

Bio-Reducing and Capping Potential of Breynia Disticha (Snowbush) Leaf Extract in Green Synthesis of Copper Oxide Nanoparticles and Antioxidant Activity

Imuetinyan Eriamiatoe

`imuetinyan.eriamiatoe@uniben.edu`

University of Benin

Loveth Ojo Oloyomeu

University of Benin

Philip Idemudia Edogun

University of Benin

Research Article

Keywords: Copper oxide, Nanoparticles, Breynia disticha, green synthesis, antioxidant activity, DPPH assay, SEM-EDX

Posted Date: March 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8810825/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Green synthesis of metal oxide nanoparticles using plant extracts has gained significant attention due to its eco-friendly, cost-effective and sustainable nature. In this research, the Copper oxide nanoparticles (CuO NPs) were successfully synthesised via a green method using *Breynia distichia* leaf extract as a natural reducing and stabilising agent. Phytochemical analysis revealed the presence of flavonoids, phenolics, tannins, alkaloids, saponins, and terpenoids, which contributed to nanoparticle formation. The synthesis was reproducible, with pH values between 6.33–6.76 and consistent dark green colouration. Characterization confirmed the nanoscale formation of CuO: FTIR identified Cu–O bonds and capping biomolecules; XRD indicated a monoclinic tenorite structure (~ 12 nm); UV–Vis showed a surface plasmon resonance peak at 290 nm and a band gap of 4.28 eV; DLS revealed an average particle size of 34.61 nm with low polydispersity (Pdl = 0.218); TGA demonstrated thermal stability up to ~ 250°C; and SEM-EDX confirmed nanosized particles with near 1:1 Cu:O stoichiometry. The nanoparticles displayed notable antioxidant activity, achieving 96.78% DPPH inhibition at 250 µg/mL with an IC₅₀ of 81.2 µg/mL. These findings highlight the potential of *Breynia distichia* for producing bioactive, thermally stable CuO NPs for biomedical and pharmaceutical applications. This work demonstrates a novel, sustainable approach for producing CuO nanoparticles with significant antioxidant potential suitable for biomedical and environmental application.

1. INTRODUCTION

Rapid population growth and the growth of industries and cities have greatly increased the need for chemicals, materials, and energy, which has made pollution and resource depletion worse around the world [1]. Green Chemistry has become an important part of sustainable development in response to this. It encourages the design of chemical processes and products that have less harmful chemicals and less of an effect on the environment [2]. In this context, creating eco-friendly nanomaterials using methods that are low in toxicity and energy-efficient has become a top research goal.

By making it possible to manipulate materials at the nanoscale, where they display special physicochemical characteristics different from their bulk forms, such as increased surface area, altered reactivity, and enhanced interactions with biological systems, nanotechnology has revolutionised the field of material science [3]. However, among various nanomaterials, metal oxide nanoparticles have garnered a lot of interest because of their chemical resistance, versatility, and wide range of applications in the industrial, biomedical, and environmental fields. Because of their notable catalytic and biological properties, small band gap energy, natural abundance, and economic affordability, copper oxide nanoparticles (CuO NPs) are particularly promising candidates. CuO nanoparticles are particularly attractive. Research on CuO NPs has focused extensively on their potential in antimicrobial treatments, sensing technologies, photocatalytic processes, and energy applications, with their enhanced nanoscale reactivity being formally recognized by regulatory bodies including the U.S. Environmental Protection Agency [4, 5]. In addition, CuO NPs exhibit promising antioxidant behavior through reactive oxygen

species (ROS) scavenging and redox cycling between Cu^{2+} and Cu^+ ions, suggesting potential biomedical relevance.

Oxidative stress, caused by excessive ROS generation and insufficient antioxidant defense, plays a crucial role in the development of chronic and degenerative diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions [6]. Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in food and pharmaceutical products, increasing evidence links these compounds to toxicity and possible carcinogenic effects, raising concerns about their long-term safety [7, 8]. Consequently, there is growing interest in identifying safer, natural, and sustainable antioxidant alternatives.

With their enhanced redox activity and high surface-to-volume ratio, nanoparticles offer an efficient platform for antioxidant applications. Because of the synergistic interactions between the nanoparticle core and phytochemicals derived from plants, green-synthesised CuO NPs have shown better antioxidant performance than their chemically synthesised counterparts [9]. However, the majority of documented CuO NP synthesis techniques still rely on physical or chemical methods involving hazardous reagents, high temperatures, and intricate waste management, which restricts their suitability for use in biomedicine and the environment [10, 11]. Furthermore, CuO NPs' antioxidant potential—especially when produced using environmentally friendly methods—remains relatively unexplored despite growing reports on their antimicrobial and photocatalytic qualities.

A practical and sustainable substitute for the production of nanoparticles is plant-mediated green synthesis. Flavonoids, phenolics, tannins, alkaloids, and terpenoids are among the many bioactive substances found in medicinal plants that concurrently function as reducing, stabilising, and capping agents during the formation of nanoparticles [12]. This method produces biocompatible nanoparticles with improved biological functionality and is straightforward, economical, scalable, and safe for the environment.

The medicinal shrub *Breynia disticha* has long been used to treat inflammatory conditions, infections, headaches, and malaria. Strong antioxidant and anti-inflammatory properties have been linked to the presence of flavonoids, phenolics, tannins, saponins, alkaloids, and sulfur-containing glycosides, according to phytochemical studies [13, 14]. *Breynia disticha* has not been thoroughly investigated as a biological resource for the environmentally friendly synthesis of copper oxide nanoparticles, despite its known pharmacological potential. To the best of our knowledge, no thorough investigation has documented the synthesis, characterisation, and antioxidant assessment of CuO NPs made from leaf extract from *Breynia disticha*.

By developing a plant-mediated green synthesis method for CuO nanoparticles using *Breynia disticha* leaf extract as a natural reducing and stabilising agent, this work fills a crucial knowledge gap. Standard analytical methods were used to thoroughly characterize the produced nanoparticles, and in vitro tests were used to assess their antioxidant activity. This research attempts to provide a sustainable, economical, and biologically active substitute for synthetic antioxidants by fusing green chemistry

concepts with nanotechnology. This will help advance environmentally friendly nanomaterials for industrial and biomedical applications.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of *Breynia disticha* leaf extract

The fresh leaves of *Breynia disticha* were collected from Uselu Shell Road, Benin City, Edo State, Nigeria (6°21'48"N and 5°36'50"E). The leaves were taxonomically identified and authenticated by Prof. Akinnibosun Henry Adewale, Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria under the specimen number UBH-B272. The fresh leaves were washed with distilled water, then 50g of the leaves was weighed and ground with an electric blender using 500mL of distilled water. The resulting extract was filtered through a funnel with a white degummed handkerchief and further filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4 °C until further use in carrying out the synthesis of nanoparticles

2.2 Synthesis of CuO nanoparticles

52mL of plant extract was mixed with 5.2g of copper sulfate pentahydrate. The extract changed Color from brown to dark green. The mixture was stirred constantly on a magnetic stirrer at 60°C, and 2.5M sodium hydroxide was added dropwise using a pipette and stirred for three hours at 24°C. The addition of sodium hydroxide changes the color from greenish black to dark green, indicating the formation of CuO NPs and increasing the pH from 4.82 to 6.76. The synthesized CuO NPs solution was centrifuged for 30 min at 4000 rpm, washed with distilled water to remove contaminants, dried in an oven at 100°C for 45 mins, and calcined in a furnace at 500°C for two hours. The green synthesis CuO NPs were subjected to UV-VIS, FT-IR, SEM-EDX, XRD, DLS, TGA and antioxidant analysis.

2.3 Analysis of CuO nanoparticles

The synthesised copper oxide nanoparticles were characterized using a range of modern analytical instruments to determine their structural, chemical, morphological, and thermal properties. The techniques employed include the following:

2.3.1. UV–Visible Spectroscopy

UV–Visible spectrophotometer instrument detects the surface plasmon resonance (SPR) of nanoparticles, which appears as an absorption band in the UV–Vis region. The position of this band gives preliminary information on the successful synthesis of CuO NPs and their optical properties.

