Research Square

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Green Synthesis, Characterisation and Antimicrobial Evaluation of Copper Oxide Nanoparticles Using Carica papaya Leaf Extract

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Abstract

The global Interest in sustainable and low-cost routes to metal nanoparticles has grown steadily, and green synthesis has become an attractive option, particularly for antimicrobial applications. Conventional chemical and physical routes often rely on hazardous reagents and consume large amounts of energy, which has encouraged the search for greener alternatives. In this work, copper oxide nanoparticles (CuO NPs) were synthesized using an aqueous leaf extract of *Carica papaya* and evaluated for antibacterial activity against two clinically relevant bacterial strains. The extract acted as both reducing and capping agent, and the products were characterised by ultraviolet–visible (UV–Vis) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). UV–Vis spectra showed strong absorption in the ultraviolet region with a maximum near 314 nm, consistent with the formation of copper oxide rather than metallic copper. FTIR identified hydroxyl, carbonyl and amide groups, indicating that phenolic compounds and proteins from the extract were involved in reduction and surface stabilisation. XRD confirmed a crystalline product with an estimated crystallite size of 35–45 nm. Antibacterial activity was assessed by agar well diffusion and minimum inhibitory concentration (MIC) against *Escherichia coli* and *Staphylococcus aureus*. The nanoparticles produced concentration-dependent inhibition, with larger zones against *S. aureus*, and an MIC of 500 µg/mL for both organisms. These findings indicate that *C. papaya*-mediated CuO nanoparticles are stable, environmentally friendly antibacterial agents with potential value in pharmaceutical and biomedical applications.

1. Introduction

Nanotechnology has reshaped much of modern materials science through the ability to design and manipulate matter at the nanoscale [1]. Among metal-based nanomaterials, copper and copper oxide nanoparticles have attracted particular interest because of their optical, catalytic and antimicrobial properties [2]. Unlike precious metals such as gold and silver, copper is abundant and inexpensive, which makes copper-based nanoparticles appealing for medical and industrial use [3]. Nanoparticles are traditionally produced by physical and chemical methods. These routes frequently depend on hazardous reducing agents such as sodium borohydride, consume considerable energy and generate by-products that can harm human health and the environment [4]. Green synthesis offers a practical alternative in which plant extracts, bacteria or fungi serve as natural reducing and capping agents [5]. *Carica papaya* (pawpaw) is a widely available medicinal plant whose leaves and peels are rich in bioactive constituents, including papain, alkaloids, flavonoids and reducing sugars. These molecules can reduce Cu^{2+} ions and stabilise the resulting particles without synthetic surfactants [6]. The spread of multidrug-resistant (MDR) bacteria has made the search for new antimicrobial agents increasingly urgent. Copper oxide nanoparticles are active against both Gram-positive and Gram-negative organisms, mainly by disrupting the cell envelope and generating reactive oxygen species (ROS) [7]. Although several groups have prepared copper-based nanoparticles from *C. papaya*, much of the existing work has focused on catalytic or dye-degradation applications, and reports that pair green synthesis with a standardised antibacterial evaluation against defined clinical pathogens remain comparatively limited. In this study we prepared CuO nanoparticles using an aqueous *C. papaya* leaf extract, characterised them by UV–Vis,

FTIR and XRD, and assessed their activity against two clinically important pathogens, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), using both agar well diffusion and MIC determination.

2. Materials and methods

2.1 Chemicals and reagents

Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99% purity, analytical grade) was used as the copper precursor. Distilled water was used throughout for preparing the plant extract and dissolving reagents, so as to avoid ionic interference. Ethanol (99%) was used for surface disinfection, and dimethyl sulfoxide (DMSO) served as the solvent for dispersing the dried nanoparticles and as the negative control in the antibacterial assays.

2.2 Culture media

Nutrient agar (NA) and nutrient broth (NB) were obtained commercially and prepared according to the manufacturer's instructions; these supported routine growth and maintenance of the test strains. Mueller–Hinton agar (MHA) was used for susceptibility testing, as recommended by the Clinical and Laboratory Standards Institute (CLSI) for diffusion methods [8].

