

Research Article

# Green Synthesized CuO Nanoparticles Using *Zanthoxylum chalybeum* Extracts: Characterization and Antibacterial Effects

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## Abstract

The green synthesis of metal oxide nanoparticles via plant extracts is an environmentally friendly, simple, inexpensive, and rapid, method for synthesizing nanoparticles for biological applications. In this study, *Zanthoxylum chalybeum* bark extracts were used to explore the biosynthesis of copper oxide nanoparticles (CuO NPs). The CuO NPs were successfully synthesized from an aqueous extract of *Zanthoxylum chalybeum* stem bark and characterized. The morphological, optical, and structural characteristics of the nanoparticles were assessed via scanning electron microscopy (SEM), X-ray diffraction (XRD), UV–visible spectrophotometer, and Fourier transform infrared (FTIR) spectroscopy. Nanocrystalline CuO NPs, with an average crystalline size of 18.26 nm and a band gap energy of 1.45 eV, were confirmed via XRD and UV–vis spectrophotometry, respectively. The SPR (surface plasmon resonance) peak was identified at wavelength of 529 nm in the UV–Vis spectrum. FT-IR analysis confirmed the presence of various functional groups that trigger the synthesis of CuO NPs. Morphological studies via SEM revealed spherical nanoparticles. Antifungal evaluation of the *Candida albicans* fungal strain and antibacterial evaluation of four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) revealed greater potency of the green-synthesized CuO NPs than the *Zanthoxylum chlamydidium* extracts and erythromycin (positive control). The green-synthesized CuO NPs obtained may be used as an antibacterial and antifungal agent for various therapeutic uses in medicine.

## Keywords

Biosynthesis, Nanoparticles, Antifungal, Antibacterial Activity

## 1. Introduction

Over the years, scientists from various disciplines have conducted research on nanotechnology, which has led to its sustainable growth. Therefore, new materials (nanoparticles) (with sizes  $\leq 100$  nm) have been fabricated [1]. NPs have recently gained attention in various disciplines in science because of their specific chemical and physical properties and

their potential uses in electronics, agriculture, medicine, and remediation of the environment. As a result, interest in developing effective and sustainable techniques for the formulation of nanoparticles has increased [2]. The development of nanoparticles is one of the prospects for the successful diagnosis and treatment of numerous illnesses, including cancer. To date,

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significant progress has been made in the manufacture of numerous medications [3]. Conventional techniques for synthesizing nanoparticles frequently entail the use of energy-intensive procedures, high temperatures, and dangerous chemicals, raising potential toxicity and environmental issues. Green synthesis has drawn a lot of interest as a potential solution to these problems [4]. Compared to chemical and physical techniques, biological synthesis is the most desirable technique for current researchers because of its simple methodology for preparation, cost effectiveness, nontoxicity, ease of availability, reduced time consumption, eco-friendly nature, and increased stability [5].

Green synthesis of nanoparticles yields a greater mass since it is more efficient technique. Several biochemical compounds found in plants can serve as reducing and stabilizing agents for creating green nanoparticles [6]. Green synthesized nanoparticles are therapeutically useful nanomaterials because of their high surface area-to-volume ratio, stability, low cost, and safety. A number of techniques for formulating both organic and inorganic NPs have recently been assessed by researchers [7]. CuO, ZnO, MgO, TiO<sub>2</sub>, SiO<sub>2</sub>, and other inorganic metal oxide nanoparticles (NPs) with strong antibacterial properties and selective toxicity suggest that these materials may find use in medical devices, diagnostics, treatments, and nano-medicine against human pathogens [8]. In contrast, the synthesis of metal oxide nanomaterials, such as gold and silver, has several disadvantages, such as high cost, whereas synthesizing CuO NPs is environmentally friendly, has useful properties, and is cheap. In addition, the specific properties, such as electrical, catalytic, optical, antifungal, and antibacterial properties, possessed by CuO NPs are desirable compared to those of other metallic nanoparticles [9]. Due to their high volume-to-surface ratio and quantum effects, metallic nanoparticles have exceptional UV sensitivity as well as antibacterial catalytic, electrical, and thermal, characteristics. Numerous NPs are employed as ways to kill microbes; metallic NPs, in particular, penetrate bacterial cell walls that lead to the production of free radicals, which have the potential to damage the cell membrane [10].

