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Original 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, rapid, inexpensive, and simple approach for obtaining nanoparticles for biological applications. In this study, copper oxide nanoparticles (CuO NPs) were successfully synthesized from an aqueous extract of Zanthoxylum chalybeum stem bark. The structural, optical, and morphological characteristics of the nanoparticles were assessed via X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, a UV–visible spectrophotometer, and scanning electron microscopy (SEM). 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. Morphological studies via SEM revealed spherical nanoparticles. Antifungal evaluation of the Candida albicans fungal strain and antibacterial evaluation of four bacterial strains (Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus) revealed greater potency of the green-synthesized CuO NPs than the Zanthoxylum chlamydium extracts and erythromycin (positive control). The bio-synthesized CuO NPs may be used as antibacterial and antifungal agents for therapeutic applications.

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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 ≤ 100 nm) have been fabricated (Gu *et al*., 2022). NPs have recently gained significant attention in various scientific disciplines because of their unique physicochemical properties and potential applications in different areas, such as medicine, agriculture, electronics, and environmental remediation. As a result, interest in developing sustainable and effective methods for synthesizing nanoparticles has increased (Osman *et al*., 2024). To date, great strides have been made in the production of many drugs, and the production of nanoparticles is one of the hopes in the effective treatment and diagnosis of many diseases, including cancer (Vinodini & Bathe, 2022). Traditional methods of synthesizing nanoparticles often involve the use of hazardous chemicals, high temperatures, and energy-intensive processes, leading to environmental concerns and potential toxicity. To address these issues, green synthesis has gained considerable attention as a promising alternative (Nzilu *et al*., 2023).

Compared with other biological, physical, and chemical methods, green synthesis is the most desirable method for current researchers because of its simple methodology for preparation, cost-effectiveness, nontoxicity, ease of availability, reduced time consumption, eco-friendly nature, and (Abbas & Fairouz, 2022)increased stability. Compared with other related methods, the green synthesis of nanoparticles via biological pathways is a more efficient technique and yields a greater mass. Plants are sources of several components and biochemicals that can act as stabilizing and reducing agents to synthesize green nanoparticles (Doman *et al*., 2024).

Owing to their low cost, high surface area-to-volume ratio, stability, and safety, bioactive nanoparticles are clinically relevant nanomaterials. Researchers have recently evaluated several methods for synthesizing organic and inorganic NPs (Vijayaram *et al*., 2024). Inorganic metal oxide NPs such as MgO, CuO, SiO_2, TiO_2, and ZnO with significant antimicrobial features, as well as their selective toxicity, point to potential applications of these materials in medical devices and diagnostics, therapeutics, and nanomedicine against human pathogens (Radhakrishnan *et al*., 2021). In contrast, the fabrication of metal oxide nanoparticles, such as silver and gold, has several difficulties, such as high cost, whereas synthesizing CuO NPs is eco-friendly, has useful properties, and is achievable at lower costs than silver and gold. Moreover, the specific properties, such as optical, catalytic, electrical, antibacterial, and antifungal properties, exhibited by CuO NPs are excellent compared with those of other metals (Prakash *et al*., 2023).

Metallic nanoparticles have excellent UV sensitivity and catalytic, thermal, electrical, and antibacterial properties due to their large volume–to-surface ratio and quantum effects. Various NPs are used as mechanisms to destroy bacteria, and metallic NPs in particular are known to use several working methods to do so. NPs penetrate the bacterial cell wall and form pores on the membrane surface, which in turn results in the formation of free radicals that can also destroy the cell membrane (D. Singh *et al*., 2023).