2.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy identifies the functional groups in *Breynia disticha* leaf extract responsible for the reduction and capping of CuO NPs. This technique helps to confirm the biomolecules (e.g., phenols,

flavonoids, proteins) that stabilise the nanoparticles and prevent agglomeration, which is crucial in green synthesis.

2.3.3. Thermogravimetric Analysis (TGA)

TGA reveals the thermal stability of CuO NPs. By monitoring weight changes as the nanoparticles were heated, this instrument revealed information about organic residues from the plant extract attached to the nanoparticle surface and the decomposition behaviour of CuO NPs under thermal stress.

2.3.4. Scanning Electron Microscopy (SEM)

SEM provided high-resolution images of the CuO NPs, allowing observation of their surface morphology, shape, and degree of aggregation. This instrument is particularly useful in confirming whether CuO NPs are spherical, rod-like, or irregular in form.

2.3.5. Energy-Dispersive X-ray (EDX) Spectroscopy

The EDX detector attached to the SEM reveals the elemental composition of the nanoparticles. It verified the presence of copper (Cu) and oxygen (O), thereby confirming the formation of CuO NPs and assessing their purity.

2.3.6. Dynamic Light Scattering (DLS)

DLS measures the hydrodynamic particle size distribution and zeta potential of CuO NPs in colloidal suspension. These measurements provide insights into the dispersion quality and stability of CuO NPs in solution, which is essential for their biological and antioxidant applications.

2.3.7. X-ray Diffraction (XRD)

XRD shows the crystalline nature and phase composition of CuO NPs. It works by directing X-rays onto the sample and detecting the diffraction pattern, which corresponds to the arrangement of atoms in the crystal lattice. This is important because the crystal size strongly influences the physical and chemical behaviour of nanoparticles.

2.4 Phytochemical screening of the *Breynia distichia* leaf aqueous extract

Phytochemical screening of the aqueous extract was performed to identify and detect the presence of various secondary metabolites, including flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, eugenols, glycosides, phenolic compounds, and reducing sugars using standard procedures established by Akindele and Adeyemi [15].

2.4.1. Test for Flavonoids

2 ml of the aqueous leaf extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two different portions, A and B, of 5ml each.

To portions A: 10% Lead Acetate solution was added in a few drops. A yellowish precipitate is required for a positive result.

To portions B: 5ml of 20% NaOH and a few drops of dilute HCl were added to the solution. Formation of a colourless solution is required for a positive result.

2.4.2. Test for Alkaloids

Dragendoff's reagent, Wagner's reagent, and Picric acid were used to test for alkaloids.

1ml of the aqueous leaf extract was transferred into three different test tubes labelled A, B, and C.

To test tube A: 2ml of Dragendoff's reagent (made of a mixture of potassium Bismuth Iodide Salt) was added. Reddish-brown precipitate is required for a positive test.

To test tube B: 2ml of Wagner's reagent was added. Reddish-brown precipitate is required for a positive test.

To test tube C: 2ml of Picric acid was added. A yellowish precipitate is indicative of a positive test.

2.4.3. Test for Saponins

0.5g of the extract was mixed with 5ml of water in a test tube and shaken vigorously. It was observed for frothing. A stable, persistent froth is required for a positive test. Saponin rein Weiss (supplied by Merck) was used as a standard.

2.4.4. Test for Tannins

To 2ml of the extract, 10ml of distilled water was added and heated for 5 minutes, and then filtered into halves. To about two drops of the filtrate, ferric chloride (FeCl_3) solution was added. The formation of a bluish precipitate is required for hydrolysable tannin. To about five drops of the filtrate, 2ml of dilute HCl was added and boiled for 5 minutes. Red precipitate is required for a positive test.

2.4.5. Test for Steroids

2ml of acetic anhydride was added to 0.5g of extract in 2ml of dilute sulphuric acid. A colour change from violet to blue or green is indicative of a positive test for steroids.

2.4.6. Test for Terpenoids

This is the Salkowski test. 5 ml of the extract was mixed with 2ml of chloroform and 3ml of conc. H_2SO_4 was carefully added down the side of the inner wall of the test tube to form a layer. A reddish-brown appearance on the interphase indicates a positive test for terpenoids.

2.4.7. Test for Eugenols

2ml of the extract was mixed with 5 ml of 5% KOH solution. The aqueous layer was separated and filtered. A few drops of HCl were added to the filtrate. A pale-yellow precipitate indicates a positive result.

2.4.8. Test for Glycosides

1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1mL of concentrated sulphuric acid. A brown ring obtained indicates the presence of a glycoside.

2.4.9. Test for Phenolic Compounds

1ml of the aqueous extract was added to 5ml of 90% ethanol. In addition, 1 drop of 10% FeCl₃ was added. A pale-yellow colour indicates a positive test.

2.4.10. Test for Reducing Sugars

2ml each of Fehling solution A and B was put in a test tube and boiled for one minute. To the test tube, 2ml of the extract was added, and the mixture was heated in a water bath for five minutes. A brick red precipitate is indicative of a positive test.

2.5 Antioxidant Assay

The antioxidant potential of the synthesised copper oxide nanoparticles (CuO NPs) was evaluated via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with minor adjustments to the method. This method is popular due to its speed, simplicity, and lack of need for complicated equipment, making it suitable for assessing the free-radical scavenging properties of plant extracts and nanomaterials. The assay operates on the principle that antioxidants can convert the deep-violet DPPH radical to the light-yellow diphenylpicrylhydrazine, leading to a quantifiable decrease in absorbance at 518 nm. The procedure was adapted from the method established by Brand-Williams *et al.* [16] to fit the experimental framework.

2.5.1 Preparation of Solutions

For the stock solution, 0.025 g of CuO nanoparticles was weighed and dissolved in distilled water and diluted to a total volume of 100mL, giving a Concentration of 250 µg/mL. From this stock solution of 250 µg/mL, diluted Solutions at concentrations of 200, 150, 100, and 50 µg/mL were subsequently prepared through serial dilution with distilled water.

- i. For the concentration of 200µg/mL, 8mL of the stock solution was measured, and 2mL of distilled water was added to make it up to 10mL.
- ii. For the concentration of 150µg/mL, 6mL of the stock solution was measured, and 4mL of distilled water was added to make it up to 10mL.

- iii. For the concentration of 100µg/mL, 4mL of the stock solution was measured, and 6mL of distilled water was added to make it up to 10mL,
- iv. For the concentration of 50µg/mL, 2mL of the stock solution was measured, and 8mL of distilled water was added to make it up to 10mL.

Ascorbic acid solutions were made at the same concentrations to act as reference standards.

2.5.2 Assay Procedure

For each of the 250, 200, 150, 100, and 50 µg/ml concentrations, 2.0 ml of 0.1mM DPPH solution was mixed with 2.0 ml of the sample solution, maintaining a 1:1 ratio (Figure 2). The same process was applied to the ascorbic acid standards. A control sample was created by combining 2.0 mL of DPPH solution with 2.0 mL of distilled water. All reaction mixtures were kept in a dark environment and incubated at room temperature for 30 minutes to allow for complete reaction.

2.5.3 Measurements

After the 30minutes incubation, the absorbance of each mixture was measured at 518 nm using a UV-Visible spectrophotometer, with methanol as the blank. The percentage inhibition or scavenging activity was calculated using the formula:

$$\text{Inhibition or Scavenging Activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c = absorbance of the control, and A_s = absorbance of the sample or standard solution.

