Green Synthesis of Copper Oxide Nanoparticles using *Microtrichia Perotitii* Dc Plant Extract: Characterization and Antibacterial Activity

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ABSTRACT

This paper describes the successful manufacture of copper oxidenanoparticles using leaf extracts from the *Microtrichia perotitii* DC plant as a chelating agent and copper tetraoxosulphate (vi) pentahydrate ($CuSO_4.5H_2O$) as a precursor. The nanoparticles were thoroughly characterized by UV-visible spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, energy dispersive X-ray analysis combined with scanning electron microscopy, and thermogravimetric analysis. X-ray diffraction examination revealed the formation of CuO NPs with an average crystallite size of 64 nm. Scanning electron microscopy pictures revealed a primarily heterogeneous sample with a combination of flaky and irregular particle morphologies, indicating some degree of nanoparticle aggregation. Fourier transform infrared spectrophotometer examination revealed a strong bond at 647 cm^{-1}, which corresponds to the stretching vibration mode of CuO nanoparticles. Thermogravimetric studies indicated that the nanoparticles are thermally stable. The synthesized nanoparticles showed antibacterial efficacy against *B. subtilis*, with a zone of inhibition measuring 21.875±3.22 mm

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1. Introduction

Nanotechnology is advancing rapidly due to the unique properties of nanoparticles and nanomaterials, which enable their application across multiple fields [1, 2]. It involves the synthesis, characterization, and study of materials at the nanometer scale (1-100 nm), where both natural and synthetic systems exhibit distinct characteristics and functionalities [3]. The extremely small size of nanoparticles results in a high surface area-to-volume ratio, which significantly enhances their biological activity, catalytic properties, mechanical strength, melting point, optical behavior, thermal and electrical conductivity, and other attributes not observed in bulk materials [4]. These exceptional properties make nanoparticles and other nanomaterials highly sought after for various applications, including biology, electronics, sensors, and optoelectronics. Among different nanomaterials, metal and metal oxide nanoparticles have been extensively studied for their biological applications, such as antibacterial, antifungal, antibiofilm, antioxidant, and anticancer treatments [5-10]. To achieve specific nanoparticle morphologies, several physical and chemical synthesis techniques have been developed, including microwave irradiation, thermal decomposition, sol-gel methods, colloidal thermal synthesis, sonochemical approaches, hydrothermal methods, and rapid precipitation [11]. However, these conventional methods often require extensive labor, high energy consumption, complex processing, and hazardous or expensive chemicals [12]. To minimize environmental risks, the use of non-toxic and eco-friendly materials in CuO nanoparticle synthesis has become a priority. As a result, biological synthesis, particularly plant-based methods, has emerged as a sustainable alternative to traditional approaches [10, 13]. Plant-derived phytochemicals, such as alkaloids, polyphenols, flavonoids, and terpenoids, have been shown to facilitate the reduction of metal ions, leading to the formation of metal nanoparticles [14, 15]. In this study, the leaf extract of *Microtrichia perotitii* DC, a plant from the Asteraceae (Compositae) family, was used for the biogenic synthesis of CuO nanoparticles. This plant is naturally found in several West African countries, including Nigeria, Senegal, Mali, Guinea, Sierra Leone, Ivory Coast, and Ghana [16]. Studies have confirmed that *Microtrichia perotitii* DC leaf extract is rich in various bioactive phytochemicals such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, and triterpenoids, which contribute to its diverse biological applications [16]. To date, numerous plant species have been investigated for the biogenic synthesis of CuO nanoparticles. For instance, Iranian propolis extracts have been used to synthesize CuO NPs, which were characterized using spectroscopic techniques such as UV-Vis

spectroscopy, XRD, and FTIR. The results indicated that the synthesized nanoparticles exhibited strong antibacterial activity, effectively inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* [17, 18]. Similarly, *Parthenium hysterophorus* whole-plant aqueous extract has been employed to produce CuO NPs, which were analyzed using FTIR, UV-Vis spectroscopy, SEM, HRTEM, XRD, TGA, and DLS techniques. These nanoparticles demonstrated an impressive ability to degrade rifampicin, achieving an optimal degradation efficiency of 98.43% under specific conditions (65°C, 50 mg CuO NPs dosage, 10 mg/L rifampicin concentration, and pH 2 within 8 minutes). These findings suggest that CuO NPs derived from *Parthenium hysterophorus* hold significant potential for the environmental remediation of antibiotic pollutants [18]. In this study, *Microtrichia perotitii* DC leaf extract was employed for the first time in the biogenic synthesis of CuO nanoparticles. Given its established biomedical significance, the synthesized CuO NPs were further evaluated for their antibacterial properties, offering a green and sustainable alternative to conventional synthesis methods.