Zanthoxylum chalybeum has been reported as an important medicinal plant for malaria treatment, fever, cough, headaches, chest pain, digestive illnesses (e.g., ulcers), diabetes, and toothache. The effectiveness of *Z. chalybeum* as an herbal medicine has been associated with the presence of bioactive compounds such as alkaloids (Kimani *et al*., 2023). Furthermore, phyto-bioactive compounds have been shown to serve as templates that guide the development of some drugs in current use, such as quinine for malaria and alkaloids for cancer. *Zanthoxylum Linn*. is a plant genus belonging to *Rutaceae,* a family that includes more than 160 genera and 2000 species, with more species still being identified (Mbinile *et al*., 2020). *Zanthoxylum chalybeum* extracts have been previously used in the synthesis of silver nanoparticles and in the assessment of the antimicrobial properties of synthesized nanoparticles (Quadri *et al*., 2024).

Thus, the present study investigated the green-synthesis potential of copper nanoparticles using *Zanthoxylum chalybeum* extracts. Physically and chemically produced NPs are less effective or have hazardous side effects. To analyse the possible antibacterial and antifungal properties of the generated CuO-NPs, we used *Zanthoxylum chalybeum* to synthesize CuO-NPs biologically.

2. Materials and methods

2.1. Materials

Cupric nitrate dihydrate ($Cu(NO_3)_2.2H_2O$) 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 $Cu(NO_3)_2 \cdot 2H_2O$ 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 eliminate contaminants before being dried in the laboratory for two weeks. After drying, the bark was crushed into a fine powder and stored in airtight containers to preserve its bioactive qualities. 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* extract was subsequently filtered through a Whatman No. 1 filter and stored in the dark for use in the synthesis of CuO nanoparticles (Khairy *et al*., 2024).

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 $Cu(NO_3)_2.2H_2O$ 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, and nanoparticle formation was monitored through color changes and UV–Vis spectrophotometry. 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 (Priya *et al*., 2023).

2.4. Antibacterial evaluation methods

The standard agar-well diffusion technique for assessing bactericidal susceptibility was used to assess the antibacterial activity of *Zanthoxylum chlamydium* bark extract and CuO-NPs against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and one fungus (*Candida albicans*). The medium was sterilized in an autoclave at 120 °C. The medium was then transferred to sterilized Petri dishes and maintained at 37 °C until solidification. The bacterial strains were distributed on Petri dishes in a loop. A gel piercing agent was used on each plate to create a single 5 mm diameter well. *Zanthoxylum chalybeum* bark leaf extract and CuO-NPs (10 µL) were added to the wells. The plates were incubated at 37 °C for 24 hours. The studies were carried out in triplicate, and the zone of inhibition was measured (Article & Erkan, 2025).

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 analysed 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 copper nanoparticles is currently a fascinating field of study. An aqueous solution of cupric nitrate dihydrate precursor salt and aqueous *Zanthoxylum chalybeum* stem bark extract was used for the synthesis of the copper nanoparticles. The mixture was uniformly mixed via a mechanical shaker at regulated temperatures and times. The formation of CuO NPs was indicated by color changes from dark black to brown (Fig. 1), as also reported by (Mohamed, 2020).

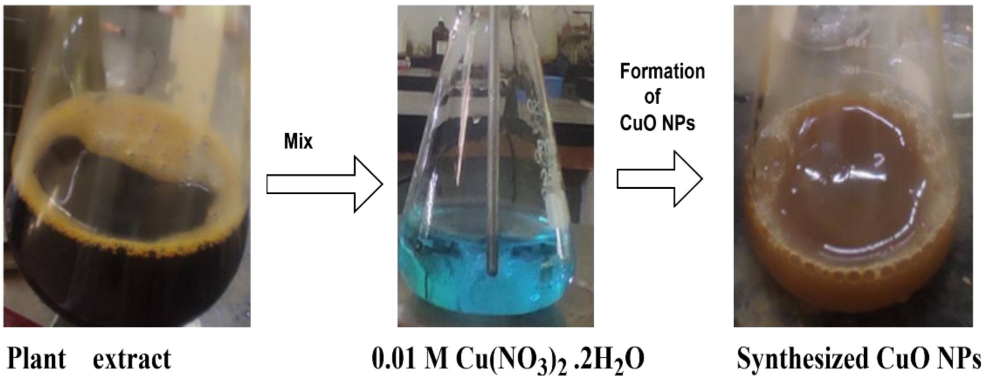

Fig. 1. The graphical flowchart for the green synthesis of CuO NPs using Zanthoxylum chalybeum stem bark extract.

