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Ecofriendly synthesis and characterization of CuO nanoparticles from *Blighia sapida* pods for antimicrobial applications

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Abstract

The increasing interest in green nanotechnology has driven research into environmentally sustainable methods for nanoparticles synthesis. In this study, *Blighia sapida* (ackee) seed pod extract was employed as a bio-reductant and stabilizing agent for the green synthesis of copper oxide (CuO) nanoparticles (NPs). The biosynthesis was indicated by a colour change during reaction and confirmed through multiple characterization techniques including UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), dynamic light scattering (DLS), and transmission electron microscopy (TEM). The nanoparticles exhibited strong absorbance at 333 nm. FTIR analysis confirmed the presence of functional groups such as O–H, C=O, C–O–C, and Cu–O bonds. XRD and TEM analyses revealed monoclinic CuO nanoparticles with crystallite sizes of 6.21 nm and 3.51 nm, respectively, while DLS revealed a hydrodynamic size of 57.38 nm with a PDI of 0.471, indicating moderate polydispersity. The biosynthesized CuO nanoparticles displayed significant antibacterial activity against both Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus penneri*) and Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) bacteria. Notably, Gram-negative strains showed greater susceptibility, attributed to their thinner and more permeable cell walls. This study demonstrates the viability of using *B. sapida* seed pods—an agricultural waste product—for eco-friendly CuO nanoparticles synthesis, highlighting its potential for sustainable antimicrobial applications.

Keywords Copper oxide, *Blighia sapida*, Nanoparticles, Characterization, Antibacterial

1 Introduction

The emergence of nanotechnology has attracted significant global attention due to its broad engagement in material synthesis, wide-ranging applications, and concerns regarding safety. The advent of nanotechnology as a transformative scientific advancement has introduced a new era of scientific exploration and technological innovation, fundamentally reshaping how researchers design, manipulate, and utilize nanoscale materials. This rapidly evolving field continues to generate interest across disciplines



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because of its extensive and diverse applications. Nanotechnology finds relevance in numerous sectors, including biomedicine, engineering, environmental science, pharmaceuticals, space exploration, cosmetics, agriculture, food processing, construction, and electronics [1–3]. The exceptional importance of nanomaterials lies in their unique physicochemical properties, which are largely attributed to their ultra-small size and extraordinarily high surface-area-to-volume ratio. These characteristics enhance their mobility, reactivity, and interaction with the environments. Materials at the nanoscale exhibit size-dependent behaviours, such as modified melting points, solubility, and mechanical properties, setting them apart from their bulk counterparts [4]. Furthermore, these features contribute to improved biodiversity, reactivity, optical, electronic, magnetic, and thermal performances [5], indicating the critical role of nanomaterials in driving innovation and enabling advanced solutions across a wide range of scientific and industrial fields.

Among nanomaterials, inorganic nanoparticles, particularly metals and metal oxides, have attracted considerable attention due to their chemical and thermal stability, structural robustness, and functional versatility [5–7]. Their high surface reactivity facilitates efficient interactions with biological and chemical systems, making them valuable for applications in catalysis, sensing, environmental remediation, and biomedical technologies [8]. The tunability of their size, morphology, and surface chemistry further enables surface functionalization, enhancing biocompatibility and application-specific performance.

Metal and metal oxide nanoparticles such as Ag, ZnO, Cu/CuO, and TiO₂ are widely recognized for their potent antimicrobial activities. Their effectiveness is attributed to mechanisms including membrane disruption, generation of reactive oxygen species (ROS), and interference with intracellular processes [9]. Consequently, these nanomaterials have been incorporated into medical devices, food packaging, textiles, surface coatings, and water treatment systems to combat microbial contamination and antimicrobial resistance [10].

In recent years, copper oxide nanoparticles (CuO NPs) have emerged as promising metal-based nanoparticles antimicrobial agents owing to their strong biocidal activity, chemical stability, and cost-effectiveness. Compared to noble-metal nanoparticles such as silver, CuO NPs offer a scalable and economically viable alternative for large-scale antimicrobial applications [11, 12]. Beyond antimicrobial uses, CuO nanoparticles have found applications in catalysis, energy storage, sensors, and agricultural systems [13]. The antimicrobial properties of CuO NPs have been reported against *Escherichia coli* [14], *Pseudomonas aeruginosa* [15], *Enterococcus faecalis* [16], *Klebsiella pneumonia* [17], *Staphylococcus aureus* [18], and *Bacillus subtilis* [19].

