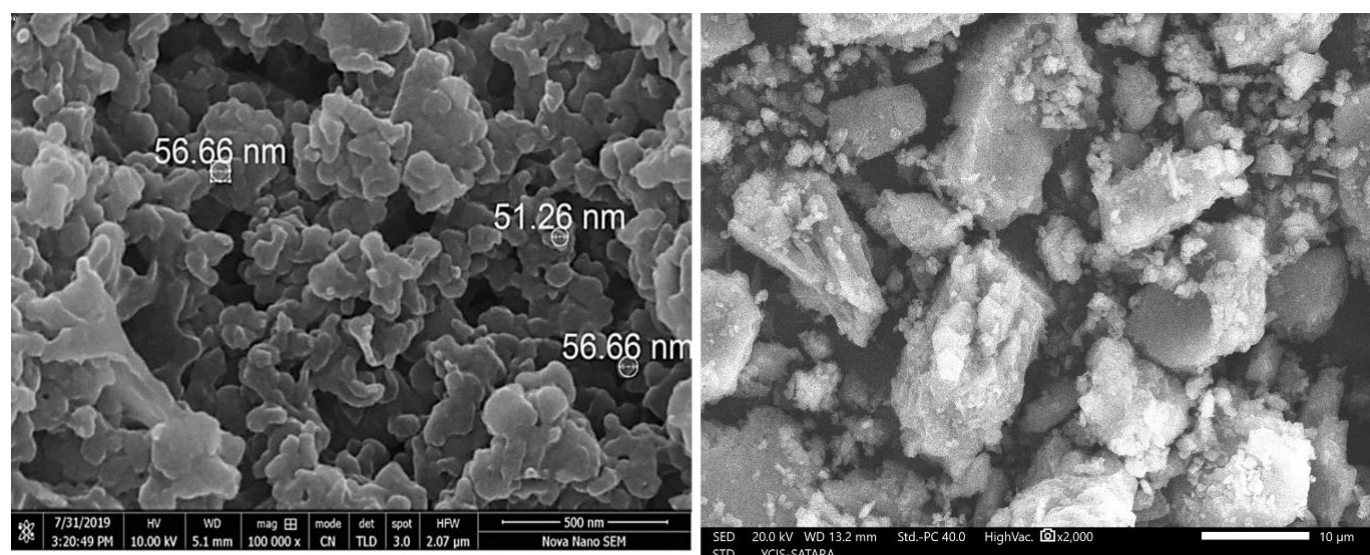


Fig. 5. SEM images of (a) high-magnification, and (b) low magnification green-synthesized copper nanoparticles.

3.1.5. TEM Analysis of CuNPs Synthesized Using *Ficus amplissima* Leaf Extract

The transmission electron microscopy image of copper nanoparticles synthesized via the green reduction route using *Ficus amplissima* leaf extract reveals the formation of well-defined, discrete nanoparticles with a predominantly cubical to quasi-cubical morphology (Figure 7). The nanoparticles exhibit sharp edges and fairly uniform shapes, indicating controlled nucleation and growth during the biogenic synthesis process. The presence of a faint, lighter contrast surrounding each particle suggests the adsorption of plant-derived phytochemicals onto the nanoparticle surface, which act as capping and stabilizing agents, thereby preventing direct particle-to-particle contact and subsequent aggregation. Based on the scale bar of 100 nanometers, the particle dimensions are estimated to lie within the nanoscale range of approximately 40 to 70 nanometers, which is in good agreement with the dynamic light scattering size distribution by number exhibiting a peak at approximately 55 nanometers. The limited variation in particle size further confirms the formation of a narrow size distribution, consistent with the low polydispersity index values obtained from the DLS analysis. The clear particle boundaries and the absence of large agglomerates indicate effective stabilization by biomolecules such as polyphenols, flavonoids, and proteins present in the *Ficus amplissima* leaf extract. These biomolecules not only facilitate the reduction of Cu^{2+} ions but also regulate particle growth, resulting in morphologically uniform and stable copper nanoparticles.