According to previous studies, *Zanthoxylum chalybeum* is a significant medicinal plant used to cure headaches, fever, malaria, cough, chest discomfort, digestive disorders (such as ulcers), diabetes, and toothaches. The presence of bioactive substances like alkaloids has been linked to its efficacy as herbal remedy [11]. Additionally, it has been demonstrated that phyto-bioactive chemicals operate as models for the development of some currently used medications, such as alkaloids for cancer and quinine for malaria. *Linn. Zanthoxylum* is a genus of plants that belongs to the *Rutaceae* family, which has about 160 genera and 2000 species, with more species yet to be discovered [12]. *Zanthoxylum chalybeum* extracts have been previously used to synthesize Ag nanoparticles and in the assessment of the antimicrobial properties of synthesized nanoparticles [13].

Thus, the present study involved the green-synthesis of copper nanoparticles using *Zanthoxylum chalybeum* stem bark extracts and testing their potential application as antifungal and antibacterial agents.

## 2. Materials and Methods

### 2.1. Materials

Cupric nitrate dihydrate (Cu (NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O) was purchased from *Precise Lab Africa Ltd.* (98%). Hydrochloric acid (HCl) (35%) and sodium hydroxide (98%) were used to monitor the pH, which were also obtained from *Precise Lab Africa Ltd., Kenya*. The bark of *Zanthoxylum chalybeum* was collected in Murkutwa, Elgeyo Marakwet, Kenya. The dissolution of 0.21 g of Cu (NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O in 100 mL of deionized water yielded a 0.01 M solution of cupric nitrate dihydrate. Bacterial and fungal cultures were grown in media that included *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. All of the chemicals and solvents utilized were of analytical grade.

### 2.2. Plant Material Collection and Preparation

*Zanthoxylum chalybeum* bark was harvested from healthy plants in the early morning to preserve its phytochemicals. The bark was cleaned with distilled water to remove contaminants before being drying in the lab for two weeks. After drying, the bark was crushed into a fine powder and stored in airtight containers to preserve its phytochemicals [14]. A botanist from the Forestry Department at the University of Eldoret recognized and authenticated the plant species. To prepare the extract, 10 g of the plant powder was dissolved in 250 mL of distilled water and then boiled at 60°C for 2 hours. The resulting solution obtained from the *Zanthoxylum chalybeum* extract was subsequently filtered through a Whatman No. 1 filter and stored in the dark for use in the synthesis of CuO nanoparticles.

### 2.3. Biosynthesis of CuO Nanoparticles

The synthesis of the copper oxide nanoparticles used cupric nitrate dihydrate as the metal precursor. A 0.01 M Cu (NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O solution was prepared by dissolving 10 g in 100 mL of distilled water. The metal mixture was then mixed with an aqueous extract of *Zanthoxylum chalybeum* at a 5: 1 ratio. The formation of the CuO NPs was monitored through color changes and UV-Vis spectrophotometry method. The colloidal solution was centrifuged at 6000 rpm for 20 minutes and then washed several times, after which the nanoparticles were dried in an oven at 70°C [15].

### 2.4. Antibacterial Evaluation Methods

The antibacterial activity of the CuO NPs and *Zanthoxylum*

*chlamydidium* extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* was evaluated using the agar-disc diffusion technique for bactericidal susceptibility. The autoclave was used to sterilize the medium at 120°C. After that, the medium was moved to sterile petri dishes and kept at 37°C until it solidified. A loop of the bacterial strains was placed on Petri dishes. A single 5 mm diameter disc was made and the CuO NPs (10 µL) and bark leaf extract from *Zanthoxylum chalybeum* were impregnated on the discs. For 24 hrs, the plates were incubated at 37°C. The tests were done in triplicates and the zones of inhibition were measured in mm [16].