2.3 Collection and authentication of plant material

Fresh, healthy leaves of *Carica papaya* L. (pawpaw; family Caricaceae) were collected on 30 August 2025 from cultivated plants grown on a private farm at Kuchikau, Karu Local Government Area, Nasarawa State, Nigeria (approximately 9.03° N, 7.60° E). The specimens were cultivated crop plants rather than wild-collected material, and the leaves were gathered with the permission of the farm owner and carried to the laboratory in sterile polythene bags. *C. papaya* is a widely cultivated, non-endangered crop species and is not listed under CITES or on the IUCN Red List; no specific permits or licences were required for its collection. The plant was identified and authenticated as *C. papaya* at the Herbarium of the Department of Botany, Mewar International University; no voucher specimen was deposited. Authentication mattered because phytochemical content can differ between closely related species and subspecies.

2.4 Preparation of the leaf extract

The aqueous extract was prepared by decoction following the method of [9] with minor modification. Leaves were washed under running water and rinsed with distilled water to remove dust and ionic contaminants, then shade-dried at room temperature for 14 days to limit thermal degradation of heat-sensitive metabolites. The dried leaves were ground to a fine powder in a sterilised electric blender. Twenty grams of the powder was dispersed in 200 mL of sterile distilled water in a 500 mL Erlenmeyer flask and heated at 60°C for 30 min on a magnetic stirrer hot-plate; mild heating promotes diffusion of water-soluble phytochemicals (phenolics, flavonoids and proteins) into the solvent. The mixture was

cooled to room temperature and filtered through Whatman No. 1 filter paper. The clear, pale-yellow filtrate was stored at 4°C and used as the reducing and capping agent.

2.5 Green synthesis of copper oxide nanoparticles

A 0.01 M stock solution of $CuSO_4 \cdot 5H_2O$ was prepared in deionised water. In a typical synthesis, 10 mL of the leaf extract was added slowly to 90 mL of the 0.01 M copper sulfate solution (1:9 v/v) in a 250 mL flask under continuous stirring. The reaction was carried out at room temperature (or at 50°C during optimisation) in the dark to limit light-induced side reactions. Nanoparticle formation was followed visually: the pale-blue copper sulfate solution changed gradually to a greenish-brown colour, indicating reduction of Cu^{2+} and formation of copper oxide. After 24 h, the suspension was centrifuged at 5000 rpm for 20 min. The pellet was washed three times with distilled water and once with ethanol to remove unbound biomolecules, dried in a hot-air oven at 60°C, and stored in an airtight container for characterisation and antibacterial testing [10].

2.6 Characterisation of the nanoparticles

Optical properties were examined with a Shimadzu UV-1800 spectrophotometer over 200–800 nm, using distilled water as the blank for baseline correction [11]. Functional groups were identified with a Shimadzu FTIR-8400 spectrophotometer over 4000–400 cm^{-1}. Crystalline structure and crystallite size were determined by X-ray diffraction using Cu Kα radiation (λ = 1.5406 Å) over a 2θ range of 3–70°. The dried powder was mounted and levelled in the sample holder. Diffraction peaks were compared with the standard monoclinic CuO reference pattern (JCPDS card no. 48-1548), and the crystallite size was estimated using the Scherrer equation.

2.7 Test organisms and inoculum standardisation

Clinical isolates of *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) were obtained from the Microbiology Laboratory, Nasarawa State University, Keffi. Identity was reconfirmed by standard biochemical tests (Gram staining, catalase and coagulase). Stock cultures were maintained on nutrient agar slants at 4°C and subcultured every two weeks [12]. For each assay, a loopful of a 24 h culture was suspended in 5 mL of sterile saline (0.85% NaCl) and adjusted to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), checked visually and by absorbance at 625 nm. Standardising the inoculum is required under CLSI guidance, since inoculum density strongly affects inhibition-zone size [13].

2.8 Antibacterial assay (agar well diffusion)

Antibacterial activity was gauged by the agar well diffusion technique. Once the poured MHA plates had set, the standardised suspension (1.5×10^8 CFU/mL) was swabbed evenly across the surface to give a confluent lawn. After the surface had dried, 6 mm wells were bored with a sterile cork borer, and equal volumes of CuO NP suspension at 250, 500 and 1000 µg/mL (in DMSO) were dispensed into separate wells. A standard antibiotic, gentamicin (40 mg/mL), acted as the positive control and DMSO as the negative control. The plates were left for 30 min to let the material diffuse and were then incubated at

37°C for 24 h, after which the inhibition-zone diameters were read in millimetres with Vernier calipers [14].