The inhibition percentages were plotted against concentrations to create a dose-response curve, from which the IC_{50} (the concentration needed for 50% inhibition) was determined through interpolation

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemical analysis revealed that *Breynia disticha* possesses various bioactive constituents, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides. These secondary metabolites are known to contribute to the plant's antioxidant, antimicrobial, anti-inflammatory, and analgesic activities. The lack of steroids indicates minimal hormonal influence. Therefore, the phytochemical screening validates the plant's traditional medicinal applications and highlights its potential for further pharmacological investigation.:

Table 1: Phytochemical screening of *Breynia disticha* leaves extracts.

Phytochemicals	Observations
Glycosides	+
Saponins	+
Alkaloids	+
Phenolics	+
Eugenols	+
Steroids	—
Terpenoids	+
Flavonoids	+
Tannins	+
Reducing sugars	+

Key: + = present, - = absent

3.2 FTIR Result of CuO-NPS

FTIR analysis (Figure 3) reveals an important functional group of the CuO NPS that was synthesised using *Breynia distichia* leaves extract. This analysis also helped to know the mechanisms of the synthesis of CuO NPs using *Breynia distichia* leaves aqueous extract.

A broad absorption band was observed at 3421.30 cm^{-1} , which corresponds to the O–H stretching vibrations of hydroxyl groups, indicating the presence of alcohol or phenolic compounds that may have served as stabilising agents. The peak at 1634.33 cm^{-1} is assigned to C=O stretching of amide or aromatic C=C stretching, suggesting the presence of carbonyl-containing biomolecules associated with the nanoparticle surface. The band at 1384.43 cm^{-1} represents C–N stretching vibrations of amines, while the peak at 1097.72 cm^{-1} corresponds to C–O stretching of alcohols or ethers. Importantly, a strong absorption band at 499.67 cm^{-1} is characteristic of Cu–O stretching vibrations, which is clear evidence for the formation of copper oxide nanoparticles. Together, these peaks confirm not only the presence of copper oxide but also the presence of organic molecules on the nanoparticle surface, indicating that the synthesised CuO NPs are capped and stabilised, which is expected in green synthesis methods.

The FTIR results obtained in this study closely agree with findings from previous reports on the green synthesis of CuO nanoparticles. Earlier studies have also shown major peaks around 3400 cm^{-1} , 1630 cm^{-1} , 1100 cm^{-1} , and 500 cm^{-1} , which correspond to O–H, C=O/C=C, C–O, and Cu–O vibrations, respectively [17-19]. This similarity confirms that functional groups from the *Breynia disticha* leaf extract, such as hydroxyl, carbonyl, and amine groups, were involved in reducing and stabilising the

nanoparticles. Therefore, the FTIR pattern of the synthesised CuO NPs supports successful green synthesis, aligning well with previous literature as shown in figure

The results align with studies by Nzilu *et al.*, Baral *et al.*, & Mohamed *et al.* [17-19] confirming successful green synthesis.

3.3 XRD Results

The crystalline structure and phase composition of the synthesised copper oxide nanoparticles (CuO NPs) were investigated using X-ray diffraction (XRD). The diffraction pattern confirmed that monoclinic CuO, known as tenorite, was the predominant phase, accounting for approximately 75% of the total crystalline content. The main diffraction peaks appeared at 2θ values of 34.65° and 37.87° , corresponding to d-spacings of 2.589 Å and 2.376 Å, respectively, which are in good agreement with standard JCPDS reference data for CuO. These observations indicate successful formation of the expected tenorite structure.

In addition to CuO, minor impurity phases were detected, including bunsenite (NiO, ~16.88%), franklinite (ZnFe_2O_4 , ~5.04%), and grossular ($\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$, ~3.12%). These secondary phases likely originated from precursor impurities or incomplete purification during synthesis and calcination. Despite these minor inclusions, CuO remained the dominant phase, demonstrating that the chosen synthesis method was largely effective in producing nanostructured CuO.

The average crystallite size of the CuO nanoparticles was estimated using the Scherrer equation applied to the most intense diffraction peaks, yielding a size range of approximately 12–13 nm. This nanoscale dimension was further supported by the calculated dislocation density of $\sim 6.1 \times 10^{-3} \text{ nm}^{-2}$, suggesting the presence of structural defects typical of nanocrystalline materials. The peak broadening observed in the diffraction pattern reflects both the small crystallite size and possible lattice strain. These nanoscale characteristics are advantageous, as they enhance the surface-to-volume ratio, which can improve the catalytic, electronic, and optical properties of the nanoparticles.

Therefore, the XRD analysis confirms that the synthesised sample predominantly consists of well-crystallised CuO nanoparticles with a tenorite structure and an average crystallite size of ~12 nm. Minor secondary phases were present but could be minimised through optimisation of synthesis parameters, such as the purity of precursors and calcination conditions. These findings indicate that the preparation method is suitable for producing nanoscale CuO with potential applications in catalysis and other nanotechnology-based fields.

The XRD findings of this work are in close agreement with previous studies on green-synthesised CuO nanoparticles. Prominent peaks observed between 35° and 38° confirm the monoclinic tenorite phase, which is consistent with standard CuO diffraction patterns reported in the literature. The estimated crystallite size of approximately 12 nm also aligns with earlier reports ranging from 10 to 25 nm [17, 18]. The few secondary phases detected are comparable to those found in other studies and are likely due to

precursor or calcination variations. The XRD results validate the successful formation of nanocrystalline CuO, in line with established findings from related works.

3.4 UV-Vis Results

The UV–Vis spectrum (200–800 nm) of CuO nanoparticles showed a strong SPR peak at 290 nm, confirming successful nanoparticle formation. Smaller peaks at 212 nm and 240 nm were due to phytochemicals from *Breynia distichia* involved in reduction and stabilisation. A weak band at 670 nm indicated d–d transitions of Cu²⁺ ions, confirming the monoclinic CuO structure. The optical band gap (E_g) was calculated as 4.28 eV ($E_g = 1240/\lambda$) – higher than bulk CuO (1.2–1.9 eV) due to quantum confinement in small nanoparticles. This value agrees with reported ranges of 3.5–4.2 eV [18, 20]. The results suggest good photocatalytic potential and confirm the nanoscale behaviour of the green-synthesised CuO nanoparticles.

3.5 DLS Results

The average particle size (Z-average) of the CuO nanoparticles was 34.61 nm, with a Polydispersity Index (Pdl) of 0.218, indicating good uniformity and stability. The majority of particles (94%) were within 9–15 nm, while only a small fraction formed larger aggregates. The intensity peak occurred at 15.27 nm (89.6%), confirming dominance of small, well-dispersed nanoparticles. The low Pdl (<0.3) signifies a narrow size distribution, suitable for stable colloidal applications. Minor larger particles (up to 4915 nm) likely resulted from biomolecule-induced aggregation during green synthesis. These results agree with previous reports of green-synthesised CuO NPs (10–50 nm, low Pdl) [17, 18]. The synthesised CuO NPs are nanosized, uniform, and stable, demonstrating effective green synthesis and size control.

3.6 TGA RESULTS

The thermal stability of the sample was assessed using Thermogravimetric Analysis (TGA) and Derivative Thermogravimetry (DTG). The TGA curve shown in **Figure 7** below depicts the percentage weight loss of the sample as temperature increases, whereas the DTG curve illustrates the rate of weight loss, allowing the determination of the temperature at which maximum decomposition occurs.

The TGA profile indicated a three-step degradation pattern for the synthesised CuO NPS.

- Stage 1 (30–150 °C): Minor weight loss due to evaporation of adsorbed moisture and volatiles.
- Stage 2 (250–450 °C): Major organic decomposition with a DTG peak at 350–370 °C, representing the main breakdown phase.
- Stage 3 (450–600 °C): Slow degradation of stable organic residues; weight becomes constant above 600 °C.

Residual mass (5–8%) attributed to inorganic oxides or mineral components, confirming good thermal stability up to ~250 °C. This degradation trend is consistent with previous findings on green-synthesised CuO nanoparticles. For instance, Mobarak et al. (2025) reported similar TGA behaviour, with initial

moisture loss below 150 °C, major organic decomposition near 350 °C, and a stable CuO residue above 600 °C. Such an agreement confirms that the synthesised CuO NPs in this study exhibit typical thermal stability and composition comparable to those obtained via plant-mediated green synthesis methods.

