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Green Synthesized Copper Nanoparticles from *Jatropha tanjorensis*: Spectroscopic Characterization and Dose-Dependent Antibacterial Activity

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Abstract

Green synthesis of nanoparticles offers a safer and more sustainable approach, with plant-mediated methods gaining attention in biomedical research. In this study, phytochemical and quantitative results of the crude aqueous extract of *Jatropha tanjorensis* leaves shows presence of 19.49 mg tannins, 25.49 mg saponins, 24.10 mg steroids, 66.54 mg flavonoids, 56.22 mg phenols and 30.59 mg alkaloids**,** the biosynthesis of the CuNPs was confirmed by the colour change from light blue to deep green. UV-Vis result of CuNPs showed absorbance at 481 nm, SEM analysis confirmed the presence of predominantly spherical CuNPs, 79% copper composition from EDX analysis, TEM results showed a distribution range of 4-19 nm with average size of 9 nm. Furthermore, XRD showed lattice planes at $\mathbf{30.22°}$, $\mathbf{32.44°}$, $\mathbf{43.05°}$, $\mathbf{54.50°}$ and $\mathbf{59.20°}$ correspounding to (111), (200), (202), (220) and (311) with crystal size range of 2-10 nm, while for FTIR analysis, metal – oxide (Cu-O) peak was confirmed in the range of 570 – 520 cm^{-1}. Inhibition zone diameter for antibactera activity was seen at 36 mm, 17 mm and 12 mm for *Moraxella catarrhalis*, *Staphylococcus aureus* and *Escherichia coli* while the MIC value was recorded for 8 mg/ml for *Moraxella catarrhalis*. These study sucessfully demonstrated the green synthesis of copper nanoparticles using *Jatropha tanjorensis* extract, with confirmed structural, morphological and antibacterial properties. The CuNPs exhibited notable antibacterial activity, particularly against *Moraxella catarrhalis,* indicating their potential as alternative antimicrobial agents. However, further studies are required to evaluate cytotoxicity, long term stability and in-vivo efficacy before clinical or pharmaceutical applications can be considered. Futur work should also focus on optimizing large-scale production feasibility and optimizing particle size control.

Keywords: Antibacterial Activity, Characterisation, Copper Nanoparticles, Green Synthesis, *Jatropha tanjorensis*

Introduction

Nanoparticles are materials with dimensions below 100 nm and exhibit unique physicochemical properties due to their high surface-to-volume ratio and quantum confinement effects [1]. These properties enable their application across medical, pharmaceutical, environmental and energy-related industries. In particular, metallic nanoparticles have demonstrated promising

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antimicrobial, catalytic and drug delivery capabilities [2]. Conventional physicochemical synthesis methods such as thermal decomposition, electrochemical reduction, laser ablation and chemical reduction of metal salts allow controlled nanoparticles production; however, they often involve toxic reagents, high energy consumption and environmentally hazardous by-products [3,4,5]. These limitations have intensified research efforts toward green synthesis approaches that utilize biological materials as reducing and stabilizing agents [3].

Plant-mediated synthesis has gained considerable attention because phytochemicals such as flavonoids, tannins, alkaloids and phenolic compounds can simultaneously reduce metal ions and stabilize the formed nanoparticles [6,7]. While gold and silver nanoparticles synthesized using plant extracts have been extensively reported and evaluated for antimicrobial activity, comparatively fewer studies have systematically investigated plant-mediated copper nanoparticles, despite copper being more cost-effective and possessing strong intrinsic antimicrobial properties [8,9]. Furthermore, although some studies report the antibacterial potential of green-synthesized CuNPs, variations in particle size distribution, crystallinity and phytochemical composition often result in inconsistent antimicrobial performance [10,11,12,13]. There remains limited data correlating structural characterization (TEM, FTIR, SEM, XRD, EDX) with antibacterial efficacy and minimum inhibitory concentration (MIC) values, particularly using *Jatropha tanjorensis* leaf extract.