CuO nanoparticles can be synthesized via physical, chemical, and biological methods [20, 21]. However, conventional physical and chemical approaches often involve high energy consumption, complex procedures, and the use of hazardous chemicals, raising environmental and sustainability concerns. These limitations have driven increasing interest in green synthesis routes, particularly plant-mediated methods, which offer simple, cost-effective, and environmentally benign alternatives. Plant extracts act as both reducing and stabilizing agents, eliminating the need for toxic reagents. Recently, CuO NPs have been synthesized using extract of plant materials such as the use of *Mussaenda frondosa* L. [22], *Moringa oleifera* [18], *Eucalyptus Globoulus* [23], *Aerva javanica* [24],

Aloe vera [25], *Psidium guajava* [26], *Carica papaya* [27], *Camellia sinensis* [28], *Allium sativum* [29], *Punica granatum* [30], and *Aegle marmelos* [31]. The utilization of biological moieties in plant as bio-reductant and stabilizing agent in nanoparticles synthesis serves as effective and alternative method to existing conventional method. In this regard, numerous plants have been investigated for the synthesis of copper oxide nanoparticles successfully.

Blighia sapida (ackee), a tropical tree native to West Africa, is widely used in traditional medicine due to its antimicrobial, anti-inflammatory, and antioxidant properties, attributed to bioactive compounds such as flavonoids, alkaloids, and saponins [32]. The arils of the ackee fruit are rich in essential fatty acids, protein, and various micronutrients [33]. The arils may be consumed fresh as a vegetable, roasted, and eaten as such, or used in soups as a meat substitute. In traditional African and Caribbean medicines, different parts of the plant including the leaves, bark, and seeds have been used to treat a wide range of ailments, such as fever, skin infections, parasitic diseases, dental decay, malaria, internal haemorrhage, dysentery, burns, eye inflammation, yellow fever, constipation, cutaneous infections, whitlow, and head lice [34, 35]. While the edible arils are nutritionally valuable, the fruit pod is commonly discarded as agricultural waste, and its potential scientific and technological applications remain largely unexplored. Harnessing this underutilized biomass for nanoparticle synthesis aligns with principles of waste valorization and sustainable nanotechnology.

Therefore, this study aims to investigate the use of *Blighia sapida* pod as a green and sustainable precursor for the biosynthesis of CuO nanoparticles. The goal is to synthesize CuO nanoparticle through an environmentally friendly and cost-effective route and evaluate their antimicrobial potencies against six bacterial strains, comprising four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus penneri*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*).

2 Materials and methods

2.1 Materials collection

Anhydrous copper (II) sulphate (CuSO_4 , 95% purity), sodium hydroxide (NaOH , 98.5%), and ethanol ($\text{C}_2\text{H}_5\text{OH}$, 99.8%) were procured from MolyChem, Nigeria. All chemicals used were of analytical reagent grade and used without further purification. Double-distilled water was used in the preparation of plant extract and all metal salt solutions. Fresh seed pods of *Blighia sapida* were harvested from a farmland located in the Ajipon area (7.6104°N, 5.2572°E), Oke-Ureje, Ado-Ekiti, Nigeria. The plant grows naturally at the location, and no formal approval was required for its use. However, it was authenticated by Dr. Jimoh Abubakar, Department of Agricultural Science, Federal Polytechnic, Ado-Ekiti, Nigeria. The plant material was transported to the Chemistry Laboratory in a clean polyethylene bag, separated from extraneous materials, and washed thoroughly under running tap water. The cleaned samples were shade-dried for 20 days, after which they were ground and sieved to obtain fine powder of 100-mesh particle size. The resulting powder was stored in an airtight container at room temperature for subsequent use.

2.2 Plant extract preparation

The *Blighia sapida* pod extract was prepared according to the procedure described by Abegunde et al. [9]. This was carried out by boiling 10 g of the dried pod powder in 100 mL of distilled water at 60 °C for 30 min, with continuous stirring on a magnetic stirrer at 200 rpm. After the heating period, the mixture was allowed to cool to room temperature, filtered using Whatman filter paper, and filtrate subsequently stored at 4 °C prior to use in the biosynthesis process.