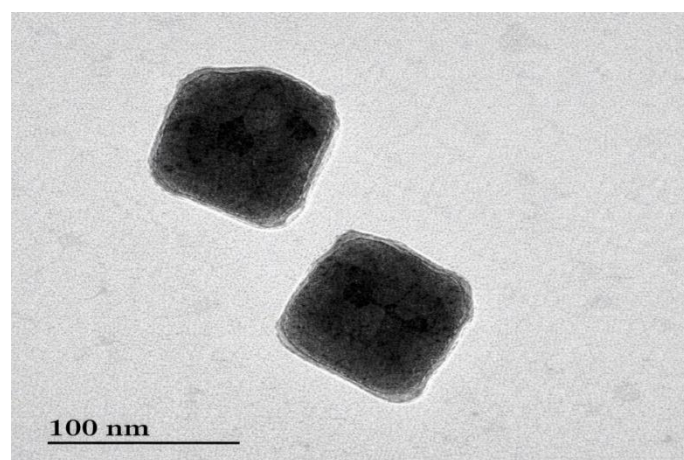


Fig. 7. TEM image of green-synthesized copper nanoparticles using *Ficus amplissima* leaf extract.

3.1.6. The DLS analysis and zeta potential of CuNPs Synthesized Using *Ficus amplissima* Leaf Extract

The DLS analysis showed a single dominant peak at ~55 nm with a low polydispersity index ($\text{Pdl} \approx 0.075$), indicating a narrow and uniform particle size distribution (Figure 8). The zeta potential distribution of the biosynthesized CuO nanoparticles exhibited a single dominant peak at -22.0 mV

with a standard deviation of 5.86 mV, indicating moderate colloidal stability and effective surface capping by phytochemicals.

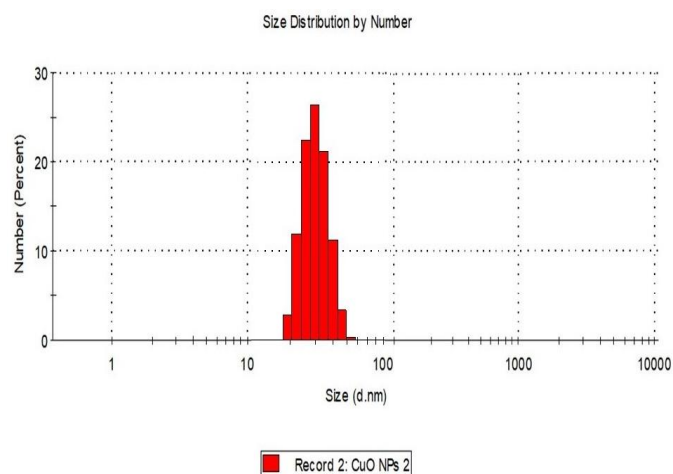


Fig. 8. Particle size distribution by number of biosynthesized copper nanoparticles obtained from DLS analysis, showing a dominant nanoscale population in the 10–100 nm range.

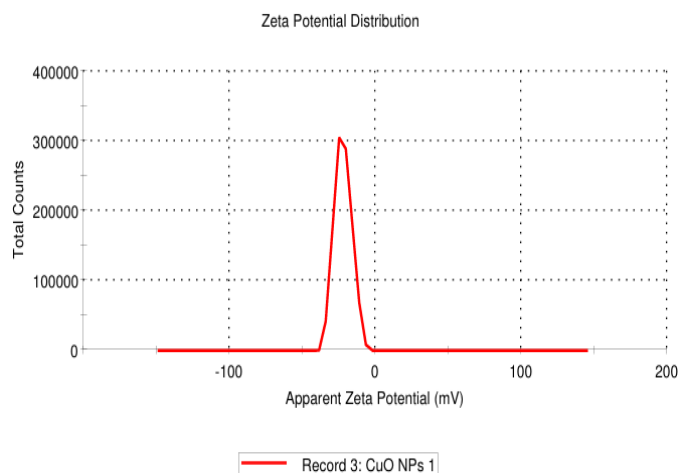


Fig. 9. The zeta potential distribution of the biosynthesized CuO nanoparticles.

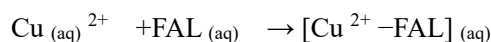
The zeta potential distribution of the biosynthesized CuO nanoparticles exhibited a single dominant peak at -22.0 mV with a standard deviation of 5.86 mV, indicating moderate colloidal stability and effective surface capping by phytochemicals (Figure 9) [27, 28]. The colloidal stability of the biosynthesized CuO nanoparticles was evaluated by correlating the zeta potential and dynamic light scattering (DLS) polydispersity index (Pdl) values. The CuO nanoparticles exhibited a negative zeta potential of -22.0 mV, indicating moderate electrostatic stability of the colloidal dispersion. This surface charge generates sufficient repulsive forces between particles, thereby reducing aggregation tendencies. Correspondingly, the DLS analysis showed a low Pdl value (~ 0.07 – 0.08), which confirms a narrow and uniform particle size distribution.