Jatropha tanjorensis is medicinal plant known for its antimicrobial and antioxidant phytochemicals [14]. However, its role in the controlled biosynthesis of copper nanoparticles and the detailed evaluation of their antibacterial potency against both Gram negative and Gram-positive pathogens remain underexplored. Therefore, this study aims to address the existing gap by synthesizing and comprehensively characterizing copper nanoparticles using *Jatropha tanjorensis* aqueous leaf extract and evaluating their antibacterial efficacy against clinically relevant bacterial isolates, including *Moraxella catarrhalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus species.*

It is hypothesized that phytochemical-mediated reduction will produce stable, crystalline CuNPs with controlled nanoscale dimensions, resulting in concentration-dependent antibacterial activity. The selected bacterial strains represent both Gram positive and Gram-negative pathogens commonly associated with respiratory and opportunistic

infections, allowing evaluation of broad-spectrum antimicrobial potential. Antibacterial activity was assessed using agar well diffusion and minimum inhibitory concentration (MIC) determination to quantitatively evaluate dose-dependent efficacy.

Materials and Methods

Reagents

The reagents used in this study were as follows: Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$) (Guangzhou JHD Chemical Reagent Co., Ltd.), nutrient agar, Muller Hinton agar, and MacConkey agar,(HiMedia Laboratories private limited company), ammonia hydroxide (CDH Chemicals Limited Company), lead acetate (Tianjin Kermel Chemical Reagent Co., Ltd), ferric chloride (Guangzhou JHD Chemical Reagent Co., Ltd), olive oil, hydrochloric acid (British Drug House BDH Chemicals Limited), glacial acid (Guangzhou JHD Chemical Reagent Co., Ltd), ethanol (Guangzhou JHD Chemical Reagent Co., Ltd), chloroform (LobaChemie Private Limited), sulphuric acid (LobaChemie Private Limited), oxidase reagent (Bio-rad Laboratories Inc.), peptone water by (HiMedia Laboratories private limited company), normal saline (Unique Pharmaceuticals Limited, Unisal), and distilled water.

Collection and Preparation of *Jatropha tanjorensis* Leaves

Fresh *Jatropha tanjorensis* leaves were obtained and authenticated at the University of Ilorin, Nigeria (Voucher number: UILH/001/1470/2022). After identification, the leaves were rinsed with distilled water and air-dried in a shaded area for 30 days. Afterwards, the dried plant samples were pulverised using a sterile blender (RENG73224: Silver Crest) and stored in a sterile air-tight container until further use.

Preparation of *Jatropha tanjorensis* Extract

The aqueous extract of *Jatropha tanjorensis* was prepared using the maceration method described by Ananda *et al*. [15]. Briefly, 20 g of the plant powder was dissolved in 400 ml of distilled water, agitated for 90 minutes. Then it was heated for an hour at 50°C, with intermittent stirring. The solution was allowed to sit at room temperature to cool and later filtered using a Whatman (11µm) filter paper. The resulting filtrate was collected and stored in a sterile container at 4°C for subsequent use in the CuNP synthesis.

Phytochemical Screening

The leaf extract of *J. tanjorensis* was screened for the presence of important phytochemical compounds, and the procedures are outlined below:

Test for Flavonoids

Three types of tests were carried out to determine the presence of flavonoids: alkali, lead, and ferric chloride. Three test tubes containing 1 ml (each) of the plant extract were labelled appropriately for the different tests. Then, 2 drops (each) of 10% ammonium hydroxide solution, 10% lead acetate solution, and 10% ferric chloride solution were added to the appropriate test tubes for alkaline, lead, and ferric chloride tests [16].

Test for Saponins

In order to identify saponins, a froth test was carried out on the extract, where 5 ml of the plant extract was vigorously shaken to produce a stable froth. After which, 6 drops of olive oil were added, and the mixture was observed for persistent frothing and emulsion [17].

Test for Tannins

Braymer′s test was carried out to detect the presence of tannins. 3 drops of 10% ferric chloride solution were added to 1 ml of the plant extract. Then, the mixture was gently agitated and observed for a colour change [17].