2.3 Biosynthesis of CuO nanoparticles

The CuO nanoparticle was prepared using modified methods reported by Kalaivani & Mathubala [36] and Sackey et al. [37]. The biosynthesis of CuO nanoparticles was carried out by boiling a mixture of 80 ml 0.1 M CuSO_4 solution and 20 ml *Blighia sapida* seed pod extract at 60 °C with continuous stirring for 2 h. A notable colour change from blue to green, followed by the appearance of a brownish-black precipitate indicated the successful formation of CuO nanoparticles. The resulting mixture was centrifuged at 4000 rpm for 10 min, filtered, and the precipitate was washed several times with double-distilled water and ethanol to remove impurities. The purified product was then dried in a hot air oven at 60 °C for 24 h to yield a black solid residue. This residue was subsequently calcined in a muffle furnace at 300 °C for 3 h to obtain CuO nanoparticle powder, which was stored in an airtight container for further use. The flow chat for the biosynthesis is presented in Fig. 1.

2.4 Materials characterization

The optical properties of synthesized CuO nanoparticles was examined using UV–Visible spectroscopy (UV-1800 series, Shimadzu, Japan). Fourier-transform infrared spectroscopy was conducted to identify the functional groups present in the nanoparticles, with spectra recorded in the range of 4000–400 cm^{-1} . The crystalline structure and phase purity was analyzed by using X-ray diffraction with Cu-K α radiation ($\lambda = 1.54 \text{ \AA}$) at ambient temperature, engaging a continuous scanning mode over a 2θ range of 20° to 80° [38]. The surface morphology of CuO nanoparticles was analyzed by using scanning electron microscopy, and elemental composition was confirmed through energy-dispersive X-ray

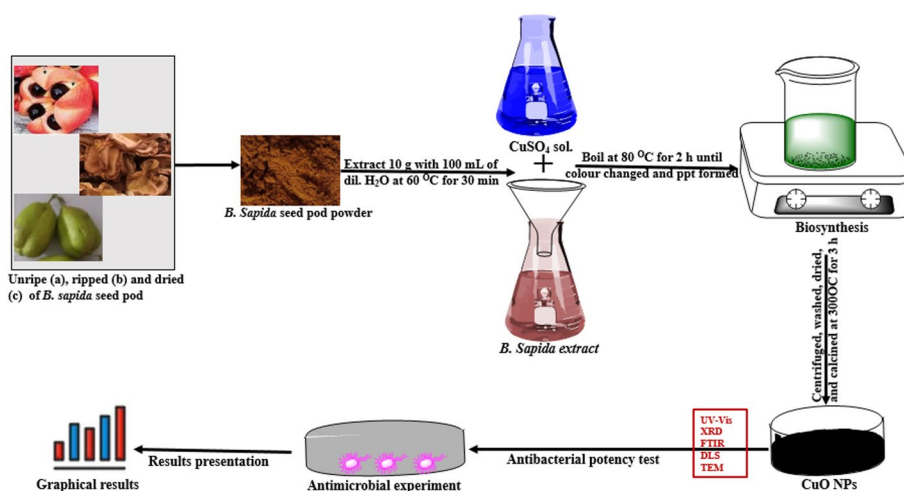


Fig. 1 Green synthesis of CuO nanoparticles using *Blighia sapida* pod extract

spectroscopy. The morphology and particle size distribution of CuO nanoparticles was evaluated using transmission electron microscopy (TECNAI F-30 HRTEM) operated at an accelerating voltage of 200 kV [16]. Furthermore, Dynamic light scattering measurements were performed using a Malvern Zetasizer Nano ZS series to determine the average hydrodynamic diameter and polydispersity index (PDI) of the CuO nanoparticles [22].

Average Crystallite Size

The average crystal size of the copper oxide crystallites was calculated using the Scherrer equation (Eq. 1) [11].

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where D represents the particle diameter, K is the shape factor (0.9), λ corresponds to the X-ray wavelength (1.5419 Å) used, β is the FWHM (full width at half maximum), and θ represents the Bragg angle.