2.9 Minimum inhibitory concentration (MIC)

MIC was determined by the broth dilution method following CLSI guidance [8]. A stock CuO NP suspension (1000 µg/mL) was prepared in sterile nutrient broth, and two-fold serial dilutions were made to give 500, 250, 125, 62.5 and 31.25 µg/mL. Each tube received 100 µL of the standardised suspension of *E. coli* or *S. aureus* and was incubated at 37°C for 24 h. A growth control (broth with bacteria, no nanoparticles) and a sterility control (broth with nanoparticles, no bacteria) were included. After incubation, tubes were examined for turbidity, and the MIC was recorded as the lowest concentration at which the broth remained clear [15].

3. Results

3.1 Visual observation of nanoparticle formation

Addition of the leaf extract to the copper sulfate solution produced a gradual colour change from pale blue to greenish-brown, the first visible indication of Cu^{2+} reduction and nanoparticle formation. The colour deepened with time and then stabilised. The suspension remained stable without rapid precipitation, suggesting that the extract biomolecules both reduced the copper ions and stabilised the particles through surface capping.

3.2 UV–visible spectroscopy

The UV–Vis spectrum (Fig. 1) showed strong absorption in the ultraviolet region, with a maximum near 314 nm. Absorbance rose from 300 nm, peaked between roughly 314 and 340 nm, and then declined towards the visible region, with no distinct band in the 550–600 nm range. The absence of a visible-region band argues against metallic copper, whose surface plasmon resonance typically appears near 560–600 nm, and is consistent with copper oxide. The relatively broad absorption suggests a distribution of particle sizes.

3.3 FTIR spectroscopy

The FTIR spectrum (Fig. 2) showed several absorption bands between 4000 and 1500 cm^{-1}. A broad, intense band near 3330 cm^{-1} is assigned to O–H stretching of hydroxyl groups in alcohols and phenolics, with weaker features near 3737 and 3853 cm^{-1} attributable to free or weakly hydrogen-bonded O–H. Bands at 1634 and 1536 cm^{-1} correspond to C = O stretching and N–H bending of amide groups, consistent with proteins such as papain. The weak features between 2100 and 2300 cm^{-1} may arise from C ≡ C or C ≡ N stretching or from atmospheric CO_2. The principal bands and their assignments are summarised in Table 1.

Table 1
Principal FTIR bands of the synthesised CuO nanoparticles and their assignments.

Wavenumber (cm^{-1})	Assignment
3330	O–H stretching (phenols, alcohols)
3737, 3853	Free / weakly hydrogen-bonded O–H
2307, 2113	C ≡ C / C ≡ N stretching (trace) or atmospheric CO_2
1634	C = O stretching (amide I)
1536	N–H bending (amide II)

3.4 X-ray diffraction (XRD) analysis

The XRD pattern (Fig. 3) was recorded over 2θ = 3–70°. Well-defined peaks appeared at 34.02°, 37.85°, 39.56°, 44.04°, 64.26° and 68.65°, with the most intense reflection at 37.85° (Table 2). The sharp, well-resolved peaks indicate a crystalline product rather than an amorphous one. The positions of the stronger reflections are broadly consistent with monoclinic CuO (JCPDS card no. 48-1548), although this assignment is treated as tentative (see Section 4.1). The crystallite size estimated from the Scherrer equation was approximately 35–45 nm. The observed peak broadening is consistent with nanoscale crystallites and may also reflect lattice strain or surface defects, which are common in green-synthesised materials.

Table 2
XRD peak list of the synthesised CuO nanoparticles.