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 (Fig. 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 (Fig. 4). A 0.01 M precursor salt concentration was used to optimize the CuO NPs by adjusting the temperature and pH. At 60 °C (Fig. 3), a sharp peak appeared at 527 nm. A pH of 8 (Fig. 5) caused a blueshift to 516 nm, indicating a reduction in nanoparticle size (J. Singh *et al*., 2019). A similar study by (JAHAN & IŞILDAK, 2020) reported the UV–Vis spectrum of fabricated copper nanoparticles, showing a characteristic maximum absorbance at 570 nm, and (Mohamed, 2020) 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 Fig. 6. A bandgap energy of 1.45 eV (Fig. 7) was determined, aligning with findings from (Sedefoglu *et al*., 2023), 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 (Lakshmanan *et al*., 2021).

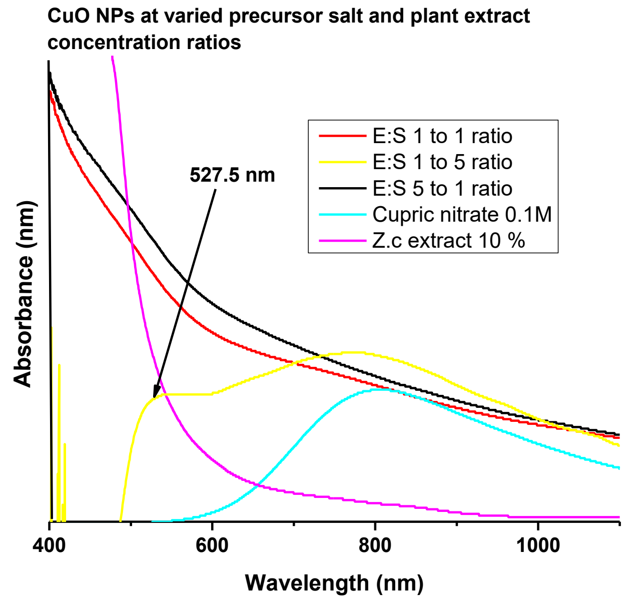

Fig. 2. Biosynthesis of CuO NPs at various concentration ratios of precursor salt to plant extract