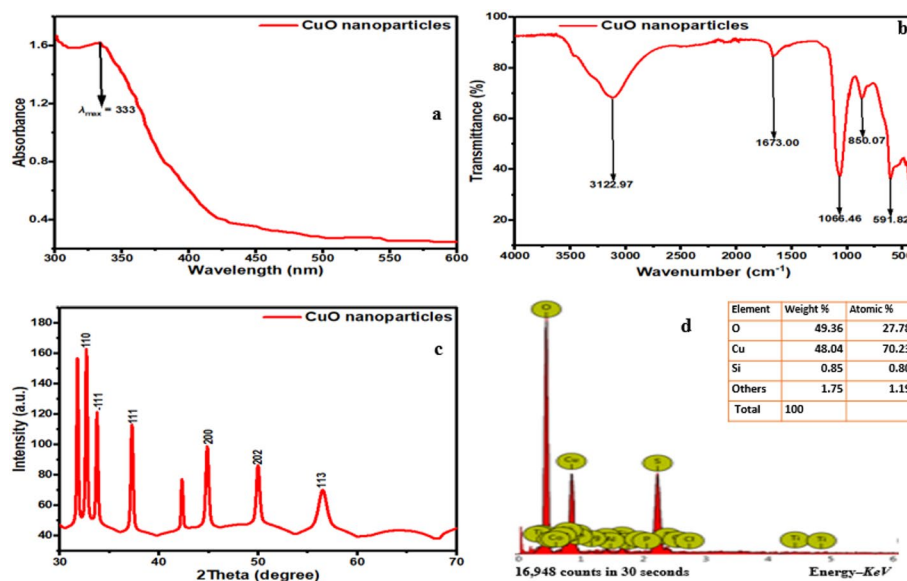
2.5 Antibacterial activity of *Blighia Sapida* mediated-CuO nanoparticles

The bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Proteus penneri* (ATCC 34419), *Staphylococcus aureus* (ATCC 12598), and *Streptococcus pyogenes* (ATCC 19615) used in this work were obtained from Biology Laboratory, Federal Polytechnic, Ado-Ekiti. The antimicrobial experiment was performed using the procedure reported by Abegunde et al. [9]. The antibacterial potency of synthesized CuO nanoparticles was evaluated using the disc diffusion method against selected Gram-negative bacteria—*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus penneri*—as well as Gram-positive bacteria—*Staphylococcus aureus* and *Streptococcus pyogenes*. Bacterial strains were cultured on Luria-Bertani (LB) medium and incubated at 25 °C for 24 h, then stored at 4 °C for subsequent use. To prepare the test inoculum, bacterial suspensions were adjusted to a concentration of approximately 10^6 colony-forming units (CFU) per millilitre following overnight incubation on nutrient agar. Two hundred microlitres of each bacterial suspension were uniformly spread onto LB agar plates. Sterile filter paper discs were impregnated with CuO nanoparticles at concentrations of 2, 4, 6, and 8 mg/l. Distilled water served as the negative control, while tetracycline was used as the positive control. The loaded discs were carefully placed on the surface of the inoculated agar, ensuring firm contact and adequate spacing between discs. The plates were incubated at 37 °C for 18–24 h, after which the zones of inhibition were measured in millimetres. All experiments were performed in triplicate, and the average values were calculated for analysis. The antibacterial evaluation results were presented using a bar chart to provide a clear and concise visual representation for effective comparison of inhibition zones across different bacterial strains and sample treatments.

3 Results and discussion

3.1 Results of CuO nanoparticles

The CuO nanoparticles synthesized using *Blighia sapida* fruit pod extract was characterized using UV–visible spectrophotometer within the wavelength range of 300–600 nm, as shown in Fig. 2a. The spectrum exhibited a distinct absorption peak at 333 nm, which



nanoparticles. The obtained results align with previous reports on the green synthesis of CuO NPs [43, 44].

Energy-dispersive X-ray analysis was carried out to assess the elemental composition and purity of synthesized CuO nanoparticles. The results, as presented in Fig. 2d, revealed that the nanoparticles primarily consist of Cu and O, with impurity levels below 3% by weight. The Cu signal in the results is likely attributed to the copper grids used during sample mounting. Additionally, the presence of C in the spectrum is associated with the carbon coating applied during transmission electron microscopy analysis. Other minor elemental signals may be ascribed to residual phytochemicals from the *Blighia sapida* pod extract used in the biosynthesis process. These findings are consistent with recent literature reports on the green synthesis of metal oxide nanoparticles [22].