2. Experimental

2.1 Preparation of *Microtrichia perotitii* DC plant extract

The *Microtrichia perotitii* DC plant used in this study was thoroughly washed with sterile distilled water to remove any surface contaminants. It was then air-dried for one week before being finely ground using a pestle and mortar. The powdered leaves of (20 g) was mixed with 100 mL of distilled water and ethanol and allowed to incubate at room temperature for 24 hours [19]. After incubation, the solution was carefully filtered using Whatman No.1 filter paper and subsequently used for antibacterial studies.

2.2 Preparation of CuO NPs using *Microtrichia perotitii* DC plant extract

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was employed as the precursor in this synthesis. A solution of $CuSO_4 \cdot 5H_2O$ (80 mL, 0.1 M) was carefully mixed with 20 mL of the *Microtrichia perotitii* DC plant extract under constant stirring. The reaction mixture was then heated to 80°C on a heating mantle equipped with a magnetic stirrer for 2 hours. Once the reaction was complete, the mixture was allowed to cool and settle. The resulting colloidal suspension was then filtered through Whatman No.1 filter paper and dried using a desiccator.

2.3.Antibacterial activities

Bacterial strains and Antibacterial Assay to assess the antibacterial activity of CuO NPs against six bacterial strains the disc diffusion method [20-22] was used with minor modifications for two Gram-positive strains (Staphylococcus aureus ATCC 33863, Bacillus subtilis ATCC 23857) and four Gram-negative strains (Pseudomonas aeruginosa ATCC 27853, Salmonella typhi ATCC 19430, Escherichia coli ATCC 25922, and Clostridium botulinum ATCC 19397). The bacterial strains listed above were purchased from the University of Ilorin Teaching Hospital Laboratory. The surface of nutrient agar media plates was swabbed slowly in three separate directions with an aliquot of 100 µL of inoculum that had been pre-adjusted (10^8 cells/mL) for seeding density. The plates' surface was covered with sterile filter paper discs containing 5 µL (20 mg/mL DMSO) of CuO NPs solution. The use of DMSO-infused discs as a negative control was contrasted with the use of ciprofloxacin (5 µg) loaded discs as a positive control. These was followed by an incubation period of 24 hrs at 37°C. After which the zone of inhibition (ZOI) was measured.

2.4.**Characterization**

The infrared spectrum of the solid CuO NPs was recorded by FTIR spectrometer (ZN- FTIR 530) to identify the functional Groups present in the nanoparticles. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (SPECORD-200). The particle shape of CuO NPs was determined by SEM. The model used is Phenom Prox model. XRD (Rigaku MiniFle 600 XRD Diffractometer) was used to determine the crystallographic structures of the nanoparticles and TG analyzer (TGA-Q500 series, TA instruments) was used to determine the thermal stability of the nanoparticles. Powder XRD analysis has been carried out to examine the crystallinity and to check the purity of the synthesized Nps. The optical properties of the nanoparticle were analyzed using UV-Vis spectroscopy. The presence of functional group was analyzed by FTIR spectroscopy. The thermal stability of the synthesized nanoparticle was analyzed by TGA. Morphology of the synthesized copper dioxide nanoparticles were analyzed using SEM.

2.5.Statistical Analysis

The data obtained from the experiment was utilized for statistical analysis. All trials were conducted in triplicate, and the findings are given as mean±standard deviation. To ascertain the statistical significance, we used SPSS with a predetermined significance level (at $p < 0.05$).