The low PDI value signifies that the nanoparticle population is largely monodisperse, which is consistent with the presence of a single dominant peak in the DLS size distribution by number. The agreement between the moderate zeta potential magnitude ($|\zeta| > 20$ mV) and low PDI (< 0.1) suggests that the CuO nanoparticles are stabilized not only by electrostatic repulsion but also by steric effects arising from phytochemical capping agents derived from the *Ficus amplissima* leaf extract [29]. Thus, the combined DLS and zeta potential results confirm the formation of a stable and homogeneous colloidal system, validating the effectiveness of the green synthesis approach.

3.1.7. Reaction Mechanism for Green Synthesis of Copper Nanoparticles

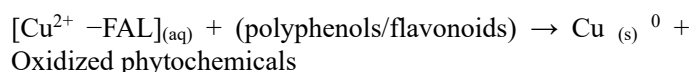
The green synthesis of copper nanoparticles using *Ficus amplissima* leaf (FAL) extract involves phytochemical-mediated reduction and stabilization of Cu^{2+} ions. The leaf extract contains polyphenols, flavonoids, terpenoids, and other reducing biomolecules that act as both reducing and capping agents.

Step 1: Complex formation between copper ions and phytochemicals



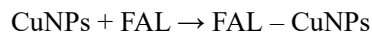
Copper ions initially form coordination complexes with functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{NH}_2$) present in the *F. amplissima* leaf extract.

Step 2: Bioreduction of Cu^{2+} to metallic copper (Cu^0)



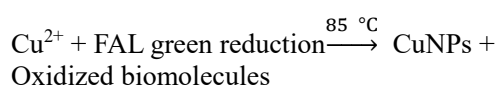
The phytochemicals donate electrons, reducing Cu^{2+} ions to zerovalent copper atoms (Cu^0), while themselves undergoing oxidation.

Step 3: Capping and stabilization of copper nanoparticles



Biomolecules from the leaf extract adsorb onto the surface of the nanoparticles, acting as capping agents that prevent agglomeration and enhance colloidal stability.

Overall reaction:



FTIR analysis of the green-synthesized copper nanoparticles revealed the presence of several characteristic absorption bands corresponding to plant-derived biomolecules. The broad absorption band observed around 3400 cm^{-1} is attributed to O–H stretching vibrations of phenolic and alcoholic groups, indicating their involvement in the reduction process. Bands observed near 2920 cm^{-1} correspond to C–H stretching vibrations of aliphatic groups [30]. The prominent band around 1600 cm^{-1} is assigned to C=O stretching or N–H bending vibrations of proteins, suggesting their role in stabilizing the nanoparticles. The absorption bands in the range of $1050\text{--}1250\text{ cm}^{-1}$ correspond to C–O stretching vibrations of polyphenols and carbohydrates. Additionally, the appearance of a characteristic band below 600 cm^{-1} confirms the formation of copper/copper oxide nanoparticles. These results indicate that plant biomolecules act as both reducing and capping agents in the green synthesis process.

3.2. Biological Activity of green-synthesized copper nanoparticles

3.2.1. Antibacterial Activity

The antibacterial activity of FAN was examined against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The test compound exhibited distinct zones of inhibition against both bacterial strains, indicating notable antibacterial potential. Against *S. aureus*, FAN produced a zone of inhibition measuring 22 mm, while streptomycin showed a zone of 26 mm (Figure 10).



Fig. 10. Agar well diffusion assay showing the antibacterial activity of FAN against *Staphylococcus aureus*. Wells correspond to negative control (DMSO), standard antibiotic (streptomycin, 1 mg/mL), and test compound FAN (1 mg/mL). Clear zones of inhibition indicate antibacterial efficacy.

Similarly, FAN demonstrated strong activity against *E. coli* with a zone of inhibition of 24 mm, compared to 29 mm for the standard antibiotic (Figure 11) [31-33]. No inhibitory zone was observed for the negative control (DMSO), confirming that the antibacterial activity was solely attributable to the test compound. The observed results suggest that FAN possesses broad-spectrum antibacterial efficacy, with activity comparable to the standard drug under the tested conditions.