The scanning electron microscopy image of synthesized CuO nanoparticles was represented in Fig. 3a. The micrograph reveals that the particles exhibit oval and platelet-like surface morphologies. The presence of pores with varying sizes and shapes indicates an interconnection among the crystallites. It is important to say that the nanoparticles appear to be agglomerated, forming larger grain clusters, although a few particles remain quite separated from each other. The observed agglomeration is likely attributable to the oxidation of the metal nanoparticles during post-processing [45, 46].

The TEM image (Fig. 3b) of CuO nanoparticles further confirms the presence of agglomeration and reveals a monoclinic crystal structure with predominantly oval and platelet-like nanostructures. The average particle size was estimated to be approximately 3.51 nm. The image also displays nanoparticles of varying shapes, indicating morphological diversity within the sample. Notably, the particle size determined from the TEM analysis closely aligns with the values obtained from XRD measurements, thereby confirming the consistency and reliability of the dimensional observations.

Figure 3c presents the result of dynamic light scattering (DLS) analysis employed to determine the average hydrodynamic diameter of the CuO nanoparticles (NPs), which was found to be 57.38 nm. It is noteworthy that the average particle size measured by

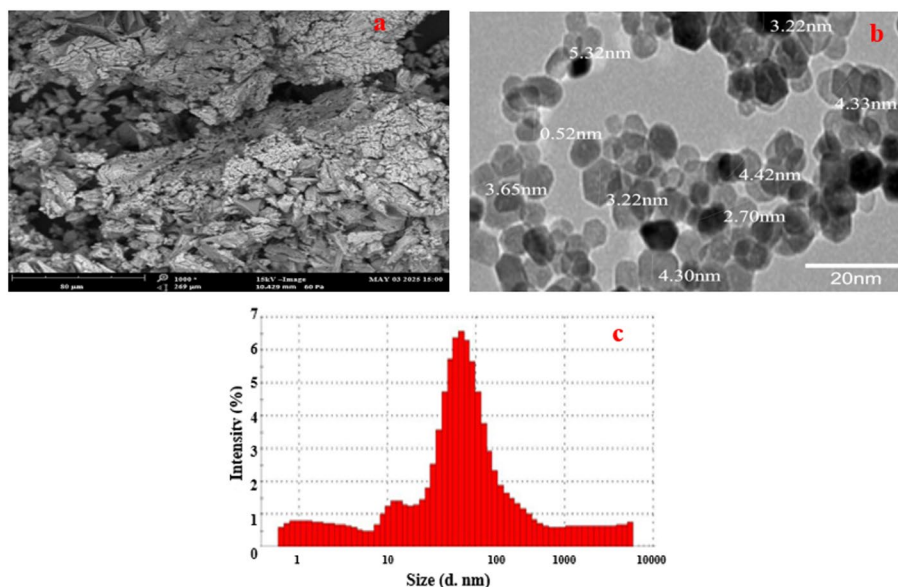


Fig. 3 SEM image (a), TEM image (b), and particle size distribution of CuO NPs

DLS is higher than that obtained from TEM and XRD. This disparity arises from the different principles on which the instrument operate. DLS measures particles in solution, accounting for their hydrated state and interactions with solvents or stabilizers, which can increase the observed hydrodynamic size [47]. In contrast, TEM and XRD analyze dry samples, providing information on the actual particle core size and crystal structure without the influence of surrounding media or surface coatings [48]. The particle size distribution profile indicates that the CuO NPs are polydisperse, potentially due to the tendency of the particles to agglomerate over time. The measured polydispersity index (PDI) was 0.471, suggesting a moderate to high degree of size variation within the sample [49]. The PDI serves as an important indicator of particle uniformity in suspension and is particularly relevant in evaluating nanoparticles for applications such as drug loading, encapsulation efficiency, bioavailability, and therapeutic efficacy.

3.2 Antibacterial activity of *Blighia Sapida* seed pods extract mediated CuO nanoparticles

The antimicrobial potency of biosynthesized CuO nanoparticles was evaluated using the disc diffusion method against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus penneri*) and Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*). The evaluation was conducted at varying concentrations of CuO NPs, and the results, as presented in Fig. 4, revealed increased in the zone of inhibition as the concentration increases. Among the tested organisms, *Escherichia coli* exhibited the largest zone of inhibition across all concentrations, whereas *Staphylococcus aureus* showed the least susceptibility. The relatively higher antimicrobial activity of the green-synthesized CuO NPs against Gram-negative