3. Results and Discussion

3.1. Fourier transforms infrared spectroscopy (FTIR) studies

Figure 1 presents the FTIR spectra of CuO NPs synthesized using *Microtrichia perotitii* DC plant extract. FTIR analysis was conducted using the KBr pellet technique within the spectral range of 4000–400 cm^{-1} to confirm the purity of the nanoparticles and identify the functional groups present. Metal-oxide absorption bands generally appear in the fingerprint region (400–1500 cm^{-1}) due to interatomic vibrations [23]. The FTIR spectra exhibited a peak at 3301 cm^{-1}, which corresponds to the O-H stretching vibration, indicating the presence of phenols and water molecules in the plant extract [18]. The peak observed at 1644 cm^{-1} is attributed to C=O stretching vibrations, which are characteristic of amides [24]. A peak at 2890 cm^{-1} corresponds to C-H stretching vibrations. A band at 1105 cm^{-1} is associated with C-O stretching in flavonoids [25].

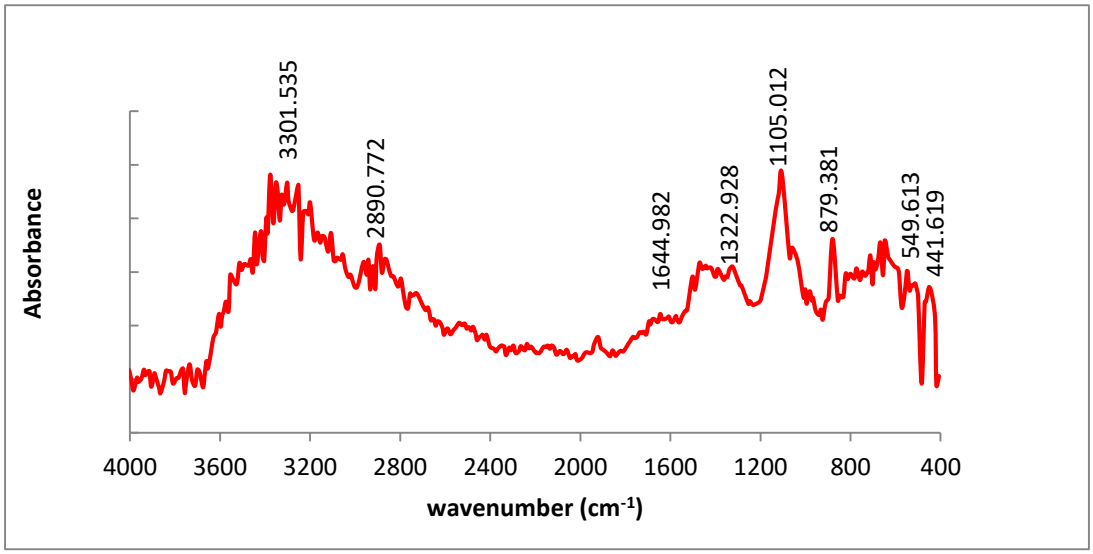

Figure 1: FTIR spectrum of *M. perotitii* synthesized CuO NPs

The presence of amines is indicated by the peak at 1322 cm^{-1}, corresponding to C-N stretching vibrations [25]. Additionally, the characteristic Cu-O stretching vibrations were identified at 441 cm^{-1} and 549 cm^{-1}, confirming the successful synthesis of CuO NPs[18].The metal-oxygen absorption frequencies obtained in this study align with previously reported FTIR spectra of CuO nanoparticles [26]. The FTIR results indicate that phytochemicals such as phenols and flavonoids in the plant extract played a crucial role in reducing and stabilizing metal ions during the synthesis of CuO NPs.

3.2. Optical properties of CuO NPs

The synthesis of CuO NPs was confirmed through UV-Vis spectroscopy, and the results are presented in Figure 2. A prominent absorption peak was observed at 375 nm, which corresponds to the surface plasmon resonance (SPR) band of CuO NPs. The presence of an SPR absorption band at this wavelength confirms the successful formation of CuO NPs [17]. Additionally, as the wavelength increased, the absorbance intensity gradually decreased, suggesting that nanoparticle formation did not occur at longer wavelengths. These findings align with previous studies, which reported the formation of CuO NPs within this spectral range [27].