Test for Alkaloids

Three types of tests were carried out to determine the presence of alkaloids. First, Mayer's test was done by adding 6 drops of Mayer's reagent to 1 ml of plant extract. Then, the mixture was observed for the formation of green precipitate. A further confirmation was done using Wagner's test, where 2 drops of Wagner's reagent were added to 1ml of plant extract, and the mixture was observed for the formation of reddish-brown precipitate. Finally, the Dragendroff's test was done by introducing 0.5 ml of the reagent into 1ml of dilute hydrochloric acid (HCl) and 1 ml of plant extract. Then, the solution was observed for the formation of an orange precipitate [18].

Test for Cardiac Glycosides

The Keller-Kiliani test was done to detect cardiac glycosides in the plant extract. In this test, 5 of the extract was mixed with 1.5 ml of glacial acetic acid, followed by a 5% ferric chloride solution drop. Subsequently, 2 ml of concentrated sulphuric acid (H_2SO_4) was added to the mixture and observed for the presence of a blue colouration at the interface [16].

Test for Phenols

A ferric chloride test was carried out to detect phenol. Three drops of 5% ferric chloride solution were added to 2 ml of the extract, and the mixture was observed for a colour change [16].

Test for Steroids

Salkowski's test was carried out for the detection of steroids. Two drops of concentrated sulphuric acid (H_2SO_4) were added to 2 ml of the plant extract for this test. The mixture was thoroughly agitated, allowed to stand for about a minute, and later observed for a colour change [16].

Test for Terpenoids

For terpenoids, 2 ml of chloroform was added to 5 ml of the extract. The mixture was evaporated in a water bath, after which 3 ml of concentrated sulphuric acid (H_2SO_4) was added and boiled in the water bath. Then, the solution was observed for the formation of a grey colour [17].

Test for Phlobatannins

Plobatannins in the plant extract were detected using the hydrochloric acid (HCl) test. For this, 2 ml of 1% HCl was added to 2 ml of the plant extract. The mixture was boiled and observed for the formation of a red precipitate [16].

Test for Anthraquinones

For anthraquinones detection using Borntrager′s test, 10 ml of 10% ammonia hydroxide solution was added to 5 ml of the plant extract. The mixture was shaken vigorously for 30 seconds, and a colour change was observed [16].

Quantitative Phytochemical Analysis

Standard quantitative phytochemical analysis was conducted for the aqueous extract of *J. tanjorensis,* following the procedure described by Shaikh and Patil [16].

Synthesis of Copper Nanoparticles

Copper nanoparticles were synthesized via a green reduction method using *Jatropha tanjorensis* extract as a bio reducing and stabilizing agent. The synthesis process involved dissolving 4 g of $CuSO_4$ in 50 ml of distilled water to make up a 0.32M solution. Afterwards, 100 ml of the prepared extract was added while continuously stirring at room temperature. The reaction mixture was maintained with consistent stirring for 4 hours, during which the noticeable color change, which gives colloidal copper nanoparticles was observed. Then, the synthesized CuNPs were centrifuged at 2,200 rpm for 10 minutes, and the supernatant was discarded. The precipitate was purified using distilled water and centrifuged, followed by ethanol precipitation. Finally, the CuNPs were dried and characterized [19].

Characterization of Copper Nanoparticles

A series of analytical techniques was employed to confirm the formation and properties of the synthesized CuNPs. The Surface Plasmon Resonance (SPR) of CuNPs was analyzed using a UV-Vis spectrophotometer (UV-Pharo 300 spectrophotometer) in the wavelength range of 200-800 nm to confirm the characteristic absorption peak [20]. Then, the surface morphology and shape of the nanoparticles were examined using Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) (using the JSM-7600F JEOL), which provided the elemental composition and characteristic energy peaks. Transmission Electron Microscopy (TEM) (FEI Verios 460L) was

used to determine the size distribution and shape of CuNPs, providing high-resolution imaging of individual particles [21]. The crystalline nature of the synthesized CuNPs was investigated using X-ray Diffraction (XRD) (using the Rigaku D/Max-IIIC and PW1800 X-ray diffractometers) to identify distinct diffraction peaks corresponding to copper. Also, the average crystallite size was calculated using the Scherrer equation, and the lattice value was determined. Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer 3000Mx spectrometer) was used to identify functional groups responsible for reducing and stabilizing the nanoparticles [22].