Pos. [°2θ]	Height [cts]	FWHM [°2θ]	d-spacing [Å]	Rel. Int. [%]
34.02	145.06	0.1476	2.6355	16.32
37.85	888.91	0.2362	2.3772	100.00
39.57	32.81	0.1771	2.2778	3.69
44.04	211.43	0.2362	2.0562	23.79
64.26	41.28	0.3542	1.4497	4.64
68.65	16.70	0.3542	1.3673	1.88

3.5 Antibacterial activity (zone of inhibition)

The nanoparticles inhibited both test organisms in a concentration-dependent manner (Table 3). For *E. coli*, the inhibition zone increased from 6 mm at 250 µg/mL to 11 mm at 500 µg/mL and 16 mm at 1000 µg/mL; the positive control gave 21 mm and the negative control produced no inhibition. For *S. aureus*, the zones were 11, 19 and 25 mm at 250, 500 and 1000 µg/mL, respectively, with 31 mm for the positive

control and none for the negative control. At every concentration the zones were larger against *S. aureus* than against *E. coli*.

Table 3
Zones of inhibition of CuO NPs against E. coli and S. aureus.

Organism	Concentration (µg/mL)	Zone (mm)
E. coli	250	6
E. coli	500	11
E. coli	1000	16
E. coli	Positive control	21
E. coli	Negative control	0
S. aureus	250	11
S. aureus	500	19
S. aureus	1000	25
S. aureus	Positive control	31
S. aureus	Negative control	0

3.6 Minimum inhibitory concentration (MIC)

No growth was observed at 1000 or 500 µg/mL for either organism, whereas growth occurred at 250 µg/mL and below (Table 4). The MIC was therefore taken as 500 µg/mL for both *E. coli* and *S. aureus*. The growth and sterility controls behaved as expected, supporting the validity of the assay.

Table 4
MIC of CuO NPs against E. coli and S. aureus.

Organism	Concentration (µg/mL)	Observation	Interpretation
S. aureus	1000	No growth	Above MIC
S. aureus	500	No growth	MIC
S. aureus	250	Growth	Below MIC
E. coli	1000	No growth	Above MIC
E. coli	500	No growth	MIC
E. coli	250	Growth	Below MIC
Both	Growth control	Growth	Viability confirmed
Both	Sterility control	No growth	Sterility confirmed

4. Discussion

The combined UV–Vis, FTIR and XRD data indicate that an aqueous *C. papaya* leaf extract can convert Cu^{2+} into crystalline copper oxide nanoparticles. The UV–Vis maximum near 314 nm, together with the absence of a visible-region plasmon band, points to copper oxide rather than metallic copper; comparable ultraviolet absorption has been reported for plant-mediated CuO nanoparticles [17]. The FTIR results clarify the role of the extract's phytochemicals. The broad band near 3330 cm^{-1} reflects hydroxyl groups from phenolics and flavonoids, which can donate electrons and reduce metal ions, while the amide bands near 1634 and 1536 cm^{-1} indicate that proteins such as papain contribute to capping and stabilisation. This dual role of plant biomolecules in reduction and stabilisation is characteristic of green synthesis and has been reported repeatedly [16, 19, 20]. The resulting capping layer helps prevent aggregation and is consistent with the visual stability of the colloid. The XRD pattern supports a crystalline product. The stronger reflections are broadly consistent with monoclinic CuO, and the estimated crystallite size of 35–45 nm agrees with values reported for biologically synthesised copper oxide [20]. A small crystallite size gives a high surface-to-volume ratio, which is generally linked to greater reactivity and biological activity. Turning to antibacterial behaviour, the nanoparticles inhibited both test organisms in a concentration-dependent manner, which is typical of nanoparticle-mediated activity and reflects increased particle–cell contact at higher doses. *S. aureus* was more susceptible than *E. coli* at every concentration. This is commonly attributed to differences in cell-wall architecture: Gram-negative bacteria possess an outer membrane that can act as an additional permeability barrier, whereas Gram-positive bacteria lack this layer, so the particles may interact more readily with the *S. aureus* surface. Stronger activity against Gram-positive organisms has been reported for copper oxide nanoparticles [21]. The MIC data are consistent with the diffusion results, with 500 µg/mL sufficient to prevent visible growth of both strains.

The antibacterial effect can be linked to the physicochemical properties observed here. The nanoscale size provides a large contact area, and the adsorbed phytochemicals identified by FTIR may promote adhesion to microbial surfaces. Copper oxide nanoparticles are generally thought to act through several routes, including the generation of reactive oxygen species, disruption of membrane integrity, and interference with intracellular proteins and DNA, with released copper ions contributing to toxicity [16]. Overall, the antibacterial performance is in line with earlier reports on plant-mediated copper nanoparticles [18, 20].