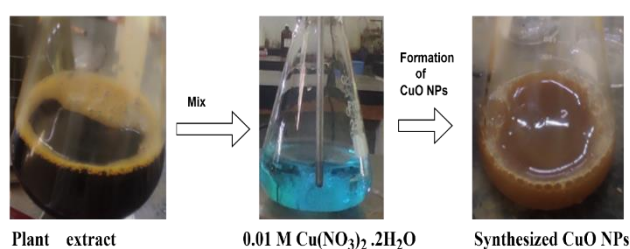
## 2.5. Statistical Analysis-one-way ANOVA

The antibacterial and antifungal assays were run three times (in triplicate). The average and standard deviation were computed. The findings were analyzed via an Excel spreadsheet program to determine whether the results were statistically significant ( $p \leq 0.05$ ).

## 3. Results and Discussion

### 3.1. Green Synthesis of CuO NPs

The green synthesis of CuO NPs is an exciting area of research. In the present study, the CuO NPs were synthesized using an aqueous solution of cupric nitrate dihydrate precursor salt and aqueous *Zanthoxylum chalybeum* stem bark extract. At controlled temperatures and incubation times, a mechanical shaker was used to evenly mix the mixture. The color shift from dark black to brown was a sign that CuO NPs were formed as shown in Figure 1 [17].

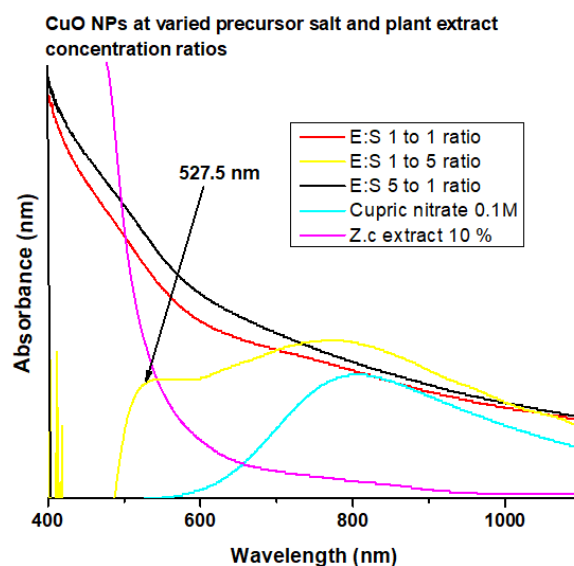


**Figure 1.** The graphical flowchart for the green synthesis of CuO NPs using *Zanthoxylum chalybeum* stem bark extract.

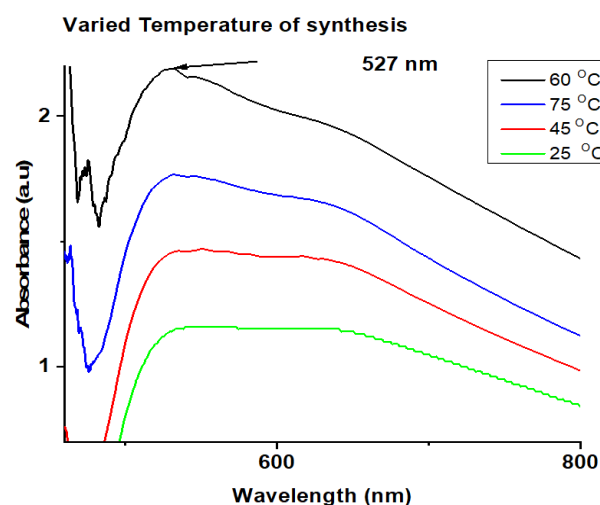
### 3.2. Optical Analysis

The UV–Vis spectrum of the CuO NPs was recorded between 200 and 800 nm using a Shimadzu 1800 Series Model. At various concentration ratios of the plant extract (10% w/v) and the precursor salt (0.01 M) from 1 to 5, a peak was observed at 527.5 nm (Figure 2). The same ratio was applied to different molar concentrations of the precursor salt, with a 0.01 M solution showing a peak at 533.5 nm. In comparison,

the 0.1 M and 1 M solutions maintained the precursor salt peak (Figure 4). A 0.01 M precursor salt concentration was used to optimize the CuO NPs by adjusting the temperature and pH. At 60°C (Figure 3), a sharp peak appeared at 527 nm. A pH of 8 (Figure 5) caused a blueshift to 516 nm, indicating a reduction in nanoparticle size [18]. A similar study by [19] reported the UV–Vis spectrum of fabricated copper nanoparticles, showing a characteristic maximum absorbance at 570 nm, and [17] noted that Cu/CuO nanoparticles have a wavelength of 576 nm; thus, the wavelength range of 500–600 nm, as shown in the optimized synthesis parameters in Figure 6. A bandgap energy of 1.45 eV (Figure 7) was determined, aligning with findings from [20] and [21], who reported bandgap energies in the 1.3–1.4 eV range. The obtained bandgap energy may be due to quantum confinement effects and the presence of intragap states in the synthesized CuO nanoparticles [22].