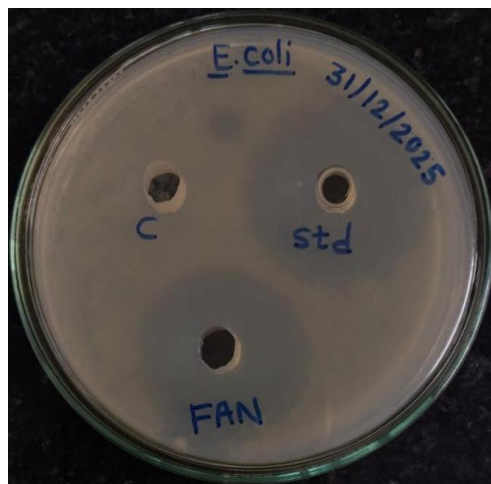


Fig. 11. Agar well diffusion assay showing the antibacterial activity of FAN against *Escherichia coli*. Wells correspond to negative control (DMSO), standard antibiotic (streptomycin, 1 mg/mL), and test compound FAN (1 mg/mL). Clear zones of inhibition indicate antibacterial efficacy.

3.2.2. Antifungal Activity

The antifungal activity of FAN was evaluated against *Candida albicans* (ATCC 10231) (Figure 12). The test compound exhibited a measurable zone of inhibition of 13 mm, indicating moderate antifungal activity. In comparison,

the standard antifungal drug miconazole produced a zone of inhibition of 24 mm, while no inhibition was observed in the negative control (DMSO). The presence of a distinct inhibition zone confirms the antifungal efficacy of FAN against *C. albicans*. Although the activity was lower than that of the standard drug, the observed results demonstrate the potential of FAN as a bioactive antifungal agent under the tested conditions.



Fig. 12. Antifungal activity of FAN against *Candida albicans* evaluated by the agar well diffusion method. Wells represent negative control (DMSO), standard antifungal agent (miconazole, 1 mg/mL), and test compound FAN (1 mg/mL). Zone diameters reflect antifungal effectiveness.

3.2.3. Cytotoxicity

The MTT assay results demonstrated that FAN exhibited very low cytotoxicity toward L929 fibroblast cells across the tested concentration range (20–100 μ g/mL) (Figures 13 and 14).

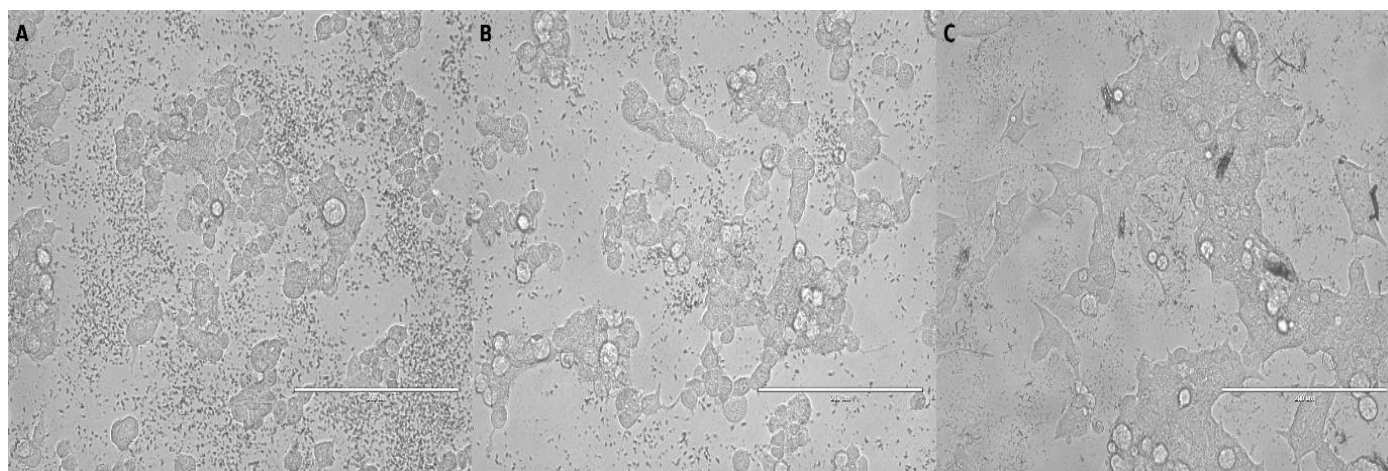


Fig. 13. Cytotoxicity profile of a) Control b) standard c) dose-dependent cytotoxicity profile of FAN against L929 fibroblast cells determined by MTT assay after 24 h exposure.