Antibacterial Activity

The antibacterial activity of the synthesized copper nanoparticles was evaluated using the agar well diffusion method against both Gram-positive (*Staphylococcus aureus*, *Moraxella catarrhalis* and *Streptococcus species*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. Fresh cultures of the identified bacterial isolates were prepared by inoculating them in the nutrient broth for 24 hours to achieve the logarithmic growth phase. Then, the bacterial cultures were adjusted to a concentration standard of 0.5×10^8 CFU/ml and inoculated on freshly prepared Muller Hinton Agar (MHA). The copper nanoparticles solution was also prepared in varying concentrations: 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml using distilled water. These concentrations were achieved by dissolving 0.2, 0.15, 0.1 and 0.05g of the dried CuNPs in 2 ml of normal saline. A sterile cork borer was used to create wells in the inoculated agar plate, and 50µL of the dissolved copper nanoparticles samples were added to the wells. Ciprofloxacin (5 mg) was used as the positive control. The plates were incubated at 37 °C for 24 hours, with *Moraxella catarrhalis* and *Streptococcus species* incubated in chocolate agar within a candle jar for anaerobic conditions [15,23]. After incubation, the zones of inhibition (clear zones around the wells) were measured to determine the antibacterial efficacy of the copper nanoparticles. The larger the clear zone, the greater the antibacterial activity exhibited by the nanoparticles [15,24].

The minimum inhibitory concentration (MIC) of the green synthesized CuNPs was determined using macro-broth dilution method. Standardized bacterial suspensions were prepared using normal saline, inoculated into MH broth, and adjusted to make a bacterial concentration of 0.5×10^8 CFU/ml. Then, 50µL of the CuNPs was introduced into the bacterial broth at concentrations ranging from 6 mg/ml to 14 mg/ml. The controls were ciprofloxacin and distilled water. The experiment was carried out at 37 °C for 24 hours, with *Moraxella catarrhalis* and

Streptococcus species incubated in chocolate agar within a candle jar for anaerobic conditions [15,24].

Results and Discussion

Phytochemical Results

The phytochemical profile (Table 1) of *J. tanjorensis* leaf aqueous extract revealed the presence of saponins, tannins, steroids, phenols, flavonoids and alkaloids, which aligned with the findings in several studies [24-26]. These were confirmed by the presence or absence of a color change in the reaction (Figure 1). Komolafe *et al.* [27] further attributed the presence of these phytochemicals to *J. tanjorensis'* antibacterial activities against *Escherichia coli, Bacillus subtilis, Bacillus aureus, Salmonella typhi, and Pseudomonas aeruginosa*. These phytochemicals have also been documented for other species within the *Jatropha* genus. Emeka *et al.,*[28] highlighted the presence of these phytochemicals in *Jatropha gossypiifolia.* Also, Daniya *et al.* [29] found alkaloids, steroids, tannin and phenol compounds in their examination of *Jatropha curcas*.

Quantitative phytochemical analysis of *Jatropha tanjorensis* leaf extract (Table 2) revealed high levels of flavonoids (66.54 mg/100 g) and phenols (56.22 mg/100 g), with tannis being the least abundant (19.49 mg/100 g). In a similar study, Komolafe *et al.* [27] also found flavonoid (98.55 mg/100 g) and phenol (69.09 mg/100 g) to be the most abundant in hot aqueous extract of *J. tanjorensis* leaves. The presence of these phytoconstituents in *Jatropha tanjorensis* supports its suitability for the green synthesis of nanoparticles; in this case, it is well-suited for the reduction and stabilization process in the synthesis of CuNPs. Also, these phytochemicals enhance the biological activity of the synthesized nanoparticles, contributing to their antimicrobial properties [30].