**Figure 2.** Biosynthesis of CuO NPs at various concentration ratios of precursor salt to plant extract.



**Figure 3.** Biosynthesis of CuO NPs at various temperatures.

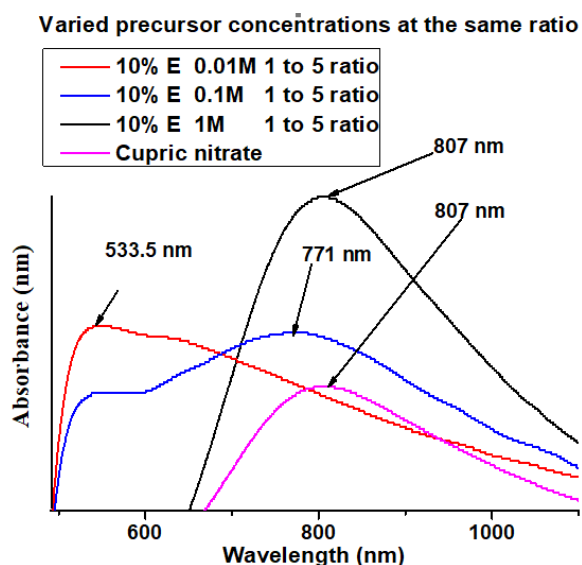


Figure 4. Biosynthesis of CuO NPs at various precursor molar concentrations.

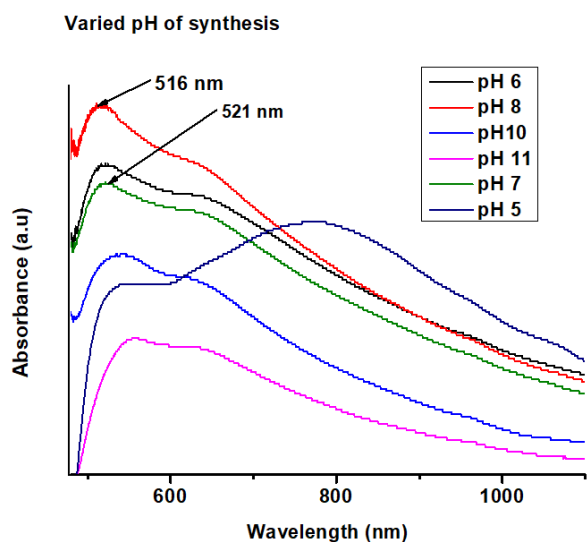


Figure 5. Biosynthesis of CuO NPs at various pH values.

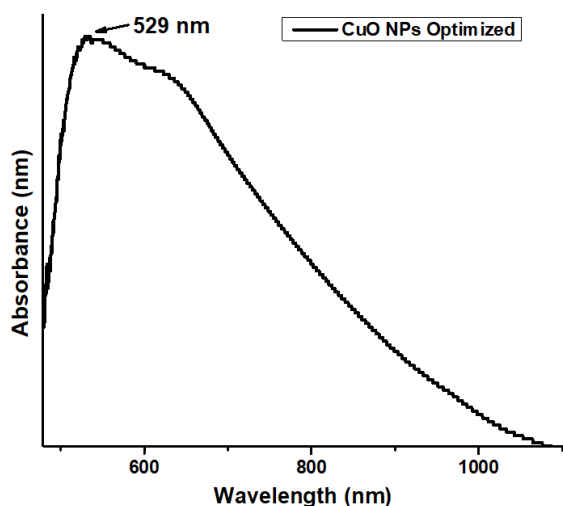


Figure 6. Optimized CuO NPs.