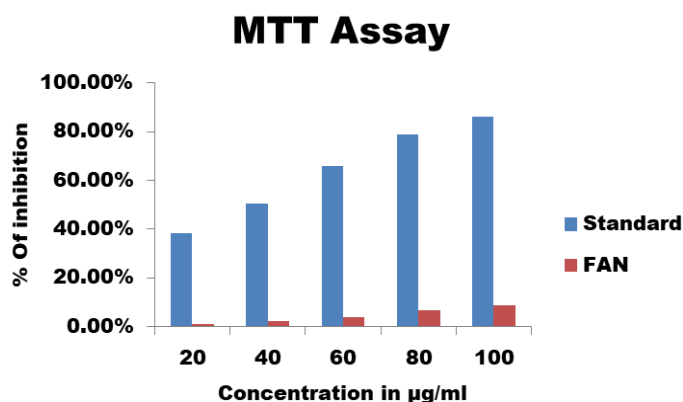


Fig. 14. MTT assay showing the cytotoxic response of FAN on L929 fibroblast cells at different concentrations after 24 h of exposure, with the standard cytotoxic agent included for comparison.

The percentage inhibition values for FAN increased marginally from 1.15% at 20 µg/mL to 8.64% at 100 µg/mL, corresponding to high cell viability levels of 98.85% to 91.36%, respectively. No IC_{50} value was observed for FAN within the tested concentration range, indicating negligible toxicity. In contrast, the standard compound showed a pronounced, dose-dependent cytotoxic effect, with inhibition values exceeding 50% at higher concentrations and an IC_{50} value of 39.33 µg/mL, confirming the sensitivity and validity

of the assay [34, 35]. The cytotoxicity assessment clearly indicates that FAN is biocompatible and non-toxic toward normal L929 fibroblast cells under the experimental conditions. The minimal increase in growth inhibition with increasing concentration suggests that FAN does not adversely affect cellular metabolic activity or membrane integrity. The absence of an IC_{50} value further supports its safety profile. The marked difference between the cytotoxic response of FAN and the standard drug highlights the selective and mild biological interaction of FAN with normal cells. Such low cytotoxicity is particularly desirable for compounds intended for antimicrobial or biomedical applications, as it reduces the risk of damage to healthy tissues. Overall, the MTT assay results confirm that FAN possesses excellent cyto-compatibility, making it a promising candidate for further in-vitro and in-vivo biological investigations.

3.2.4. Anticancer Activity

The anticancer activity of FA and FAN was further evaluated against the human triple-negative breast cancer cell line MDA-MB-231 (Figures 15 and 16). Both samples exhibited concentration-dependent cytotoxic effects; however, FAN consistently showed higher inhibition than FA at all tested concentrations. FAN achieved 62.35% inhibition at 100 µg/mL with an IC_{50} value of 78.99 µg/mL, whereas FA showed 57.15% inhibition with an IC_{50} of 92.85 µg/mL.