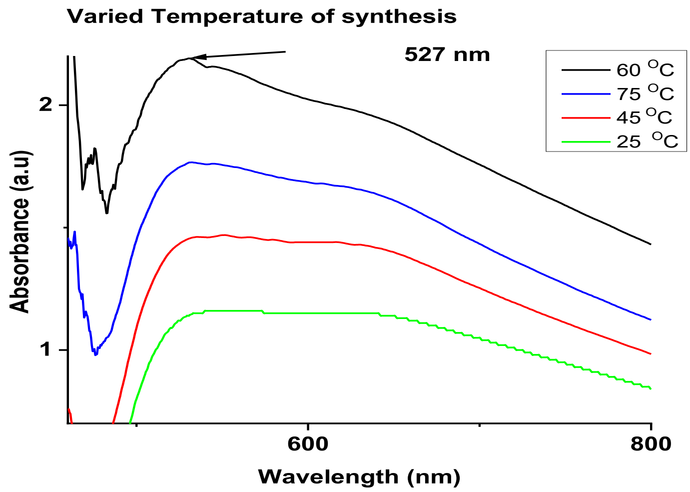

Fig. 3. Biosynthesis of CuO NPs at various temperatures

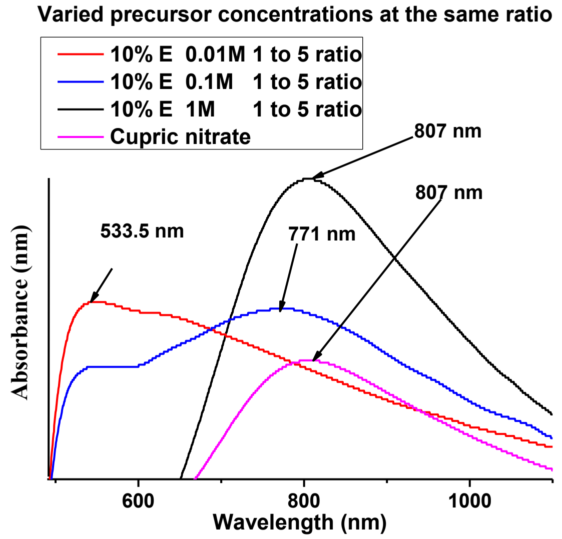

Fig. 4. Biosynthesis of CuO NPs at various precursor molar concentrations

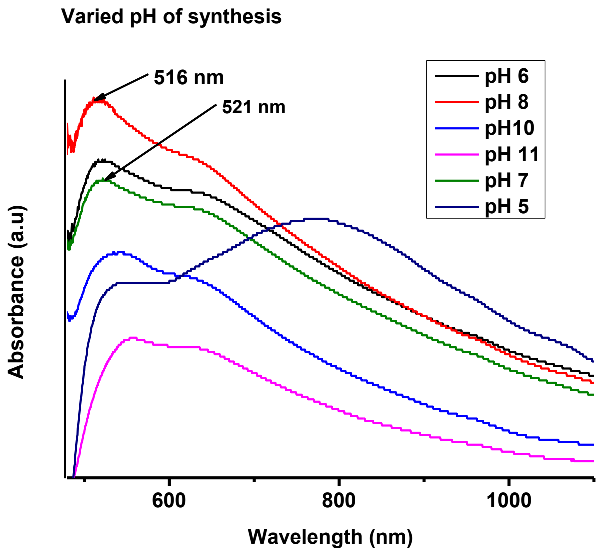

Fig. 5. Biosynthesis of CuO NPs at various pH values

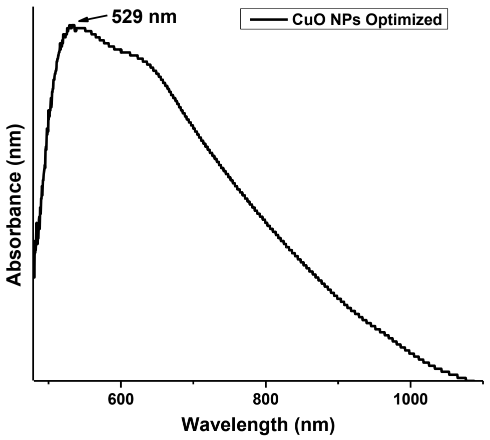

Fig. 6. Optimized CuO NPs

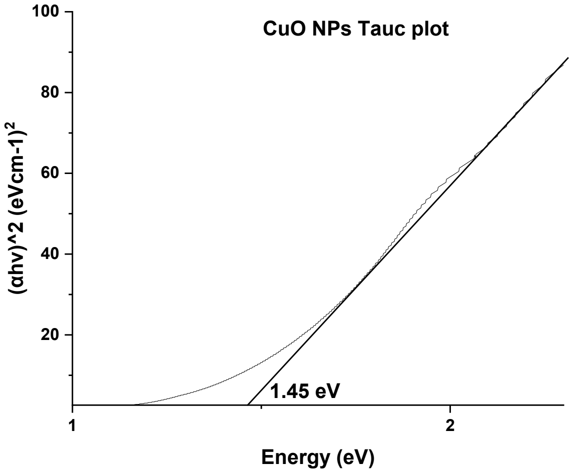

Fig. 7. Tauc plot of CuO NPs

3.3. FTIR analysis

FT-IR analysis was carried out between 400 and 4000 cm^{-1} to identify the functional groups present in the synthesized CuO NPs (Fig. 8). The broad, intense absorption band at 3,448 cm^{-1} corresponds to the stretching vibrations of hydroxyl (-OH) groups, suggesting the presence of phenols and alcohols (Thirumoorthy *et al.*, 2024). The peak at 2103 cm^{-1} indicates C–H stretching in alkanes, confirming the presence of saturated hydrocarbons (Faridatul *et al.*, 2024). The signal at 1659 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 (Labaran *et al.*, 2024). The absorption at 782 cm^{-1} points to substituted aromatic compounds. The peak at 422 cm^{-1} may suggest the presence of trace amounts of inorganic or metal complex substances (Sahib *et al.*, 2025). 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, flavonoids, and terpenoids.

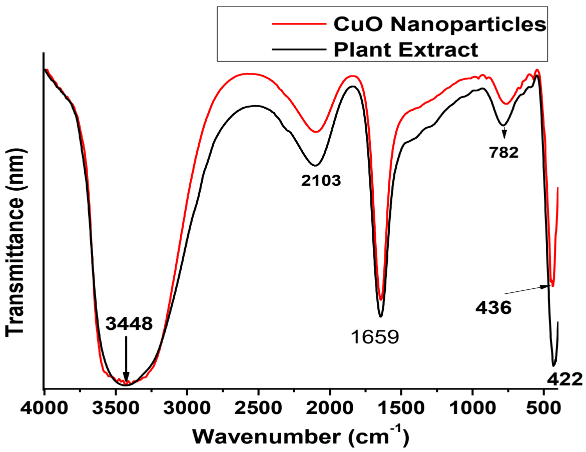

Fig. 8. FTIR spectrum of CuO NPs