Table 1: Phytochemical Constituents of *Jatropha tanjorensis* Leaf Extract

Phytochemicals	Test Performed	Observation	Results
Alkaloids	Dragendorff's test Mayer's test Wagner's test	Orange precipitate Green precipitate Reddish-brown precipitate	Present
Flavonoids	Alkaline test Lead test $FeCl_2$ test	Yellow fluorescence Yellow precipitate Green precipitate	Present
Phlobatannis	HCl test	No red precipitates	Absent

Anthraquinones	Borntrager's test	No colour observed	Absent
Terpenoids	Liebermann Burchard test	No colour observed	Absent
Tannins	Braymer's test	Greenish-black colour	Present
Phenol	$FeCl_2$ test	Dark green colour	Present
Saponins	Froth test	Froth and emulsion	Present
Steroids	Salkowshi's test	Reddish lower layer	Present
Cardiac glycosides	Keller-Killani test	No colour observed	Absent

Biosynthesis of Jatropha tanjorensis CuNPs

In this study, J. tanjorensis sleaf extract was employed as both a reducing and stabilizing agent for the synthesis of the CuNPs. Studies have shown that this is possible due to the presence of various secondary metabolites, which synergistically reduce Cu^{2+} to Cu^0, with flavonoids, alkaloids, phenols, and terpenoids acting as the best reducing agents [31]. This reduction was visibly detected by the color change from a light blue solution to a deep, dark green solution. Such noticeable color change, which gives colloidal copper nanoparticles was noticed 10 minutes into the experiment. This color change observation agrees with the report of Amer and Awwad [19].

Characterization of Jatropha tanjorensis CuNPs

UV-Vis Analysis

The UV-Vis spectra was shown for the initial (CuSO4 +Jatropha tanjorensis extract) (Figure 2A) and final (CuNP colloidal solution) (Figure 2B) solution. This distinct SPR band for the CuNPs confirms the reduction of Cu^{2+} to Cu^0. The initial stage of green synthesis and its interaction with Cu^{2+} was seen at 206 nm and 271 nm, while 338 nm is the intermediate signature before full formation of CuNPs. The peak at 481nm for the CuNPs is around the blue shift region, indicating the presence of smaller-sized nanoparticles [20]. Additional peaks 599-754 nm with subsequent red shifts, indicating presence of large size nanoparticles. These results signifies the stabilization and uniform dispersion of the green synthesized nanoparticles in solution, which shows slight agglomeration similar to results obtained in SEM and TEM [32].

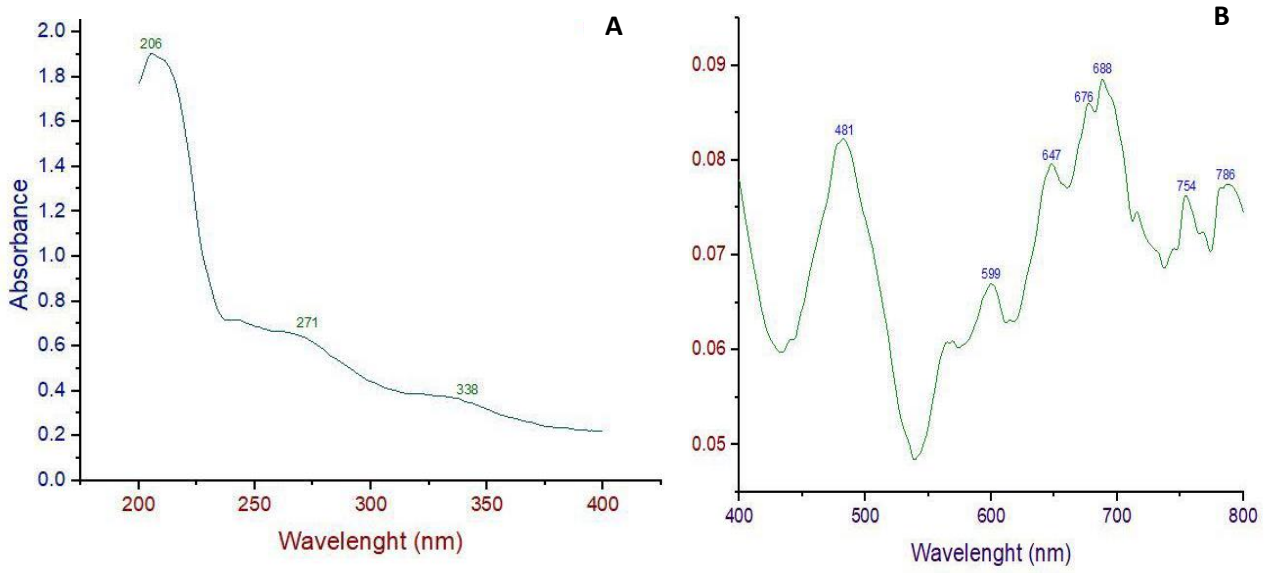


Figure 2: Spectrum of UV-Vis Analysis

(Fig A shows initial Cu^{2+} and phytochemicals interaction peaks: Fig B confirms CuNP formation with SPR at 481nm).