Several limitations should be acknowledged in this study. First, particle morphology and size distribution were not examined directly; the reported size rests on the Scherrer estimate from XRD rather than on electron microscopy (SEM or TEM), which would give a more reliable picture of shape and dispersity. Second, elemental composition and copper oxidation state were not confirmed by energy-dispersive X-ray spectroscopy (EDX) or X-ray photoelectron spectroscopy (XPS). The XRD pattern is broadly consistent with monoclinic CuO, but the reflection near 44° and the small angular shift of the lower-angle peaks mean that a mixed or oxygen-deficient copper phase cannot be excluded; the phase assignment is therefore tentative and would benefit from complementary analysis. Third, colloidal stability was judged

only visually, whereas zeta-potential measurement would give a quantitative measure. Fourth, the antibacterial assays were based on single determinations, without replicate measurements or statistical analysis, so the values should be regarded as indicative; future work should include replicates with appropriate statistics, together with time-kill kinetics and minimum bactericidal concentration (MBC) testing. Finally, the antibacterial mechanism was inferred from the literature rather than measured directly (for example, by ROS assays), and only two bacterial species were examined. Addressing these points would strengthen the conclusions and support broader application.

5. Conclusion

An aqueous leaf extract of *C. papaya* was used to prepare copper oxide nanoparticles by a simple, eco-friendly route, with the extract acting as both reducing and capping agent. Taken together, the UV–Vis, FTIR and XRD data indicate a crystalline copper oxide product with an estimated crystallite size of 35–45 nm and a surface layer of plant-derived biomolecules. The nanoparticles showed concentration-dependent antibacterial activity against *E. coli* and *S. aureus*, with greater activity against *S. aureus* and an MIC of 500 µg/mL for both organisms. These results support green synthesis as a practical approach to biologically active copper oxide nanoparticles, while the limitations noted above indicate clear directions for further characterisation and validation.

Declarations

Ethics approval and consent to participate: This study did not involve human participants, human data, or animals, and ethical approval was therefore not required. The plant material used, *Carica papaya*, is a common cultivated crop and is not a protected, endangered, or CITES-listed species. Collection of the plant material complied with the relevant institutional, national, and international guidelines and legislation. The leaves were obtained from cultivated plants with the permission of the landowner, and no specific permits or licences were required for the collection of this cultivated species.

Consent to participate: Not applicable. This study did not involve human participants.

Consent to publish: Not applicable. This manuscript does not contain data or identifying information relating to any individual person.

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Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability: All data generated or analysed during this study are included in this article. The raw datasets (UV–Vis, FTIR and XRD files) are available from the corresponding author on reasonable request.

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CRediT author contributions: Fadimatu Isah: conceptualisation, methodology, formal analysis, investigation, visualisation, writing – original draft, review and editing. Uwaisu Nura Kani: writing – review and editing. Mujahid Abubakar Wudil: writing – review and editing. Aisha Muhammad: supervision, writing – review and editing.