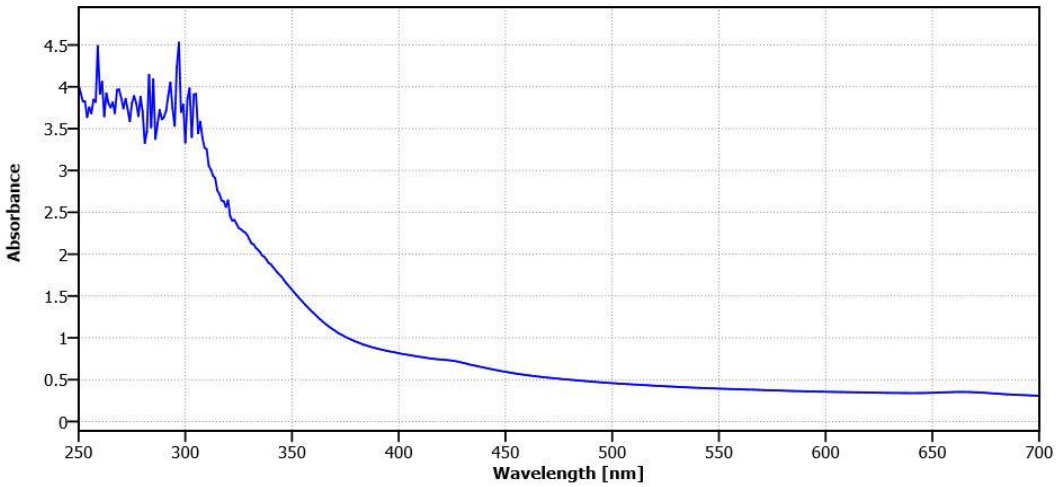

Figure 2: Absorption spectrum of *M. perotitii* synthesized CuO NPs

3.3. X-ray diffractometry (XRD) analysis

The structural characteristics and crystallinity of CuO nanoparticles synthesized using *Microtrichia perotitii* DC plant extract were examined through X-ray diffraction (XRD), as shown in Figure 3. The sharp and intense diffraction peaks observed in the XRD pattern indicate that the synthesized CuO NPs possess a high degree of crystallinity. The diffraction peaks appeared at 2θ = 19.30° and 31.72°, corresponding to the (111), (220), and (311) crystal planes, as referenced in the JCPDS file no. 00-063-0093. The crystallite size of the CuO nanocrystals was determined using Debye-Scherrer's equation (1).

$$D = \frac{0.89\lambda}{\beta \, x \cos\theta} \qquad (1)$$

which considers parameters such as the average crystal size (D), X-ray wavelength (λ), and the full width at half maximum (β). The average crystallite size of CuO NPs in the most intense diffraction plane was 64 nm. XRD analysis confirmed that the CuO NPs exhibited a face-centered cubic structure. These results align with previous studies that have reported the green synthesis of CuO NPs within this size range [28], further supporting the findings of this research.