SEM-EDX Analysis

The SEM analysis investigated the morphology and shape of the CuNPs. SEM micrographs (Figure 3) revealed that the CuNPs were mostly spherical, with mild agglomeration. Based on this analysis, the agglomerated size was 12 nm. As a commonly encountered phenomenon in nanoparticle synthesis, agglomeration can be linked to the bioactive molecules from *Jatropha tanjorensis* extract acting as capping agents [33]. EDX analysis confirmed the elemental composition, showing a high copper content of 79%, along with traces of oxygen, carbon, and other minor elements [23].

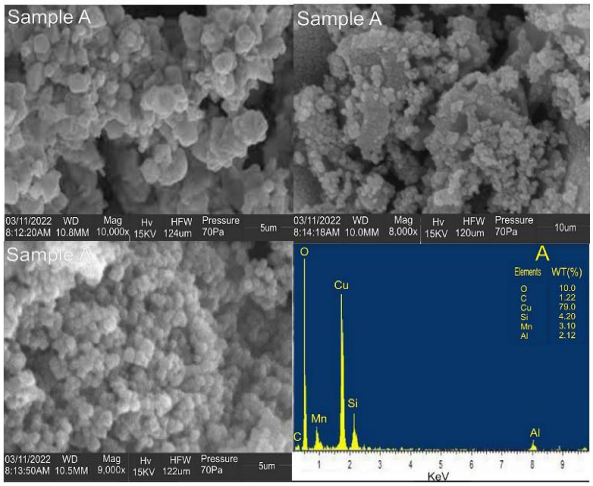


Figure 3: SEM Results Showing the Shape of Copper Nanoparticles and EDX Analysis Displaying Elemental Composition

Transmission Electron Microscopy (TEM)

The average size and distribution of the green synthesized CuNPs was determined using TEM. The distribution range was 4–19 nm, and the average size was 9± 2nm (n=120). A histogram of the nanoparticle distribution is shown alongside the TEM micrograph (Figure 4).

This size, estimated by TEM, reveals the exact particle diameter that was measured in a dry state, and may likely reveal a larger hydrodynamic volume when dispersed in a solvent. Due to efficient reduction and capping of the Jatropha tanjorensis extract, slight agglomeration was also observed from TEM micrograph, further suggesting localized interactions among the nanoparticles during synthesis and due to their high surface energy [34,35].