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Figures

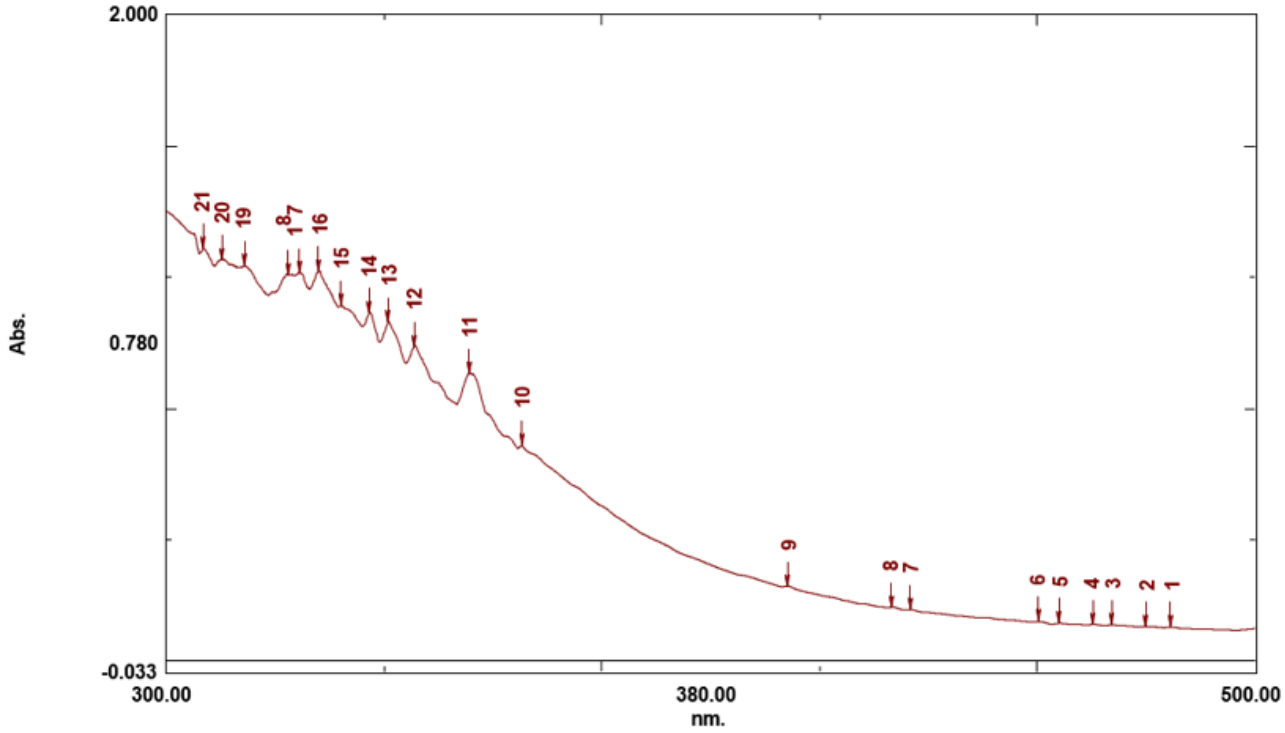


Figure 1

UV–visible absorption spectrum of the synthesised CuO nanoparticles.