3.7 SEM-EDX Results Analysis

The EDX spectrum revealed strong peaks for Copper (Cu) and Oxygen (O), verifying that CuO is the main constituent. Quantitative data showed Cu (75.52 wt%, 54.80 at%) and O (24.48 wt%, 45.20 at%), giving a Cu:O atomic ratio $\approx$ 1:1, which matches the theoretical stoichiometry of CuO. The SEM micrographs (8000 $\times$ –9000 $\times$) showed irregularly shaped nanoparticles aggregated into clusters due to their high surface energy. The individual nanoparticles are nanosized, while clusters extend into the micrometre range, exhibiting a rough and uneven texture that increases surface area. These morphological and compositional features confirm successful green synthesis, with high purity, nanoscale formation, and good surface characteristics ideal for catalytic, sensing, and antimicrobial applications. The SEM–EDX analysis confirms the formation of pure CuO nanoparticles with the correct Cu:O ratio (1:1), uniform nanoscale morphology, and functional surface properties. These results align with the findings of Khan *et al.* (2023) [22], supporting the successful green synthesis of CuO NPs using *Breynia disticha* extract.

3.8 Antioxidant Potential Assay Results

The table below shows the percentage inhibition of DPPH radicals by CuO nanoparticles and the percentage inhibition of ascorbic acid at different concentrations.

The antioxidant activity of the green-synthesised CuO nanoparticles was evaluated using the DPPH radical scavenging method and compared with ascorbic acid as a standard. The results showed a clear dose-dependent increase in radical scavenging activity with increasing nanoparticle concentration. The CuO nanoparticles exhibited an IC₅₀ value of 81.2 µg/mL, while ascorbic acid showed a lower IC₅₀ of 40.4 µg/mL, indicating higher antioxidant efficiency for the standard. Although the CuO NPs displayed moderate antioxidant efficiency compared to ascorbic acid, they still demonstrated significant free-radical scavenging capacity, likely due to the phytochemicals from *Breynia disticha* acting as capping and stabilising agents during synthesis. These findings confirm that the synthesised CuO NPs possess meaningful antioxidant potential and suitability for biomedical and catalytic applications.

4.0 CONCLUSION

This study demonstrated the successful green synthesis of copper oxide nanoparticles (CuO NPs) using *Breynia disticha* leaf extract as a natural reducing and stabilizing agent. Characterization analyses (UV–Vis, FTIR, XRD, TGA, SEM-EDX, and DLS) confirmed the formation of stable, nanosized CuO particles. The synthesized nanoparticles showed notable antioxidant activity, slightly lower than that of ascorbic acid, indicating their effective free radical scavenging potential. Finally, *Breynia disticha* proved to be a sustainable source for CuO NP synthesis, suggesting potential applications in pharmaceutical, biomedical, and catalytic fields.

Declarations

Acknowledgement

The authors are grateful to the staff of the Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City, Nigeria. They also appreciate Mrs Deborah Oghomwen Unoko and Dr Bukola Ovoranmwun for providing financial support and guidance during the research.

Funding

The authors received no financial or organizational support for the research submitted in this publication.

Data Availability Statement

All data generated and analyzed during this study are included in this article.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this publication.

Consent to publish

Not applicable.

The Authors' Declaration

The research work is original and has not been published previously, non under consideration for publication elsewhere and does not infringe any copyright or other rights of others.

Plant Guidelines:

The scientific name of the plant is ***Breynia Disticha*** and the local/common name is Snowbush. To ensure responsible collection, documentation and publication, the plant leaves of ***Breynia Disticha*** were collected from the authors' garden along Uselu Shell Road, Benin City, Edo State, Nigeria (6°21'48"N and 5°36'50"E). Following proper identification, a voucher specimen of the plant was prepared and deposited in the departmental herbarium of Plant Biology and Biotechnology (PBB) of the University of Benin, Benin City, Edo State, Nigeria under the specimen number UBH-B272.

Permission to collect the Plant Part:

Not applicable.

Ethic and Consent to Participate Declaration

Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

To improve the language and readability, the authors used CHATGPT during the research. After using this tool/service, they also reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

1. Kurul, F., Doruk, B. & Topkaya, S.N. (2025). Principles of green chemistry: building a sustainable future. *Discov. Chem.* 2, 68. <https://doi.org/10.1007/s44371-025-00152-9>
2. Ratti, R. (2020). Industrial applications of green chemistry: status, challenges and prospects, *SN applied sciences*. <https://DOI.org/10.1007/s42452-020-2019-6>
3. Beliyani, N. 2020, *Nanochemistry and technology*, random publications. New Delhi (India).
4. Arendsen, L. P., Thakar, R., & Sultan, A. H. (2019). The use of copper as an antimicrobial agent in health care, including obstetrics and gynecology. *Clin Microbiol Rev* 32(4):e00125–e00118. <https://doi.org/10.1128/CMR.00125-18>
5. Munanader, N., Kasim, S., Arfah, R., Basir, D. N., Hala Y., Zakir M., & Natsir H. (2022). Green synthesis of copper oxide nanoparticles using *Anredera cordifolia* leaf extract and their antioxidant activity. *Communications in science and technology* 7(2)(2022)127-134, <https://doi.org/10.21924/CST.7.2.2022.1004>.
6. Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., ... & Abete, P. (2020). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 13, 757–772. <https://doi.org/10.2147/CIA.S158513>
7. Sharma, A., Sharma, P., & Singh, J. (2021). Natural antioxidants and their therapeutic potential: A review. *Future Journal of Pharmaceutical Sciences*, 7, 76. DOI:10.1186/s43094-021-00226-7. Publisher/article page Springer Open: <https://fjps.springeropen.com/articles/10.1186/s43094-021-00226-7>.
8. Ribeiro, J. S., Santos, M. J. M. C., Silva, L. K. R., Oliveira, S. B. S., & Costa, J. R. (2022). Natural and synthetic antioxidants used in the food industry: A review. *Journal of Functional Foods*, 95, 105110. <https://doi.org/10.1016/j.jff.2022.105110>
9. Bhattacharya, S., Das, S., & Banerjee, A. (2022). Copper oxide nanoparticles: Green synthesis and biomedical applications. *Materials Today: Proceedings*, 62, 3092–3098. <https://doi.org/10.1016/j.matpr.2022.03.211>
10. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2020). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7(1), 17–28. <https://doi.org/10.1016/j.jare.2015.02.007>
11. Rai, M., Deshmukh, S. D., Ingle, A. P., Gupta, I., & Gade, A. (2021). Green synthesis of metallic nanoparticles: Applications and limitations. *Journal of Applied Research and Technology*, 19(1), 3–13. <https://doi.org/10.1016/j.jare.2021.03.002>