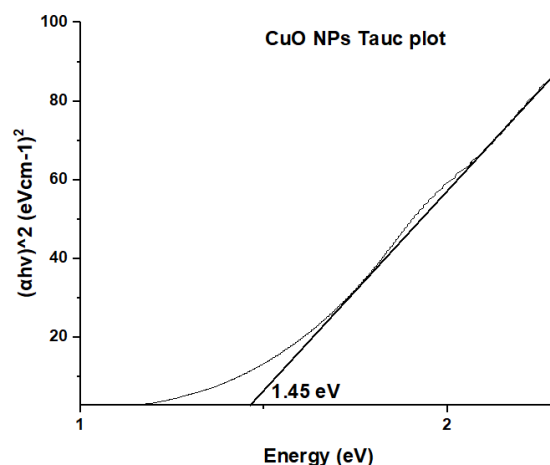


Figure 7. Tauc plot of CuO NPs.

### 3.3. FTIR Analysis

FT-IR analysis was carried out at the range of 400 and 4000  $\text{cm}^{-1}$  to identify the functional groups present in the synthesized CuO NPs (Figure 8). The stretching vibrations of hydroxyl (-OH) groups are shown by the wide, strong absorption band at 3,448  $\text{cm}^{-1}$ , which suggests the presence of alcohols and phenols [23]. The presence of saturated hydrocarbons is represented by the peak at 2103  $\text{cm}^{-1}$  due to C-H stretching in alkanes [24]. The signal at 1659  $\text{cm}^{-1}$  is associated with carbonyl (C=O) stretching vibrations and C=C stretching vibrations, which are often linked to esters, ketones, aldehydes, alkenes, or aromatic compounds [25]. The absorption at 782  $\text{cm}^{-1}$  points to substituted aromatic compounds. The peak at 422  $\text{cm}^{-1}$  may suggest the presence of trace amounts of inorganic or metal complex substances [26]. Overall, the FTIR analysis of the aqueous extract of *Zanthoxylum chalybeum* revealed hydroxyl, carbonyl, aromatic, and alkane groups, indicating a diverse profile of bioactive chemicals, including phenolics, terpenoids and flavonoids.

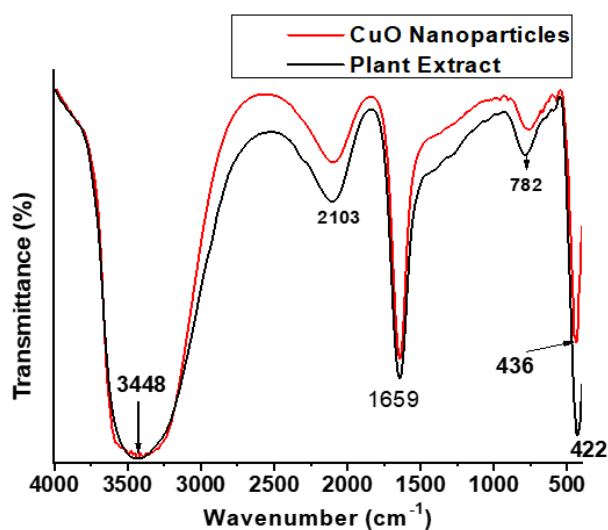


Figure 8. FTIR spectrum of CuO NPs.

### 3.4. XRD Analysis

The crystallinity and phase purity of the prepared CuO NPs were studied via XRD. The XRD pattern of the prepared sample is depicted in Figure 9. The XRD pattern exhibited sharp, well-defined high peaks. The XRD pattern of the synthesized CuO-NPs showed distinct diffraction peaks at  $2\theta$  values of  $31.87^\circ$ ,  $36.37^\circ$ ,  $47.66^\circ$ ,  $62.99^\circ$ , and  $68.08^\circ$  (Figure 8), which corresponded to 101, 111, 020, 113, and 220, respectively. These results confirm the face-centered cubic structure of the obtained CuO-NPs, as indexed with the standard powder diffraction card of the Joint Committee on Powder Diffraction Standards database (JCPDS card no. 48 - 1548) [27]. The Debye–Scherrer equation  $D = k\lambda/\beta\cos\theta$ , where  $\beta$  is the full width at half maximum,  $\lambda$  is the X-ray wavelength ( $1.5418 \text{ \AA}$ ), and  $k$  is a constant, was used to estimate the average crystallite size. The size of the crystallites was estimated using all of the study's peaks, and the average size of the crystallites discovered was  $18.26 \text{ nm}$  [28].