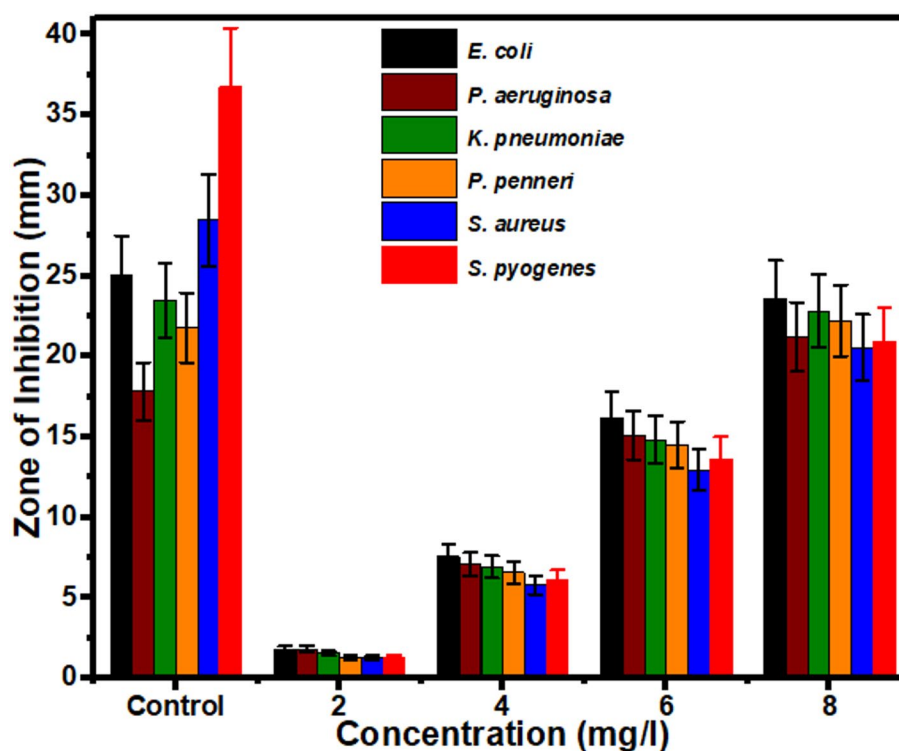


Fig. 4 Antibacterial activity of CuO nanoparticles against selected bacterial strains at concentrations of 2, 4, 6, and 8 mg/L. Negative control is distilled water and exhibited no antimicrobial activity against all tested strains, while the control in the chart is tetracycline (30 µg) used as positive control

bacteria compared to Gram-positive strains may be attributed to the structural differences in their cell walls [50]. Gram-negative bacteria possess a more porous outer membrane, which may facilitate greater nanoparticles permeability and intracellular disruption. In contrast, Gram-positive bacteria have a thicker peptidoglycan layer that acts as a barrier, limiting nanoparticles penetration. These observations are consistent with previously reported findings in the literature [51, 52].

4 Mechanisms of antibacterial activity of CuO nanoparticles

The antibacterial activity of CuO nanoparticles is majorly influenced by the structural and compositional differences between Gram-negative and Gram-positive bacteria [53]. Gram-negative bacteria possess a relatively thin peptidoglycan layer located between an inner cytoplasmic membrane and an outer membrane composed of lipopolysaccharides, phospholipids, and proteins [54]. This outer membrane, although protective, is relatively porous, allowing for easier interaction of nanoparticles with the bacterial cell wall. As a result, CuO NPs can more readily disrupt the outer membrane, increasing permeability and leading to the leakage of intracellular contents. Additionally, the generation of reactive oxygen species (ROS)—including hydroxyl radicals, superoxide anions, and hydrogen peroxide—plays a critical role in damaging cellular components such as lipids, proteins, and DNA [9, 55]. The partial dissolution of CuO NPs releases copper ions (Cu^{2+}), which interfere with vital enzymatic processes and disrupt metabolic functions. These ions, along with ROS, can impair the bacterial electron transport chain, inhibit ATP synthesis, and induce DNA damage, leading to cell death. In contrast, Gram-positive bacteria are characterized by a much thicker peptidoglycan layer embedded with teichoic acids and the absence of an outer membrane. This thick cell wall offers a more formidable barrier to nanoparticles penetration, reducing the overall susceptibility of Gram-positive bacteria to CuO NPs. Nonetheless, CuO nanoparticles can still adsorb onto the bacterial surface, disrupting membrane potential and ion exchange. Similar to Gram-negative bacteria, the generation of ROS and the release of copper ions contribute to oxidative damage and enzyme inhibition. Furthermore, the interaction of CuO NPs with the peptidoglycan matrix may interfere with cell wall synthesis, compromising the structural integrity of the bacterial cell. The possible antimicrobial mechanism of synthesized CuO NPs is presented in Fig. 5.