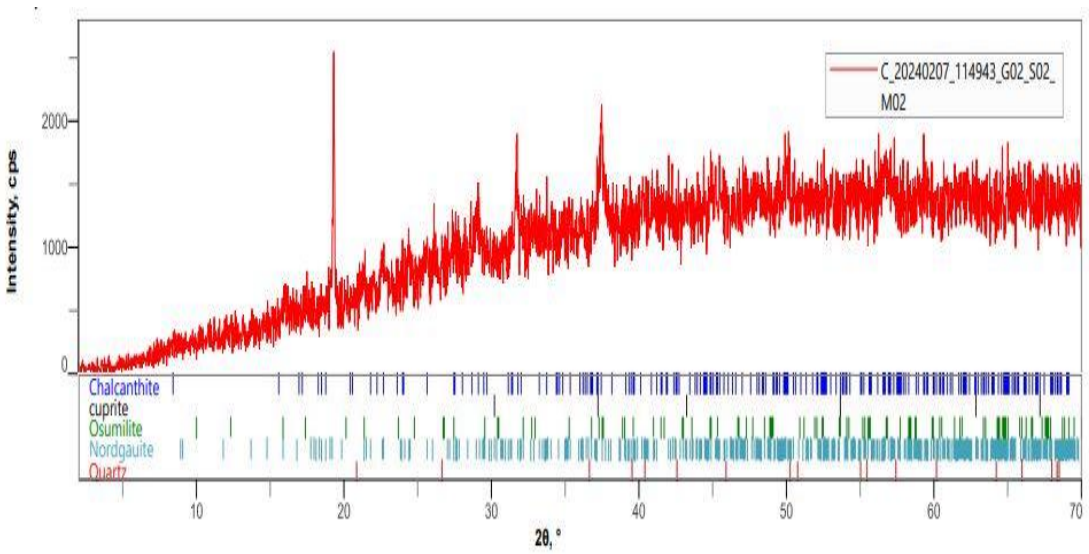

Figure 3: XRD pattern of *M. perotitii* synthesized CuO NPs

3.4. Surface morphology characterization

The shape and surface structure of the synthesised CuO NPs were analysed using SEM, and the resulting micrograph is shown in Figure 4. The SEM image depicts a non-homogeneous sample with a mixture of flaky and irregular particle morphologies packed together to form aggregate structures, showing nanoparticle agglomeration. Previous experiments yielded similar results, as reported by [29]. Cracks and rough surfaces may indicate stress during the synthesis or drying processes, which could be due to temperature fluctuations or solvent evaporation.

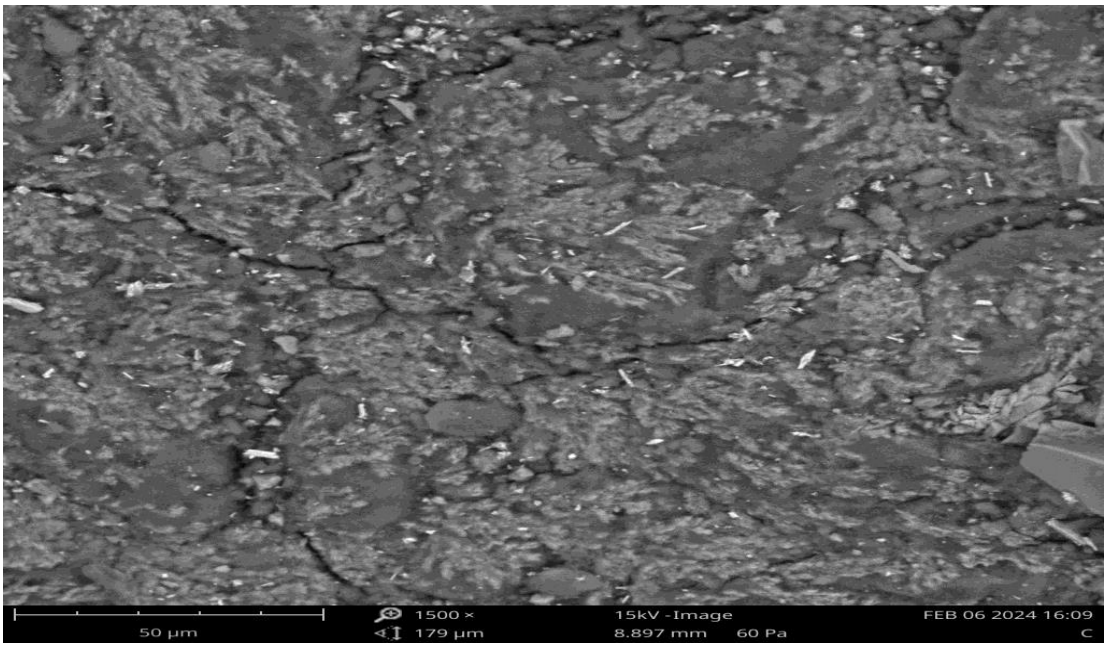

Figure 4: SEM micrograph *M. perotitii* synthesized CuO NPs

3.5 Thermogravimetric Analysis (TGA).

Thermogravimetric analysis (TGA) was conducted on *Microtrichia perotitii* DC plant extract-derived CuO NPs to evaluate their thermal stability and degradation pattern. The TGA spectra indicate a significant decomposition of the sample as the temperature increases. The initial weight loss between 25°C and 100°C is attributed to the evaporation of ethanol and water present in the sample [30]. As shown in Figure 5, a substantial weight loss of approximately 70–80% occurs when the temperature reaches 500°C, likely due to the presence of volatile organic compounds from the plant extract. However, beyond 450°C up to 900°C, no further mass loss was observed, suggesting that CuO NPs remain thermally stable within this temperature range. The thermogram clearly demonstrates that CuO NPs exhibit high thermal stability up to 900°C.