12. Agarwal, H., Menon, S., Kumar, S. V., & Rajeshkumar, S. (2020). Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. *Chemico-Biological Interactions*, 311, 108775. <https://doi.org/10.1016/j.cbi.2019.108775>
13. Saadullah, M., Rehman, T., Khan, M., Mehmood, A., & Ahmed, N. (2022). Phytochemistry and pharmacological properties of the genus *Breynia*: A review. *Heliyon*, 8(7), e09366. DOI: <https://www.sciencedirect.com/science/article/pii/S2405844022005976>
14. Nakashima, K. I., Abe, N., Oyama, M., Murata, H., & Inoue, M. (2023). Sulfur-containing spiroketals from *Breynia disticha* and evaluations of their anti-inflammatory effect. *Beilstein Journal of Organic Chemistry*, 19, 1604–1614. <https://doi.org/10.3762/bjoc.19.117>
15. Akindele, A. J., & Adeyemi, O. O. (2007). Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. *Fitoterapia* 78(1), 25-28 <https://doi.org/10.1016/j.fitote.2006.09.002>
16. Band-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT Food Science and Technology*, 28(1) 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
17. Nzilu, D.M., Madivoli, E.S., Makhanu, D.S., Wanakai, S.I., Kiprono, G. K., & Kareru, P. G. (2023). Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. *Sci Rep* 13, 14030. <https://doi.org/10.1038/s41598-023-41119-z>
18. Baral, J., Pokharel, N., Dhungana, S., Tiwari, L., Khadka, D., Pokhrel, M. R., & Poudel, B. R. (2025). Green Synthesis of Copper Oxide Nanoparticles Using *Mentha* (Mint) Leaves: Characterization and Its Antimicrobial Properties with Phytochemicals Screening. *Journal of Nepal Chemical Society*, 45(1), 111–121. <https://doi.org/10.3126/jncs.v45i1.74491>.
19. Mohamed E. A. (2020). Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. *Heliyon*, 6(1), e03123. <https://doi.org/10.1016/j.heliyon.2019.e03123>
20. Vinothkumar, P., Manoharan, C., Shanmugapriya, B., et al. (2019). Effect of reaction time on structural, morphological, optical and photocatalytic properties of copper oxide (CuO) nanostructures. *Journal of Materials Science: Materials in Electronics*, 30, 6249–6262. <https://doi.org/10.1007/s10854-019-00928-7>
21. Mobarak, M. B., Mashrafi Bin Mobarak, Sikder, M. F., Muntaha, K. S., Islam, S., Fazle Rabbi, S. M., & Chowdhury, F. (2025). Plant extract-mediated green synthesis of copper oxide nanoparticles: A review of synthesis, characterization and applications. *Nanoscale Advances*. <https://doi.org/10.1039/D5NA00035A>
22. Khan, S. A., Zafar, A., Almarhoon, Z., & Alam, M. M. (2023). Green synthesis of copper oxide nanoparticles using *Azadirachta indica* leaf extract: Characterization and biological applications. *Materials Today: Sustainability*, 23, 100370. <https://doi.org/10.1016/j.mtsust.2023.100370>

Figures

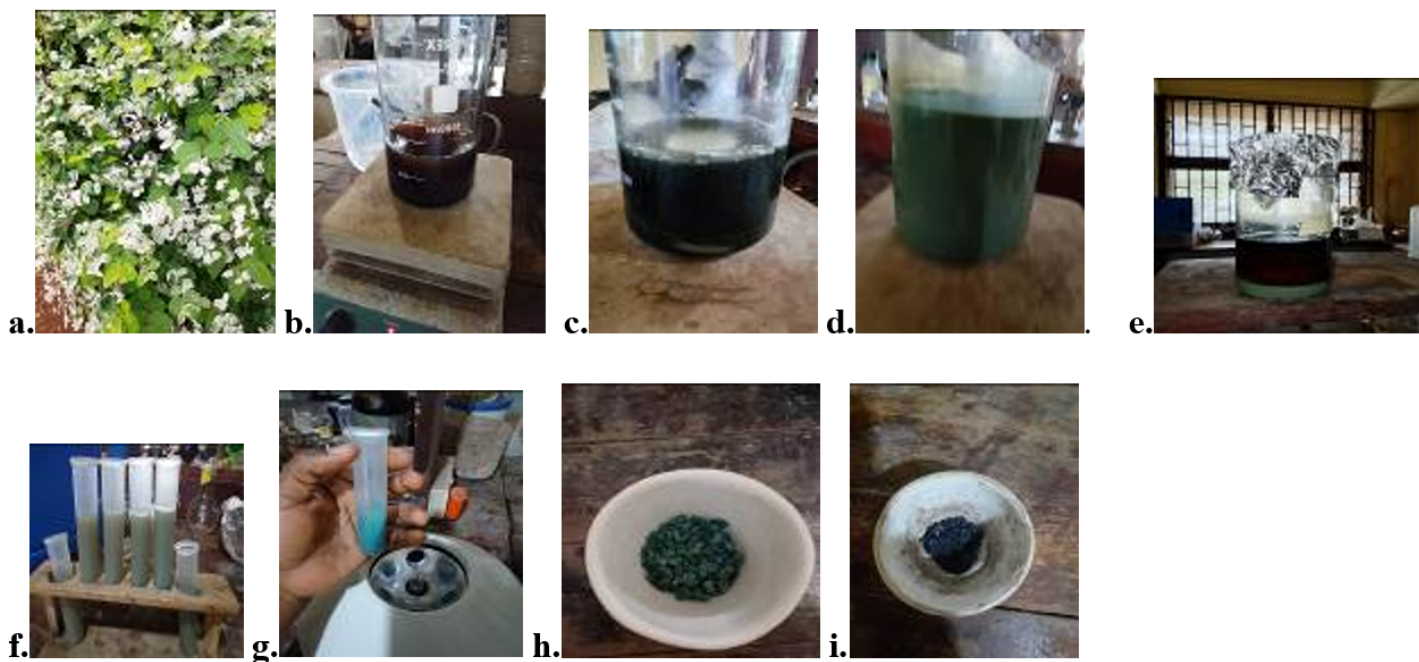


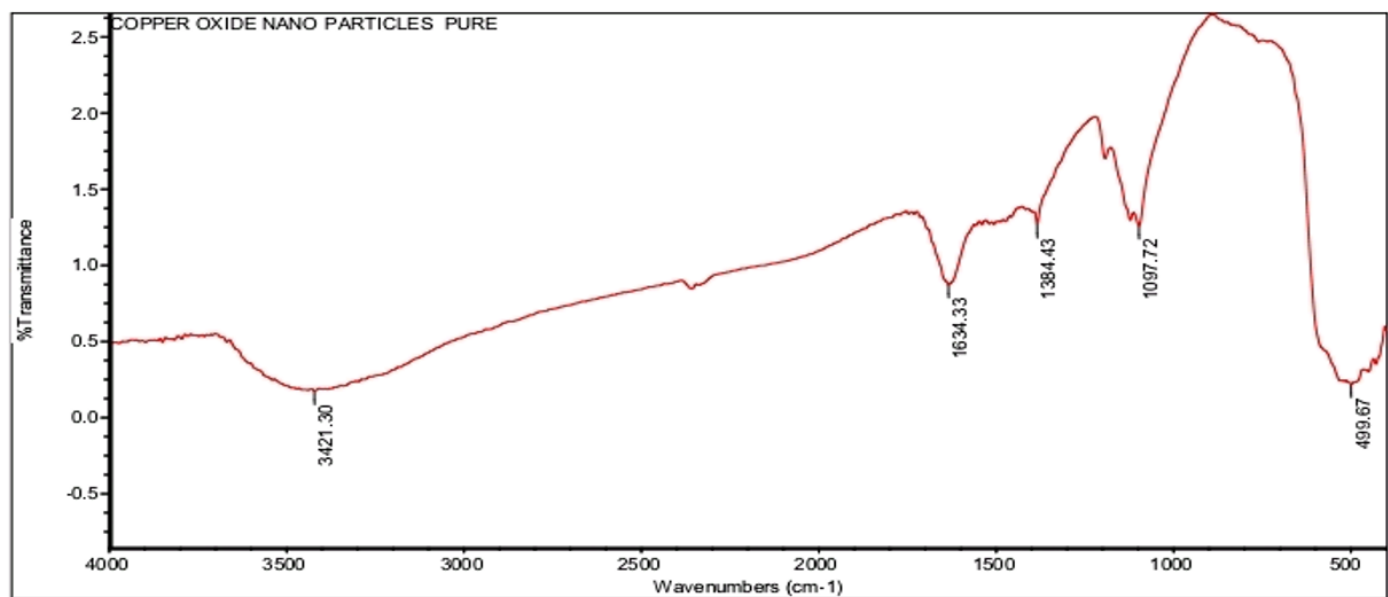
Figure 1

Scheme of synthesis of CuO Nanoparticles (a – i)