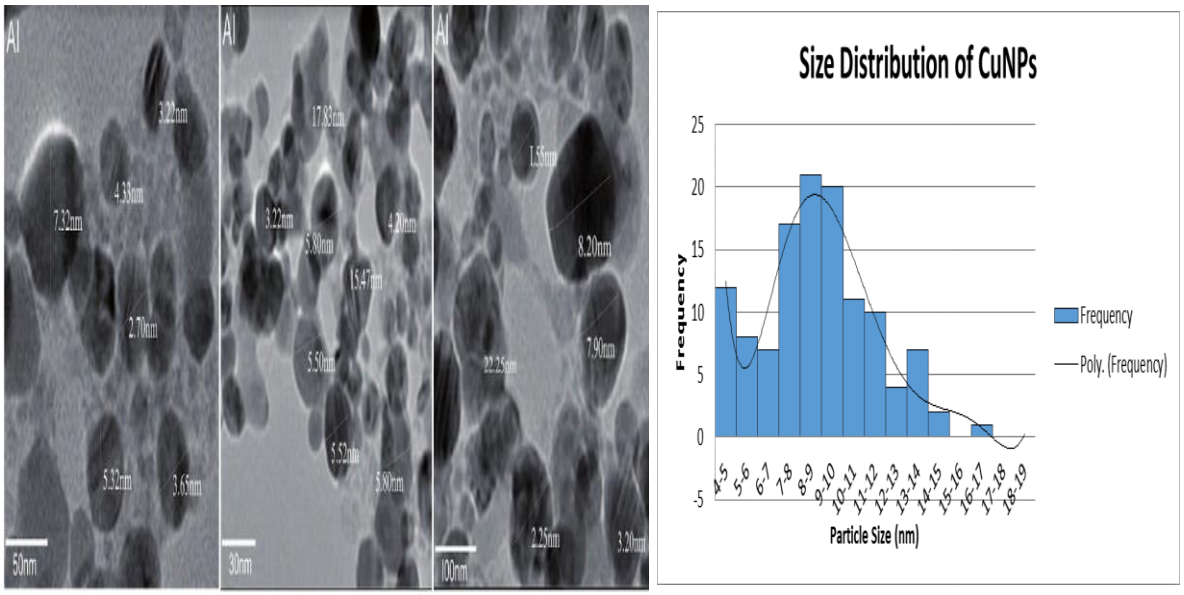


Figure 4: TEM Results Showing CuNP Size and Distribution

XRD Analysis

X-ray diffraction was used to determine the structure as well as particle size of the green synthesized CuNPs. The XRD pattern, shown in Figure 5, revealed distinct diffraction peaks at 2θ values of $30.22°\,(111)$, $32.44°\,(200)$, $43.05°\,(202)$, $54.50°\,(220)$and$59.20°\,(311)$which correspond to the crystal planes of the copper, as identified by Joint Committee of Power Diffraction Standards (JCPDS) card (21-1272). The crystallite size estimated using the Debye-Scherrer equation ranges from 2 to 10 nm. The sharp nature of these peaks indicates a well-defined crystalline structure, a characteristic necessary for enhanced stability and reactivity. These findings are consistent with the report of Naradala *et al,.*[36].

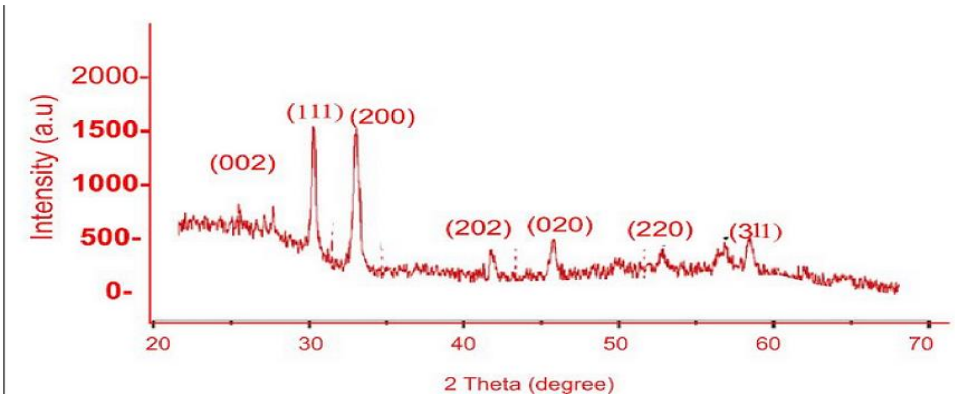


Figure 5: XRD Spectrum Confirming the Crystalline Nature of the synthesized CuNP

FTIR Analysis

The FTIR spectrum for the synthesized CuNPs (Figure 6) revealed peaks at 3320 cm^{-1} (corresponding to N-H stretching), 3362 cm^{-1} (corresponding to O-H stretching), and 1182 cm^{-1} (corresponding to C-O stretching) also in (Table 3). Thus, the mechanistic pathway for the synthesis of these CuNPs involves amine (N-H), carboxylate (COOH) and hydroxyl (O-H) groups, which are found in *J. tanjorensis* phytochemical compounds, as the reducing agents. These served as electron donors for the reduction and stabilization (capping) of Cu2+ ions Cu^0 (copper nanoparticles) [37]. Amines and carboxyl groups can be found in alkaloids and amino acids. Also, the hydroxyl groups are present in tannins, flavonoids, and polyphenols of plant extracts.38 There were additional peaks corresponding to the C-H, C=C and C≡C stretching vibrations, indicating the presence of organic compounds such as alkanes, alkenes and alkynes, which are likely involved in the capping process. Similar results were obtained by Chandraker *et al,.*[39] and Tsilo *et al*. [40].