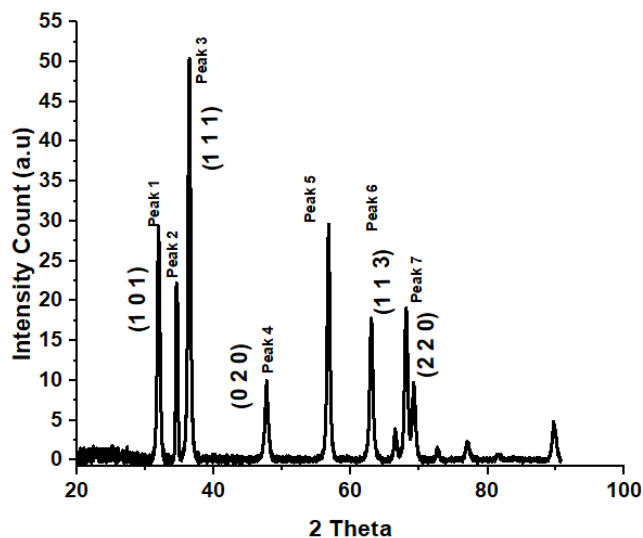


Figure 9. XRD spectrum of CuO NPs.

Table 1. CuO nanoparticle XRD data.

Peak NO	$2\theta$ values ( $^\circ$ )	FWHM	Particle size $(0.9*0.154)/(\beta \sin \theta)$ (nm)
peak 1	31.87	0.46	17.95
peak 2	34.53	0.33	25.20
peak 3	36.37	0.46	18.17
peak 4	47.66	0.58	14.97
peak 5	56.73	0.5	18.05
peak 6	62.99	0.55	16.93
peak 7	68.08	0.58	16.52
Average Particle size (nm)			18.26

### 3.5. SEM Analysis

SEM was performed on green copper oxide nanoparticles. The SEM images, presented in Figure 10, revealed a uniform and spherical morphology of the copper oxide nanoparticles, revealing no agglomeration due to good separation. The SEM images also indicate that the plant extract contains bioactive compounds that can effectively reduce and stabilize copper oxide nanoparticles. A study by [29] reported similar results on the synthesis of CuO NPs from *Aegle marmelos* leaf extracts.