Figure 2

sample bottles containing sample solutions for the antioxidant assay



Mon Aug 25 10:11:36 2025 (GMT+01:00)
 FIND PEAKS:
 Spectrum: COPPER OXIDE NANO PARTICLES PURE
 Region: 4000.00 400.00
 Absolute threshold: 1.413
 Sensitivity: 50
 Peak list:

Position	Intensity
499.67	0.216
1097.72	1.259
1384.43	1.270
1634.33	0.871
3421.30	0.174

Figure 3

FTIR Result shows the FTIR spectrum of CuO NPs samples that were synthesised

CuO NPS_20250904_153404_G02_S02_M02-Evaluation report (CuO NPS_20250904_153404_G02_S02_M02)

General information

Analysis date	2025-09-06 12:19:34	Measurement start time	2025-09-04 15:38:59
Analyst	Default	Operator	Default
Sample name	CuO NPS	Comment	
Measured data name	C:\WallPaper\04-09-2025\CuO NPS_20250904_153404_G02_...	Memo	

Peak list

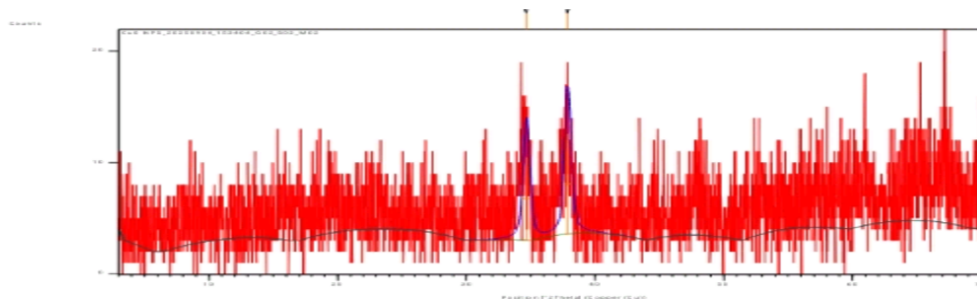
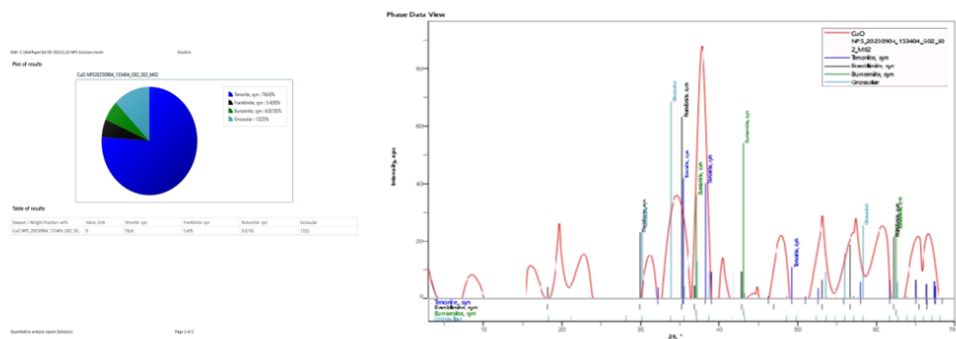
No.	2θ, °	d, Å	Height, cps	FWHM, °	Int. I, cps	Int. W, °	Asymmetry	Decay(η)/mL	Decay(η)/mH	Size, Å
1	34.55(12)	2.594(9)	79(15)	0.80(9)	67(12)	0.9(3)	1.6(9)	0.0(6)	0.0(9)	108(12)
2	37.85(10)	2.375(6)	87(19)	0.86(10)	89(13)	1.0(4)	1.9(10)	0.5(5)	0.0(8)	102(11)
3	60.99(16)	1.518(4)	21(10)	2.5(10)	100(22)	5(3)	5(7)	1.5(4)	0.0(19)	39(16)

No.	2θ, °	Phase Name	Chemical Formula	Card No	Norm. I	Profile Type	Distributi...	Degree of Orientation
1	34.55(12)	Tenonite, syn 1 1 -1 (Franklinite,...	Cu ₂ O,Zn Fe ₂ O ₄ ,Ca...	01-089-2531,01-08...	67.14	Split pseudo-Voigt	-	-
2	37.85(10)	Tenonite, syn 1 1 1 (Burnsenite,...	Cu ₂ O,Zn O,Ca ₂ 96...	01-089-2531,01-07...	88.26	Split pseudo-Voigt	-	-
3	60.99(16)	Tenonite, syn 1 1 -3 (Franklinite,...	Cu ₂ O,Zn Fe ₂ O ₄	01-089-2531,01-08...	100.00	Split pseudo-Voigt	-	-

No.	2θ, °	Ring Factor	β Cluster
1	34.55(12)	-	-
2	37.85(10)	-	-
3	60.99(16)	-	-

Qualitative Analysis Results

Phase name	Formula	Figure of merit	Phase ref. detail	Space Group	DB Card Number
Tenonite, syn	Cu ₂ O	1.001	S/M/POF-4 Minerals 2025)	15 : C 12/c 1	01-089-2531
Franklinite, syn	Zn Fe ₂ O ₄	0.906	S/M/POF-4 Minerals 2025)	227 : Fd -3m:2	01-089-4926
Burnsenite, syn	Ni O	0.984	S/M/POF-4 Minerals 2025)	225 : Fm -3m	01-071-4750
Grossular	Ca ₂ 96 Mn _{0.04} Fe _{0.05} Al _{1.95} ...	1.175	S/M/POF-4 Minerals 2025)	230 : Ia -3d	04-008-8411



Peak List: (Bookmark 3)

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
34.6474	7.44	0.6298	2.58903	82.76
37.8677	8.99	0.6298	2.37594	100.00

Figure 4

XRD Results show the XRD patterns of CuO NPs samples that were synthesised

TEST SETUP
GENESYS 10S v1.200 2L7J355002

Scanning	4:51pm 5Sep25
Test Name	Sample CUO NPS[Saved]
Measurement Mode	Absorbance
Start Wavelength	200nm
Stop Wavelength	800nm
Sample Positioner	1-Cell Platform
Scan Speed	Fast
ID# (0=OFF)	1
Auto Print	Off