3.4. XRD analysis

The phase purity and crystallinity of the prepared CuO NPs were studied via X-ray diffraction (XRD). The XRD pattern of the prepared sample is depicted in Fig. 9. The XRD pattern exhibited sharp, well-defined high peaks. The XRD pattern of the synthesized CuO-NPs showed distinct diffraction peaks at 2θ values of 31.87°, 36.37°, 47.66°, 62.99°, and 68.08° (Fig. 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) (Jabeen *et al*., 2024). The average crystallite size was estimated via the Debye–Scherrer equation: $D = k\lambda/\beta\cos\theta$, where β is the full width at half maximum, λ is the X-ray wavelength (1.5418 Å), and k is a constant. All the peaks obtained in the study were used to estimate the size of the crystallites, and the average size of the crystallites found was 18.26 nm, which corresponds to that previously reported for CuO NPs by (Ikhioya *et al*., 2023).

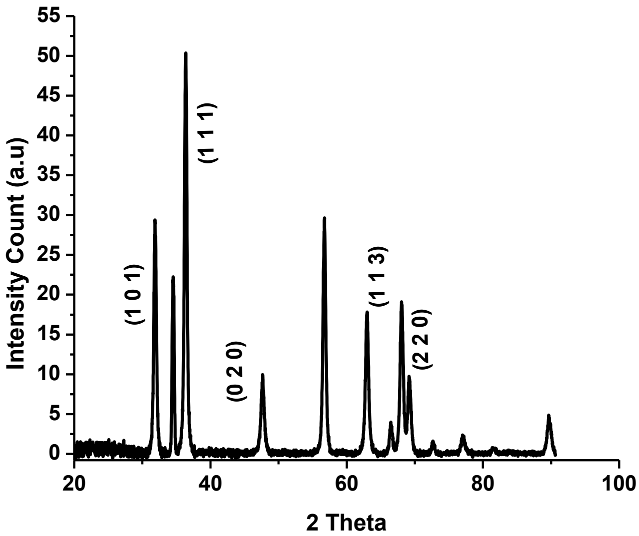

Fig. 9: XRD spectrum of CuO NPs

Table 1: CuO nanoparticle XRD data

Peak NO	2θ values (°)	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

Scanning electron microscopy (SEM) was performed on green copper oxide nanoparticles. The SEM images, presented in Fig. 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 (Ali *et al*., 2023) reported similar results on the synthesis of CuO NPs from *Aegle marmelos* leaf extracts.