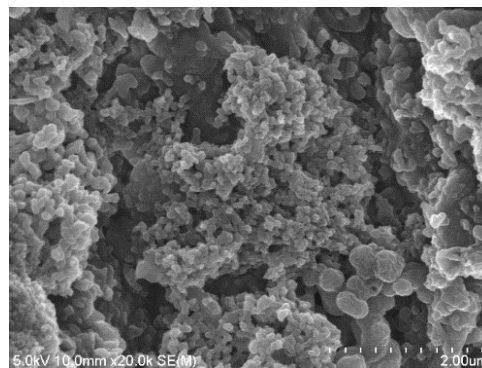
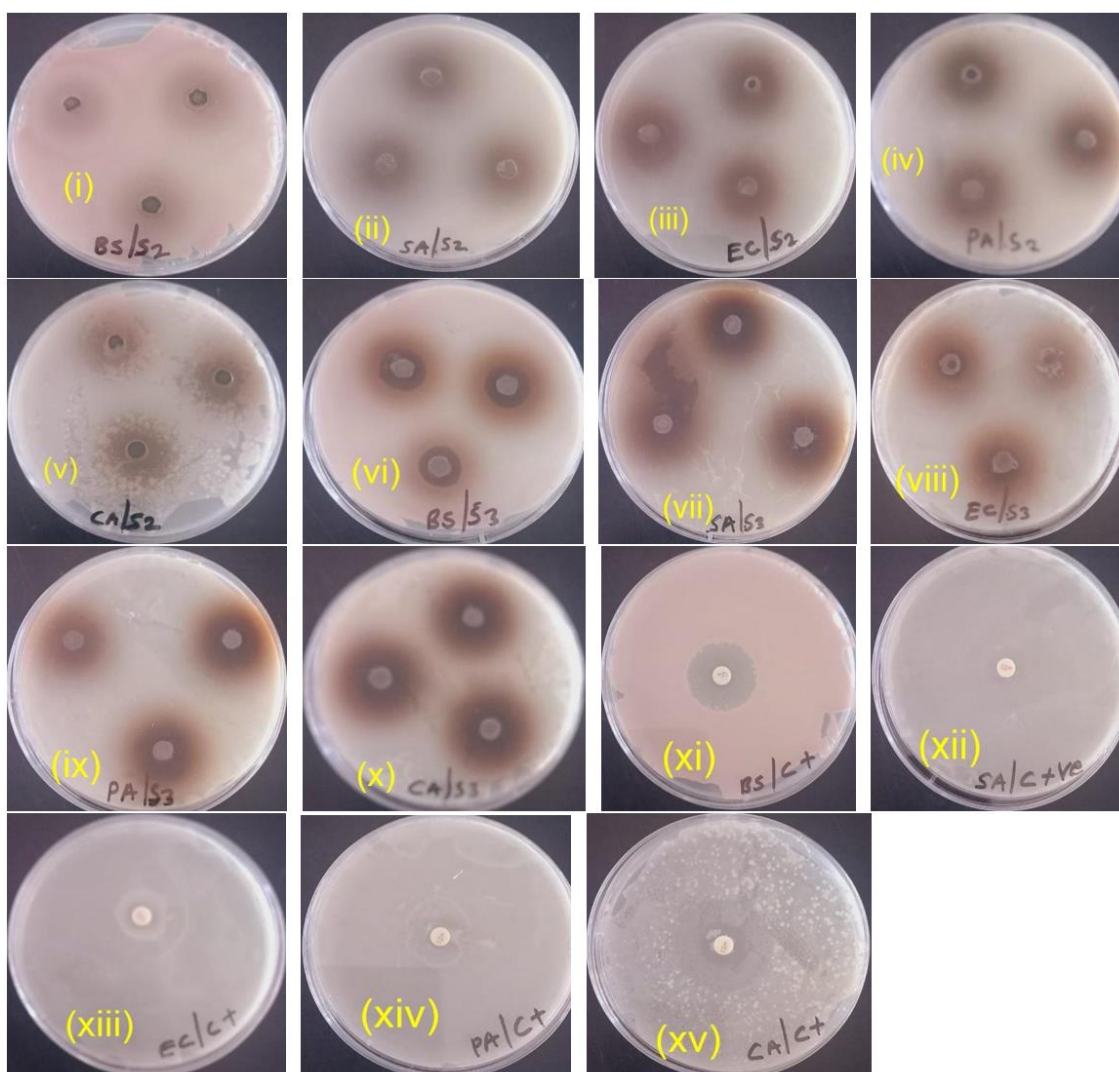


Figure 10. SEM image of CuO NPs.

### 3.6. Antimicrobial Activity

The fresh microbial cultures were swiped evenly on sterilized petri dishes with nutrient agar. For the clean discs, 15  $\mu$ L of synthesized CuO NPs, a positive control (erythromycin), or an aqueous extract of *Zanthoxylum chalybeum* stem bark was added and incubated overnight for 24 hours at 37°C. The development of a bacterial inhibition zone surrounding the discs was identified, as shown in Figure 11. CuO NPs were found to be more effective antimicrobial agents against all the examined pathogens than erythromycin and the plant extracts were, as recorded in Table 2. Hence, CuO NPs have remarkable antifungal and antibacterial activities against pathogenic fungal and bacterial strains. The compound (CuO NPs) showed less activity against *Staphylococcus aureus* bacteria ( $6.67 \pm 0.47$  mm). This

happened because different bacteria react differently to metal oxide nanoparticles depending on the thickness of their cell walls. Compared to gram-positive bacteria like *S. aureus*, which have thicker cell walls, gram-negative bacteria like *E. coli* are more susceptible to copper nanoparticle penetration [30]. The antibacterial and antifungal mechanisms of CuO NPs depend on their structure, size, and concentration. The main mechanism of action involves (i) oxidative stress, (ii) infiltration and cellular disruption, and (iii) degeneration of the membrane and cell wall [15, 31]. The occurrence of inhibition zones (Figure 11) was an indication of the absence of microbial growth on the plate. The interaction of pathogens and CuO ions released from nanoparticles causes adhesion and bioactivity due to electrostatic forces when they are adsorbed on the cell surfaces of the microorganisms. This damages the cell wall by killing the pathogens, resulting from the resistance of the synthesized nanoparticles to microorganisms [32].