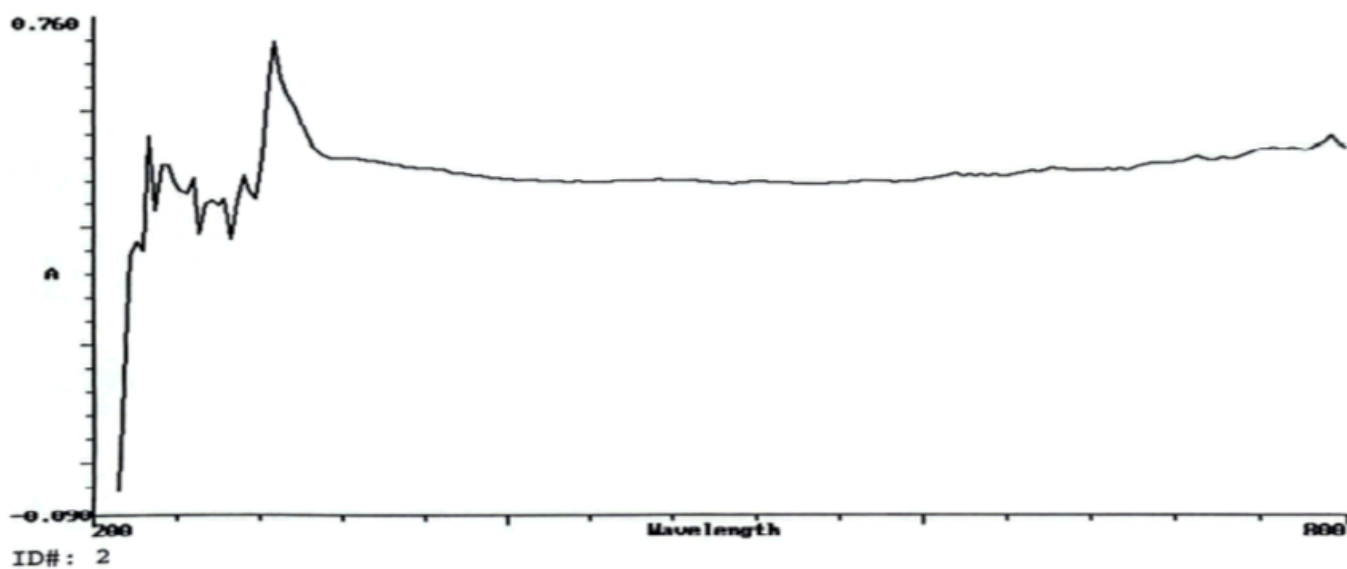


Figure 5

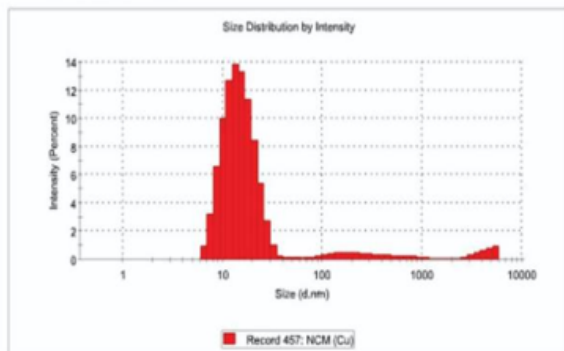
UV-Vis Results show the Peaks of CuO NPs samples that were synthesised

System

Temperature (°C): 25.0 Duration Used (s): 70
 Count Rate (kcps): 209.9 Measurement Position (mm): 4.65
 Cell Description: Disposable sizing cuvette Attenuator: 7

Results

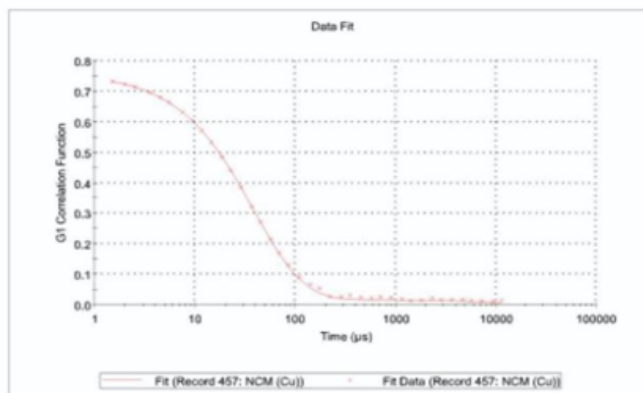
Size (d.nm) % Intensity St Dev (d.nm)
Z-Average (d.nm): 34.61 Peak 1: 15.27 89.8 5.605
Pd: 0.218 Peak 2: 345.5 6.7 318.7
 Intercept: 0.757 Peak 3: 4304 3.5 1055
 Result quality **Good**



System

Temperature (°C): 25.0 Duration Used (s): 70
 Count Rate (kcps): 209.9 Measurement Position (mm): 4.65
 Derived Count Rate (kcps): 16640.2 Attenuator: 7
 Cell Description: Disposable sizing cuvette

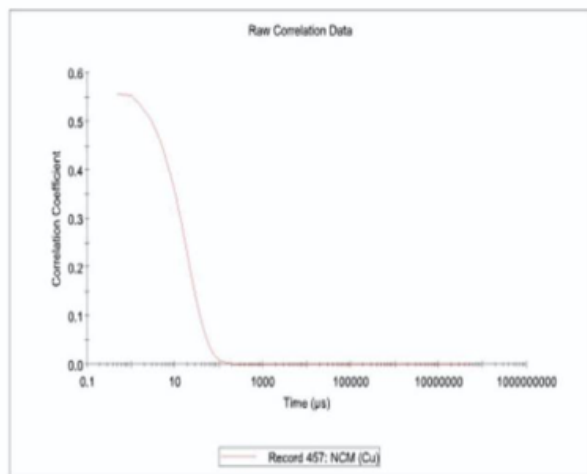
Results



System

Temperature (°C): 25.0 Duration Used (s): 70
 Count Rate (kcps): 209.9 Measurement Position (mm): 4.65
 Cell Description: Disposable sizing cuvette Attenuator: 7

Results



System

Temperature (°C): 25.0 Duration Used (s): 70
 Count Rate (kcps): 209.9 Measurement Position (mm): 4.65
 Cell Description: Disposable sizing cuvette Attenuator: 7

Results

Size (d.nm) % Volume St Dev (d.nm)
Z-Average (d.nm): 34.61 Peak 1: 11.59 94.9 6.095
Pd: 0.218 Peak 2: 261.6 2.8 217.0
 Intercept: 0.757 Peak 3: 4915 1.6 1011
 Result quality **Good**

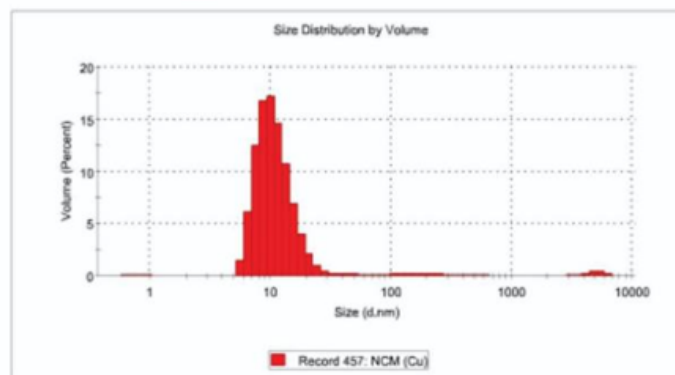


Figure 6

DLS Results show the average particle size of CuO NPs samples that were synthesised

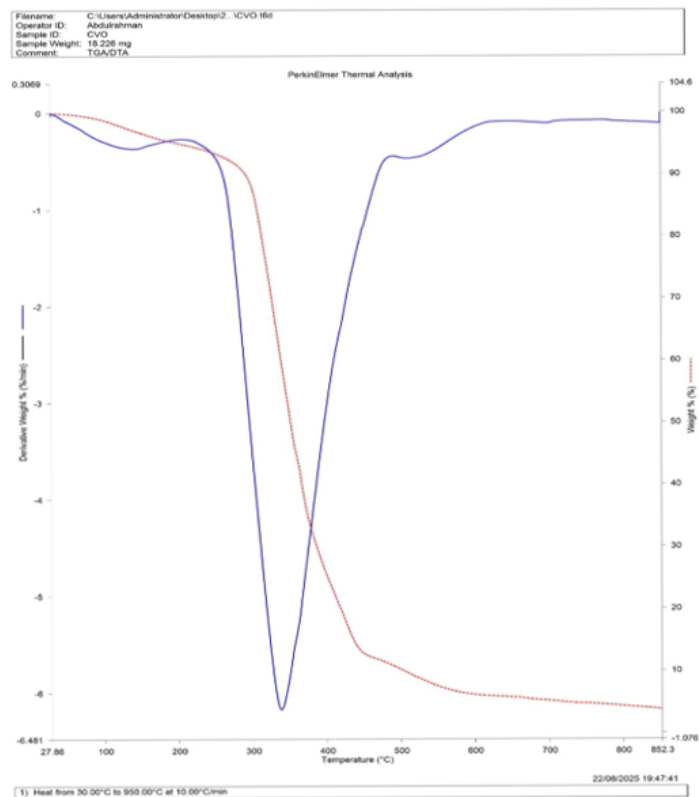
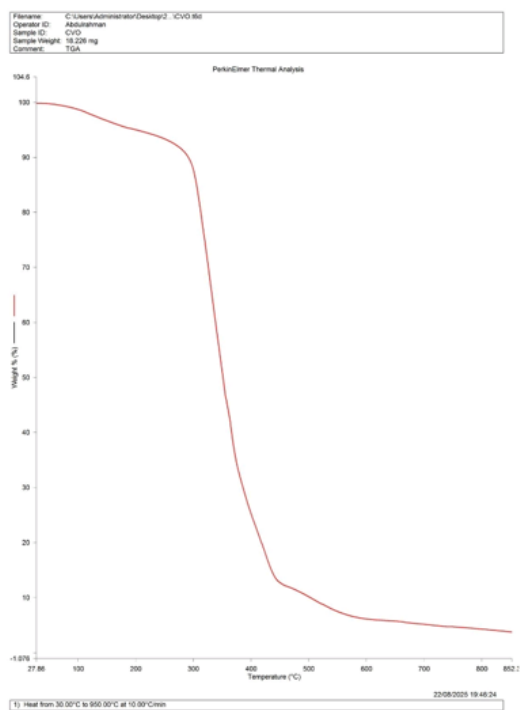