5 Conclusion

This study successfully demonstrated the green synthesis of CuO nanoparticles using *Blighia sapida* seed pod extract, offering an eco-friendly, cost-effective, and sustainable alternative to conventional physical and chemical methods. Characterization results affirmed the production of pure, crystalline, and morphologically diverse CuO nanoparticles with nanoscale dimensions and desirable physicochemical attributes. The synthesized nanoparticles exhibited significant antibacterial activity, particularly against Gram-negative bacterial strains, due to their enhanced susceptibility stemming from structural differences in their cell walls. The antimicrobial efficiency is largely attributed to nanoparticle-induced generation of reactive oxygen species, release of copper ions, and subsequent disruption of bacterial membranes and metabolic functions. These findings show the dual role of *B. sapida* extract as both reducing and stabilizing agent, supporting its application in green nanotechnology. Moreover, the valorization

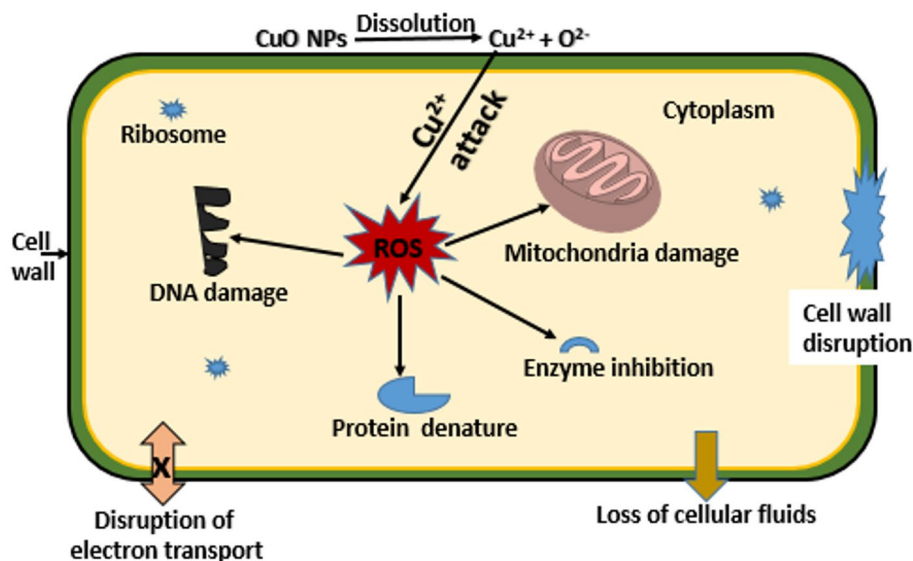


Fig. 5 Antimicrobial mechanism of *S. sapida* mediated CuO NPs

of agricultural waste, such as ackee seed pods, aligns with global sustainability goals and waste-to-resource strategies. Finally, this study provides a foundational approach for leveraging indigenous plant materials in the development of next-generation antimicrobial agents, and sets the stage for further research into biomedical, environmental, and industrial applications of biosynthesized CuO nanoparticles.

The results of this study confirm the effectiveness of plant-based extracts as natural reducing and stabilizing agents. While the present investigation was limited to the green synthesis of CuO nanoparticles using *Blighia sapida* pod extract for antibacterial activity, further studies are needed to assess other possible functionalities such as antioxidant, anti-inflammatory, and catalytic properties. Future research will focus on optimizing synthesis parameters to enhance nanoparticles yield and stability, as well as evaluating their applicability in areas such as wastewater treatment, dye degradation, and catalytic oxidation. This work aligns with the United Nations Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being), SDG 6 (Clean Water and Sanitation), and SDG 12 (Responsible Consumption and Production), contributing to sustainable innovation and environmental preservation through ecofriendly synthesis and the valorization of agricultural waste.

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Author contributions

M.O. participated in experimental and drafted the original manuscript. S.M. participated in experimental, drafted the original manuscript, and edited the manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

No ethical approval is required (Not applicable).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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