**Figure 11.** Zones of inhibition of (a) plant extract against (i) *Bacillus subtilis*; (ii) *Staphylococcus aureus*; (iii) *Escherichia coli*; (iv) *Pseudomonas aeruginosa*; (v) *Candida albicans* (Fungus), and (b) AgNPs against (vi) *Bacillus subtilis*; (vii) *Staphylococcus aureus*; (viii) *Escherichia coli*; (ix) *Pseudomonas aeruginosa*; (x) *Candida albicans* (Fungus); and (c) erythromycin against (xi) *Bacillus subtilis*; (xii) *Staphylococcus aureus*; (xiii) *Escherichia coli*; (xiv) *Pseudomonas aeruginosa*; and (xv) *Candida albicans* (Fungus).

**Table 2.** Zones of inhibition values of CuO NPs, plant extracts, and erythromycin against four bacterial strains (*E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus*) and one fungus (*Candida albicans*) according to the disc diffusion method.

Sample Name	Microbial Strains	CuO NPs (S1)	<i>Zanthoxylum chalybeum</i> extract (S5)	Positive Control (erythromycin)
Bacillus subtilis		13.33±0.47	7.67±0.47	10.67±0.47
<i>Pseudomonas aeruginosa</i>		11.33±0.94	7.50±0.41	7.67±0.47
<i>Escherichia coli</i>		11.33±0.47	5.83±0.24	7.83±0.24
<i>Staphylococcus aureus</i>		6.67±0.47	5.33±0.47	7.33±0.47
<i>Candida albicans</i>		15.33±1.25	9.67±0.94	9.83±0.24

### 3.7. Statistical Analysis: One-way ANOVA

**Table 3.** ANOVA of the antibacterial and antifungal (zones of inhibition) results.

Anova: Single Factor						
Groups	Count	Sum	Average	Variance		
Cuo NPs	5	57.99	11.598	10.33912		
Plant extract	5	36	7.2	2.9464		
Positive control	5	43.33	8.666	2.21168		
ANOVA						
Source of Variation	SS	df	MS	F	P value	F crit
Between Groups	50.14697	2	25.07349	4.85381	0.028537	3.885294
Within Groups	61.9888	12	5.165733			
Total	112.1358	14				

The acquired antibacterial and antifungal results are significant because the p value is less than 0.05 (Table 3).

## 4. Conclusions

The green synthesis of CuO NPs has been investigated using the aqueous bark extract of *Zanthoxylum chalybeum*. It has been well characterized by UV-Vis, XRD, FT-IR, and SEM analyses. The CuO NPs were confirmed to have formed by color changes from dark black to brown. FT-IR has revealed the major functional groups present in the plant extract as well as their chemical interactions. SEM images revealed spherical shapes. The antimicrobial activity of the extracts was investigated against the bacterial species *S. aureus* and *E. coli*. The extracts showed significant zones of inhibition against five microbial isolates, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. *Staphylococcus aureus* has the lowest average activity, whereas *Candida albicans* (a fungus) has the highest average activity on CuO NPs. Therefore, *Zanthoxylum chalybeum*-

metal oxide-formulated nanoparticles can be recommended for biomedical applications.

## Abbreviations

CuO NPs	Copper Oxide Nanoparticles
HCl	Hydrochloric Acid
XRD	X-ray Diffraction
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
SPR	Surface Plasmon Resonance

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## Author Contributions

**Posla Chelang'a:** Conceptualization, Visualization, Writing – original draft

**Kiplagat Ayabei:** Methodology, Supervision, Writing – review & editing

**Paul Tarus:** Formal Analysis, Investigation, Supervision

**Lemeitaron Njenga:** Conceptualization, Writing – review & editing

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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