Figure 7

TGA Results show the thermal stability patterns of CuO NPs samples that were synthesised

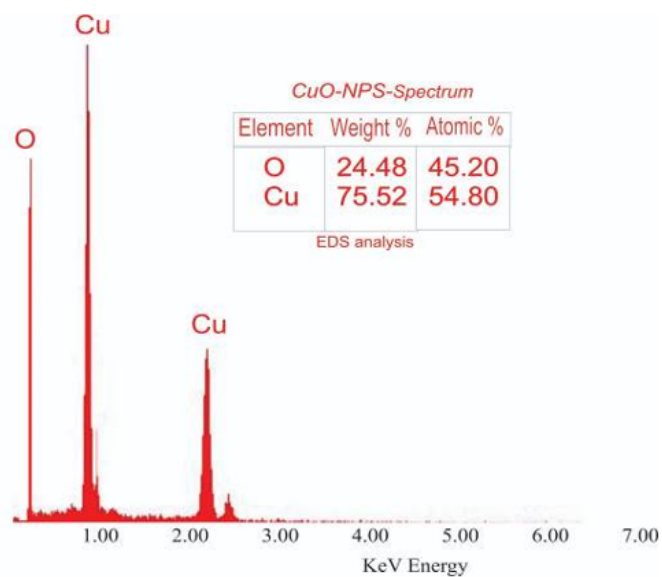
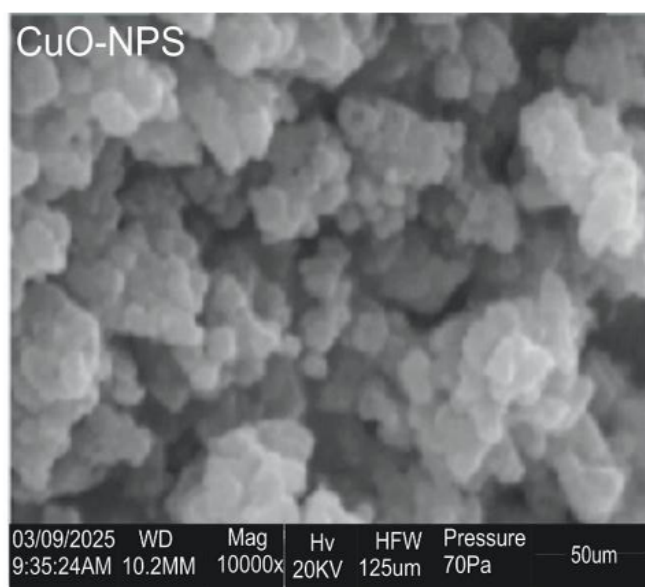
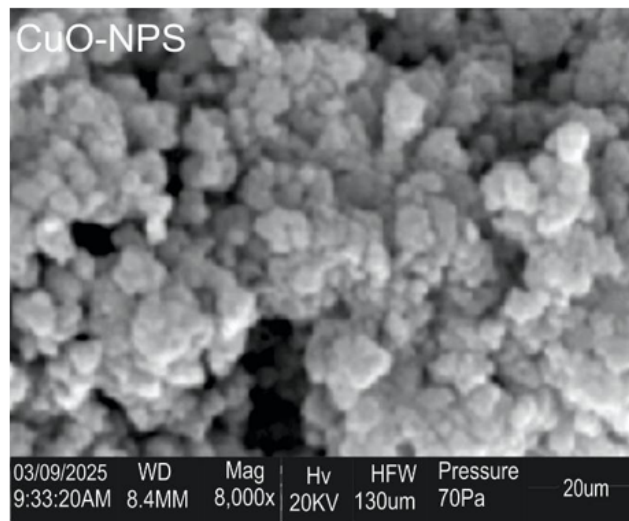
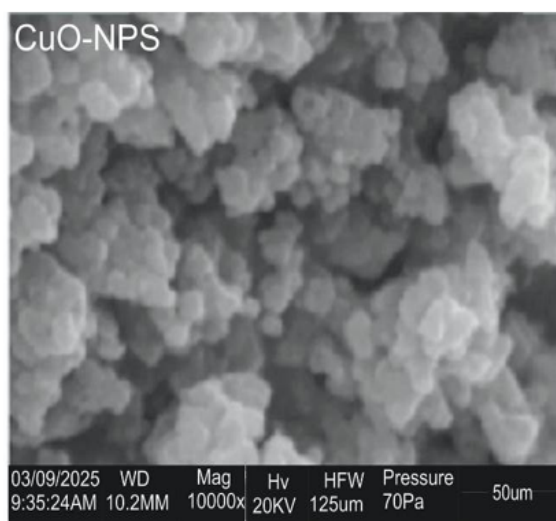


Figure 8

SEM-EDX Results show the morphology and peaks of CuO NPs samples that were synthesised

Concentrations ($\mu\text{g/mL}$)	Absorbance (Control)	Absorbance (Samples)	Absorbance (Ascorbic acid)	Inhibition or scavenging activity (%) (CuO NPs)	Inhibition or scavenging activity (%) (Ascorbic acid)
50	0.807	0.441	0.392	45.353	51.425
100	0.807	0.381	0.332	52.788	58.851
150	0.807	0.296	0.254	63.321	68.525
200	0.807	0.199	0.149	75.341	81.537
250	0.807	0.026	0.020	96.778	97.522

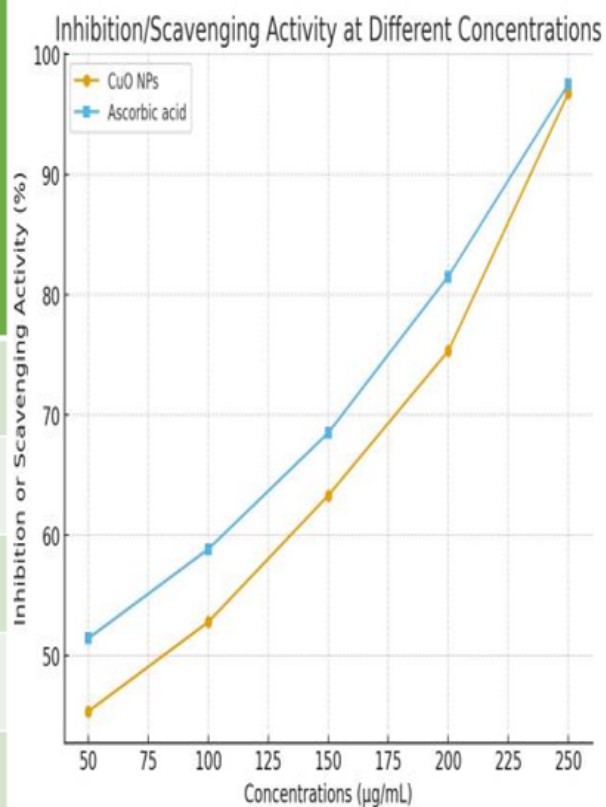


Figure 9

Antioxidant assay shows the percentage inhibition curve of CuO NPs samples that were synthesised.