Table 4: Antibacterial activity of the synthesized CuNPs

S/N	Isolates	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	Control
1	*Escherichia coli*	12 mm	11 mm	09 mm	06 mm	06 mm
2	*Moraxella catarrhalis*	36 mm	25 mm	21 mm	20 mm	06 mm
3	*Streptococcus species*	10 mm	09 mm	06 mm	06 mm	06 mm
4	*Staphylococcus aureus*	17 mm	15 mm	14 mm	11 mm	40 mm
5	*Pseudomonas aeruginosa*	09 mm	08 mm	06 mm	06 mm	37 mm

Table 5: Minimum Inhibitory Concentration of the Green-Synthesized CuNPs

Sample	**MIC (mg/ml)**				
	Escherichia coli	***Moraxella catarrhalis***	***Streptococcus species***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
CuNPs	12	8	14	10	14
Ciprofloxacin	-	-	-	-	-
Distilled Water	-	-	-	-	-

Key: "-" = no inhibitory activity

The antibacterial activity of the biogenic CuNPs is attributed to their small size and high surface area, which enhances their interaction with bacterial membranes. The hypothesized mechanisms of action include cell wall disruption, reactive oxygen species (ROS) generation, and protein inactivation. CuNPs attach to bacterial cell walls, causing permeability changes that lead to leakage of cellular components [42]. Also, Molonry and Cotter [43] discussed that CuNPs induce oxidative stress, leading to lipid peroxidation, protein oxidation, and DNA damage in bacterial cells. Furthermore, CuNPs interact with thiol groups of proteins; thus, disrupting essential enzymatic activities and decreasing the chances of bacterial survival [44]. These mechanisms collectively enhance the antimicrobial potential of CuNPs, making them promising candidates for biomedical applications such as wound dressings,

coatings, and drug delivery systems. The results obtained in this study align with previous research on plant-mediated CuNPs, reinforcing the role of green-synthesized nanoparticles in combating bacterial infections [45,46]. The use of *Jatropha tanjorensis* extract not only enhances the sustainability of nanoparticles synthesis but also imparts additional bioactivity due to the phytochemicals present in the extract. These findings demonstrate that green-synthesized CuNPs are effective antimicrobial agents with potential applications in biomedicine and environmental disinfection.

Conclusions

This study successfully demonstrated the green synthesis of copper nanoparticles (CuNPs) using *Jatropha tanjorensis* extract as both a reducing and stabilising agent. The phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, alkaloids, saponins, steroids and phenols, which played a crucial role in nanoparticles formation. The synthesised CuNPs were characterised using spectroscopy and microscopy analysis, such as UV-Visible spectroscopy, SEM-EDX, TEM, XRD, and FTIR, validating the successful synthesis of CuNPs with a spherical morphology, small size, and well-defined crystalline nature. The UV-Vis spectroscopy results confirmed the presence of surface plasmon resonance at 481 nm, indicative of CuNP formation, while XRD analysis verified their crystalline structure. EDX provided insight into their elemental composition, having 79% copper. The spherical shape of the synthesised copper nanoparticles was confirmed using SEM, and the distribution (4-19 nm) and average size (9nm) was also confirmed by TEM. The antibacterial activity of the synthesised CuNPs was rigorously evaluated against multiple pathogenic bacterial strains, demonstrating remarkable efficacy. The highest inhibitory effect of the CuNPs was recorded for *Moraxella catarrhalis* (36 mm). Notable results were also observed for *Staphylococcus aureus* (17 mm) and *Escherichia coli* (12 mm). Also, least MIC value was recorded for *Moraxella catarrhalis*(8mg/ml). These findings align with previous research supporting the use of plant-mediated CuNPs and emphasise the potential of CuNPs as potent antimicrobial agents capable of combating bacterial infections.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this study are available at **https://zenodo.org/records/16410424**

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