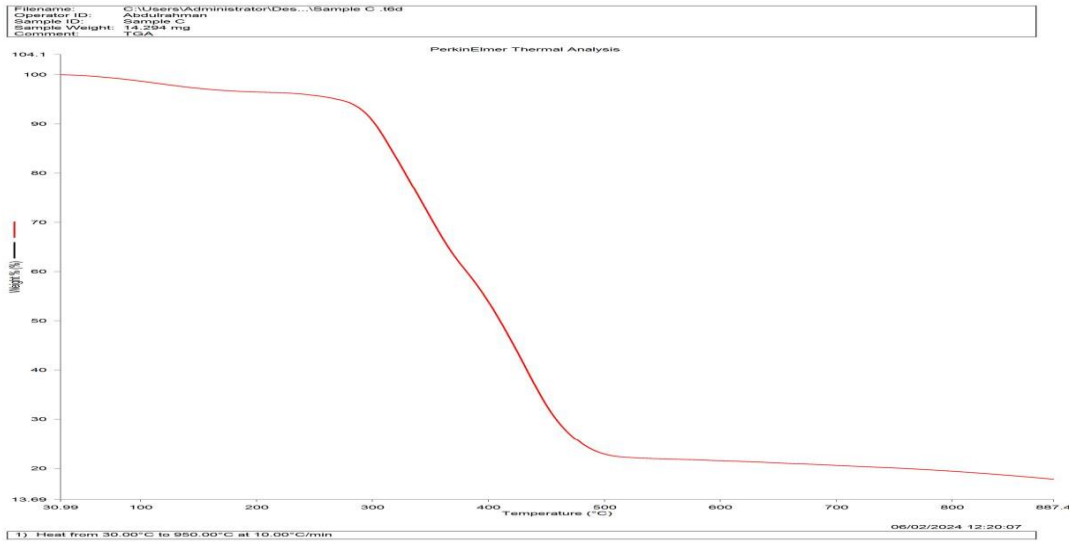

Figure 5: TGA curve of *M. perotitii* synthesized CuO NPs.

3.5 Evaluation of antibacterial activity using CuO NPs prepared using *Microtrichia perotitii* DC plant extract

The antibacterial potential of CuO nanoparticles synthesized from *Microtrichia perotitii* DC plant extract was assessed against various bacterial pathogens, including *Staphylococcus aureus* (Gram-positive), *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Salmonella typhi* (Gram-negative), *Escherichia coli* (Gram-negative), and *Clostridium botulinum* (Gram-negative). The zone of inhibition (ZI) was determined as presented in Table 1, demonstrating that CuO NPs exhibited antibacterial activity against certain bacterial strains compared to the positive control. Among the tested pathogens, CuO NPs were most effective against *Bacillus subtilis* (21.875±3.22 mm ZI) and *S. aureus* (11.875±1.03 mm ZI), showing a stronger inhibitory effect on Gram-positive bacteria. In contrast, CuO NPs exhibited moderate activity against *P. aeruginosa* (14.225±1.58 mm ZI) but had no antibacterial effect against *S. typhi*, *E. coli*, and *C. botulinum*. These results suggest that Gram-negative bacteria demonstrate greater resistance or tolerance to CuO NPs compared to Gram-positive strains. This difference in susceptibility could be attributed to structural and compositional variations in bacterial cell walls [31-33]. Gram-negative bacteria possess an outer membrane that acts as a barrier against antimicrobial agents, contributing to their higher resistance. Furthermore, Gram-negative strains

are widely recognized for their increased resistance to antibiotics [34-36], which has led to the emergence of numerous antibiotic-resistant infections [37].