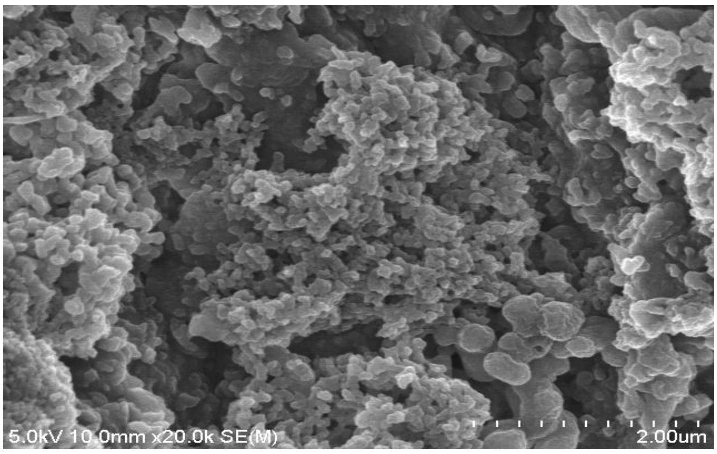

Fig. 10. SEM image of CuO NPs

3.6. Antimicrobial activity

The fresh microbial cultures were swiped evenly on sterilized Petri dishes with nutrient agar. For the clean discs, 15 µL of synthesized CuO NPs, a positive control (erythromycin), or an aqueous extract of *Zanthoxylum chalybeum* stem bark was added and incubated overnight at 37 °C for 24 hours. The development of a bacterial inhibition zone surrounding the discs was identified, as shown in Fig. 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 bacterial and fungal strains. The compound (CuO NPs) showed less activity against *Staphylococcus aureus* bacteria (6.67 ± 0.47 mm). This occurred because cell wall thickness affects how effectively different bacteria respond to metal oxide nanoparticles.

Gram-negative bacteria, such as *E. coli,* have thinner cell walls, making them more vulnerable to copper nanoparticle penetration than gram-positive bacteria, such as *S. aureus*, which have thicker cell walls (Lopez-miranda *et al*., 2023). The antibacterial and antifungal mechanisms of CuO NPs depend on their concentration, structure, and size. The main mechanism of action involves (i) oxidative stress, (ii) infiltration and cellular disruption, and (iii) degeneration of the membrane and cell wall (Priya *et al*., 2023), (Khan *et al*., 2021). The occurrence of inhibition zones (Fig. 1) 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 surface of the microorganism’s cell wall. This damages the cell wall by killing the pathogens, resulting from the resistance of the synthesized nanoparticles to microorganisms (Bayat *et al*., 2021).