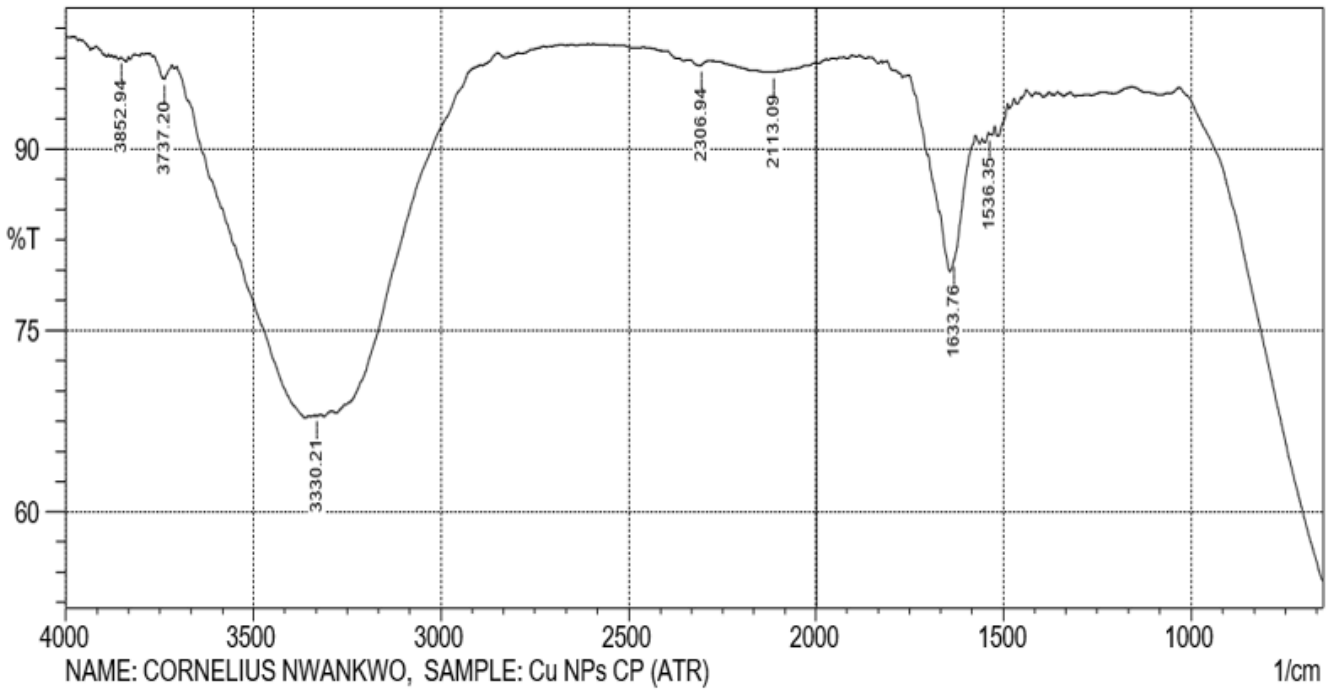


Figure 2

FTIR spectrum of the synthesised CuO nanoparticles.

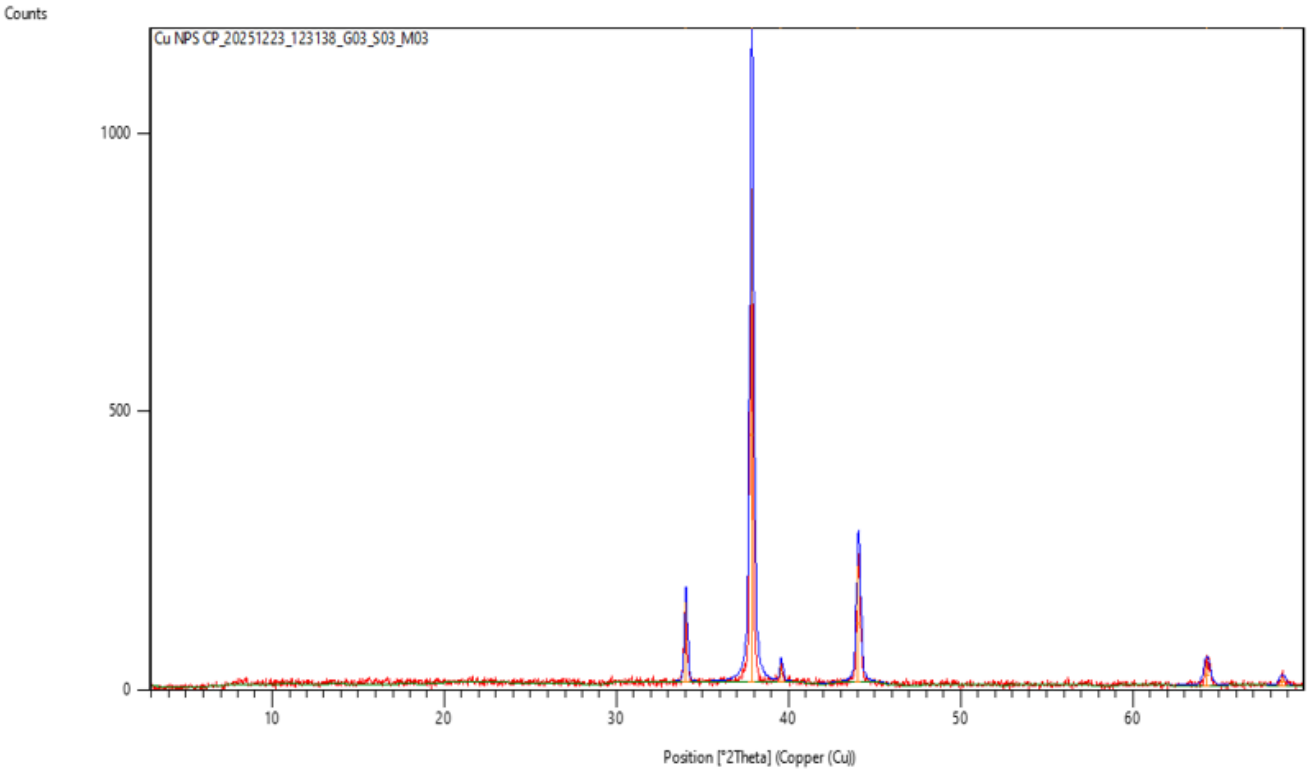


Figure 3

X-ray diffraction pattern of the synthesised CuO nanoparticles.