Table 1: Antimicrobial activity of *M. perotitii* synthesized CuO NPs

Test organisms	Activity of CuO NPs (mm)	Ciprofloxacin (5 µg disc)
S. aureus	11.875±1.03^{b}	25.06±3.26^{d}
P. aeruginosa	14.225±1.58^{c}	21.55±1.50^{c}
S. tyhi	0.000±0.00^{a}	18.55±2.30^{a}
E. coli	0.000±0.00^{a}	20.70±3.57^{c}
C. botulinum	0.000±0.00^{a}	19.55±0.79^{f}
B. subtilis	21.875±3.22^{d}	21.38±2.10^{d}

The superscript lettered (a-f) indicate significant difference (at $p < 0.05$) when subject to SPSS test. The findings are given as mean ± standard deviation

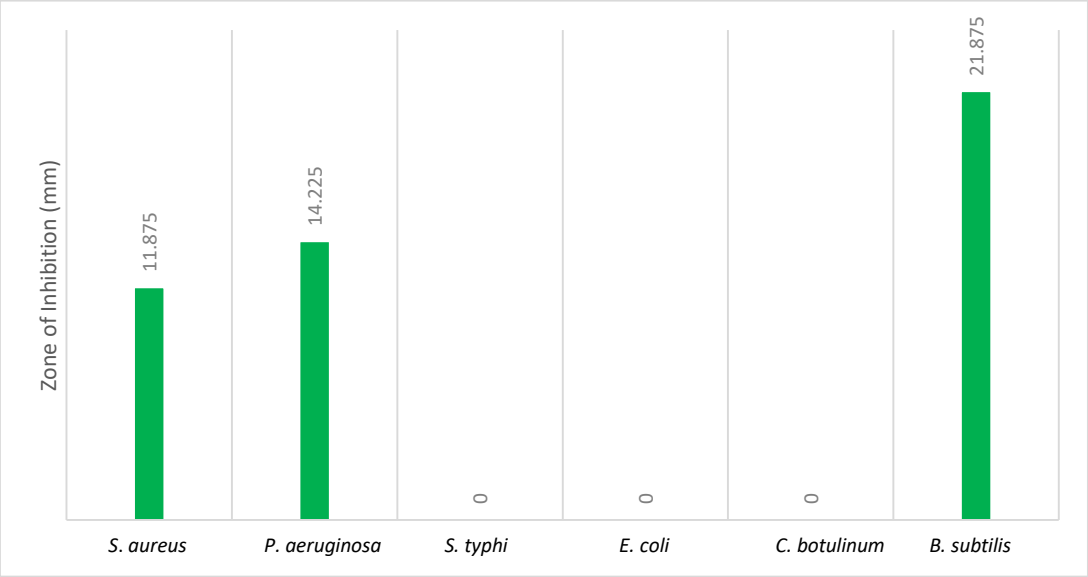

Figure 6: Antimicrobial activity of *M. perotitii* synthesized CuO NPs

4. Conclusions

The current study offers a green synthesis of CuO NPs that is both cost-effective and environmentally friendly, using *Microtrichia perotitii* DC plant extracts as reducing and capping agents. UV-Vis spectroscopy, FTIR, SEM, XRD, and TGA were employed to characterise the CuO NPs. The UV-Vis spectrum revealed an absorption band at 375 nm from CuO NPs. The FTIR analysis showed that metal oxide nanoparticles were reduced from copper ion solutions to CuO NPs with a stretching vibration at 647 cm^{-1}. SEM micrographs show the uneven form of CuO NPs. XRD analysis validated the crystallinity of CuO NPs, with a predicted average crystallite size of 64 nm. The zone of inhibition statistics indicate that the produced nanoparticle was more effective against Gram-positive bacterial strains than Gram-negative pathogens. CuO NPs are expected to be used in biomedical applications in the future since they are biosynthesised from natural chemicals in an environmentally friendly manner. However, the precise antibacterial and synergistic actions of *Microtrichia perotitii* DC in combination with CuO NPs may need to be determined. As a result, clinical trials must be developed to assess the function of green biosynthesised CuO NPs utilising *Microtrichia perotitii* DC in order to determine the long term efficacy of this approach and *Microtrichia perotitii* DC extract.

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