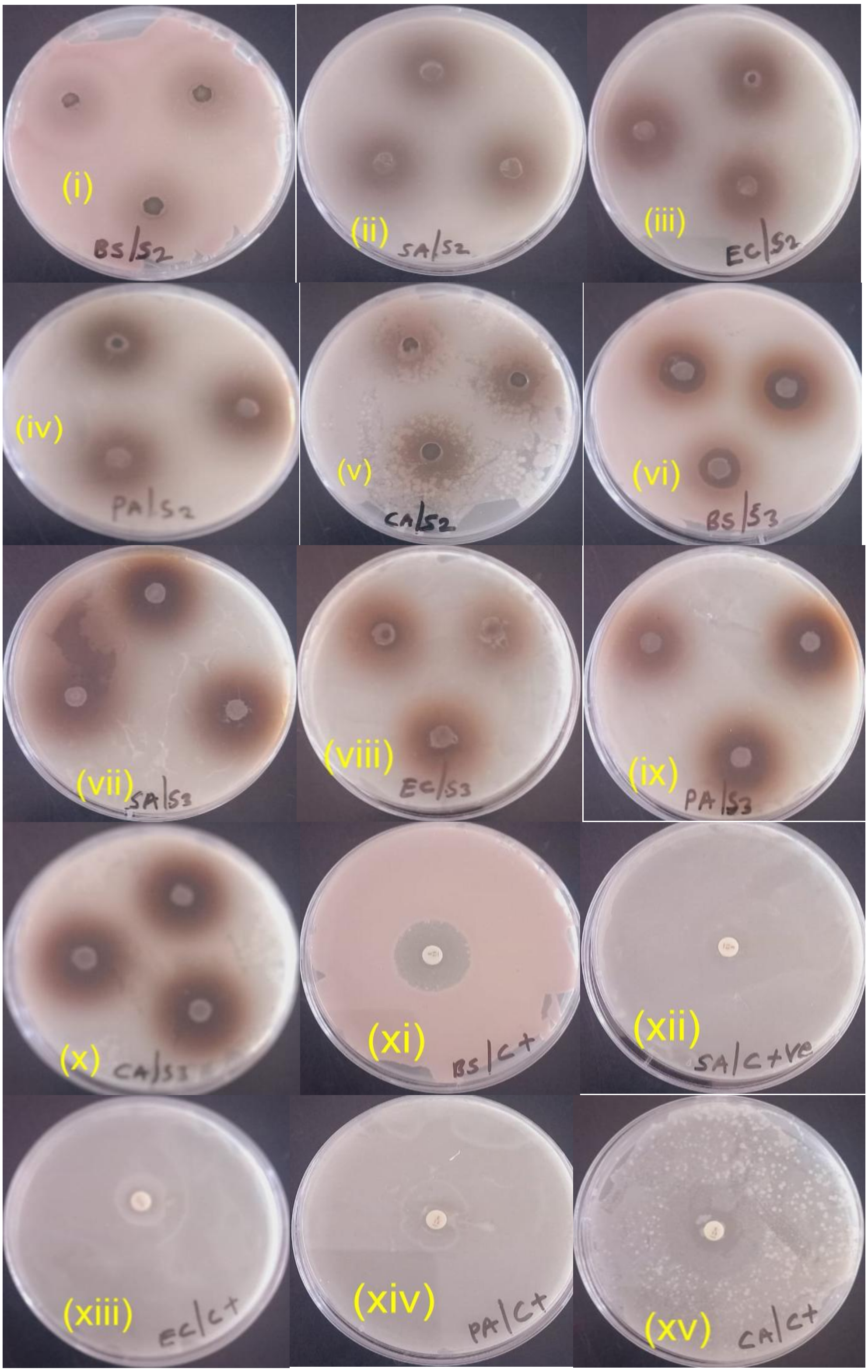

Fig. 11. Zones of inhibition of (a) plant extract against (i) *Bacillus subtilis*; (ii) *Staphylococcus aureus*; (iii) *Escherichia coli*; (iv) *Pseudomonas aeruginosa*; (v) *Candida albicans* (Fungus), and (b) AgNPs against (vi) *Bacillus subtilis*; *(vii) Staphylococcus aureus*; (viii) *Escherichia 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)	*Zanthoxylum chalybeum* extract (S5)	Positive Control (erythromycin)
Bacillus subtilis	13.33±0.47	7.67±0.47	10.67±0.47
Pseudomonas aeruginosa	11.33±0.94	7.50±0.41	7.67±0.47
Escherichia coli	11.33±0.47	5.83±0.24	7.83±0.24
Staphylococcus aureus	6.67±0.47	5.33±0.47	7.33±0.47
Candida albicans	15.33±1.25	9.67±0.94	9.83±0.24

3.7. Statistical analysis: one-way ANOVA

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

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				

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.

Declaration of conflict of interest:

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