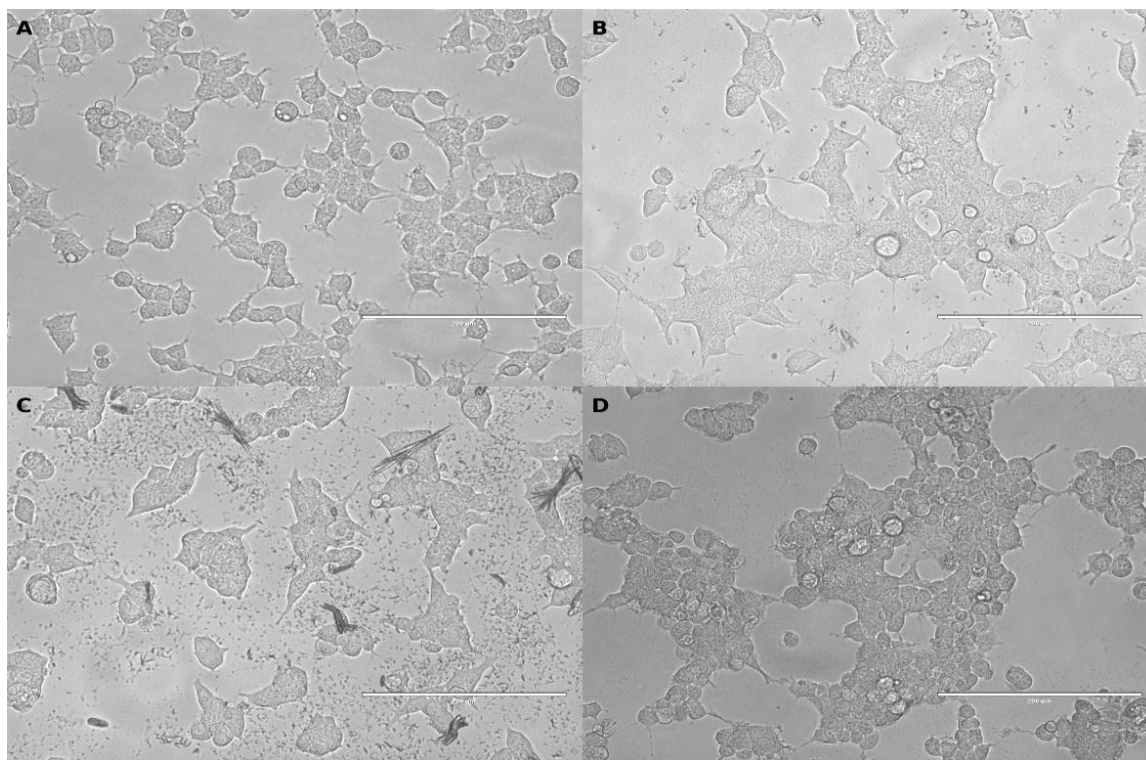


Fig. 15. Phase-contrast micrographs of MDA-MB-231 cells showing morphological changes after treatment with (A) control, (B) *Ficus amplissima* Smith Leaves Extract, (C) *Ficus amplissima* Leaves, and (D) standard anticancer drug, as observed in

the MTT assay. Images were captured after 24 h of treatment. Scale bar = 200 μ m.

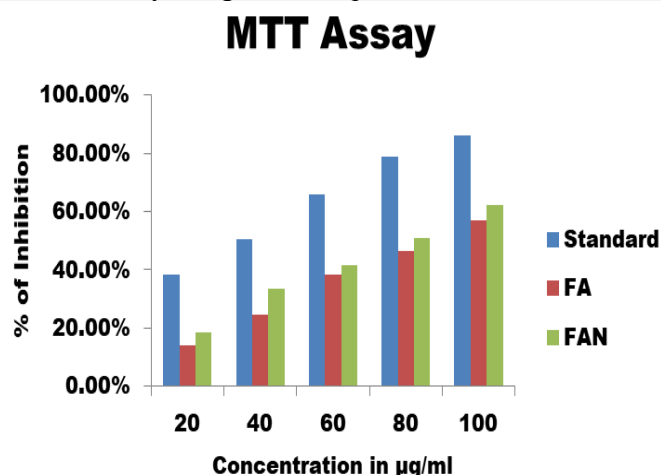


Fig. 16. Effect of *Ficus amplissima* Leaves Extract and *Ficus amplissima* copper nanoparticle on the viability of MDA-MB-231 cells assessed by MTT assay at various concentrations after 24 h incubation.

The standard anticancer drug (5-fluorouracil) displayed the highest cytotoxicity, validating the assay. The enhanced activity of FAN compared to FA suggests that modification or formulation significantly improved anticancer efficacy against aggressive breast cancer cells. Overall, the combined results demonstrate that FAN exhibits effective antimicrobial activity, negligible toxicity toward normal cells, and promising anticancer potential against triple-negative breast cancer cells. The favorable balance between biological activity and biocompatibility highlights FAN as a strong candidate for further mechanistic studies and advanced therapeutic evaluation.

4. CONCLUSION

An environmentally benign method for the synthesis of copper nanoparticles using *Ficus amplissima* leaf extract was successfully developed. Structural and morphological analyses confirmed the formation of crystalline CuNPs with an average size of ~ 55 nm, low lattice strain, and minimal defect density. Phytochemical-assisted reduction and stabilization were verified by FTIR analysis, while DLS and zeta potential measurements demonstrated good colloidal stability. The synthesized nanoparticles showed strong antibacterial efficacy, moderate antifungal activity, negligible cytotoxicity toward normal fibroblast cells, and appreciable anticancer activity against triple-negative breast cancer cells. The combination of structural integrity, biological effectiveness, and biocompatibility suggests that these green-synthesized CuNPs are promising candidates for future biomedical and therapeutic applications.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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