

Evaluation Of Antioxidant And Antimicrobial Effects Of Iron-Doped Copper Oxide Nanoparticles Against Some Selected Organisms

Abiola Olanike Adesina^{a*}, Olusegun Richard Adeoyo^b, Owolabi Mutolib^a
Bankole

^aDepartment Of Chemical Sciences, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria

^bDepartment Of Microbiology, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria
Correspondence e-mail: abiola.adesina@aaua.edu.ng

Abstract

Treatment of infectious diseases has become more challenging and has had a major influence on treatment rates due to the advent of new transmissible diseases and the rise in resistance of pathogens to available antibiotics. The use of nanoparticles as an antibiotic replacement has also gained attention due to the multidrug resistance of antibiotics to bacterial infections. This study investigated the synthesis and characterization of iron doped copper oxide nanoparticles (Fe-CuO Nps). Different characterization techniques which include X-ray diffraction (XRD), Scanning Electron Microscopy (SEM)/Energy Dispersive X-ray Spectroscopy (EDX) and Transmission electron microscopy TEM methods of analysis were used to confirm the successful synthesis of the nanoparticles. Thereafter, plate agar diffusion assays were used to determine the antimicrobial efficacy of the synthesized nanoparticles against ten different organisms which include *Klebsiella ornithinolytica*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Enterococcus faecalis* (bacteria), *Candida albicans*, *Geotrichum candidum* (fungi). The antioxidant efficiency of the synthesized Fe-CuO Nps was analyzed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. The obtained results from this investigation revealed that all the test organisms were susceptible to the synthesized nanoparticles. The zones of inhibition (ZOI) ranged between 22.00 and 32.00 mm which an indication that the synthesized nanoparticles exhibited excellent antimicrobial potency. Results obtained from this study revealed that the synthesized Fe-CuO Nps exhibited a strong antimicrobial and antioxidant property. Therefore, Fe-CuO nanoparticles have the potential for controlling microbial infections.

Keywords: Antimicrobial activity; Antioxidant activity; Fe-CuO Nps; Microbial infections

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I. Introduction

One of the leading causes of morbidity and death today is microbial infections. Additional bactericidal and fungicidal methods must be developed in light of the growing worry over biofilm-associated infections and multidrug-resistant microbial strains. As a result, new and developing materials based on nanoparticles have received particular interest in the field of antimicrobial chemotherapy (Zinjarde, 2012; Tasnim et al., 2024).

The development of microbial pathogen resistance to currently used synthetic antibiotics has been the main focus of study in recent years. Human illness is caused by a number of species, including *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermidis*. Appropriate antibiotic treatment is necessary to remove illnesses brought on by these microbes. Pathogens may not always be killed or have their growth inhibited by the antibiotic that is given. It has been demonstrated that pharmaceutical companies' antimicrobial agents are not effective against bacteria and some yeasts that are resistant to many drugs. Finding and creating nano-based medications or agents to treat microbial infections is crucial in light of these pressing issues (Jinu et al., 2017, 2016).

Several researchers have investigated the antimicrobial potency of some nanoparticles. Adesina et al. (2021), reported the use of alginate stabilized iron oxide nanoparticles against *Bacillus subtilis*, *S. aureus*, *Candida albicans*, *Vibrio cholerae*, *P. vulgaris*, and *Micrococcus luteus*. The author also investigated the antibacterial activity of silver conjugated magnetic iron oxide nanoparticles against *Serratia marcescens* and *S. aureus*. The reports from these studies revealed the susceptibility of the test organisms to the synthesized

nanoparticles which is an indication of the strong potency of the nanoparticles as an antibacterial agent (Adesina et al., 2021; Adesina, 2022).

More so, huge attention has been given to semiconductor and metallic nanoparticles in the field of nanomedicine. This interest relates to physicochemical features of size and form. A key component of these characteristics is the nanoparticles' surface area to volume ratio (Æ and Hofmann, 2008; Wu et al., 2008; Kalbassi et al., 2019). Copper oxide (CuO) and its alloy on a nanoscale are among the key application materials in this industry (Huang et al., 2010; Longano et al., 2012; Taran et al., 2016). A growing number of people are interested in copper oxide nanoparticles because of their special optical, thermal, electrical, chemical, and biological characteristics (Bhattacharjee, 2016; Qamar et al., 2020). These characteristics allow for a broad range of applications in several industries, including the development of sensors, storage devices, super capacitors, and infrared filters as well as the environment and health sectors (Dagher et al., 2014; Qamar et al., 2020).

Since transition metal oxide nanoparticles have fascinating physical and chemical properties, there have been numerous attempts to create them. Out of all of them, CuO, a p-type semiconductor with a low 1.2eV band gap, has been studied the most because of its potential applications in gas sensors, solar cells, batteries, field transistors, semiconductors, and magnetic storage media (Chen et al., 2013; Shrestha et al., 2010; Wu et al., 2010; Xi et al., 2012; Yang et al., 2013). Additionally, CuO nanomaterials are excellent candidates for application as therapeutic agents due to their antibacterial properties (Wages et al., 2015). The fight against drug resistance in the healthcare industry is currently posing a significant challenge to researchers. Generally, metal oxide nanoparticles are known for their excellent antibacterial and antifungal activities (Shahzad et al., 2022; Usman et al., 2013).. Different metal oxide nanoparticles like zinc oxide, iron oxide, have been explored for their various applications. For biological applications, inorganic nanocrystalline metal oxides are more suited since they may be made with incredibly large surface areas (Fathima et al., 2019). In contrast to organic and microbiological agents, CuO nanoparticles are stable, durable, and have a longer shelf life. Copper oxide possesses antimicrobial, antibacterial, antifungal, gas sensing, ultraconductive, catalytic, optical, magnetic phase change, and biocidal qualities (Rajeshkumar et al., 2021).

A lot of investigations have been carried out on the effect of metal dopant treatment on nanoparticles. For instance, the antibacterial activity of ZnO nanoparticles, and Mn-doped ZnO nanoparticles were tested against both Gram-negative and Gram-positive organisms by Rekha et al. in 2010. Results from the investigation revealed that the Mn-doped ZnO nanoparticles exhibited better antibacterial activity than the undoped ZnO (Rekha et al., 2010). According to (Guo et al., 2015), Ta-doped ZnO nanoparticles exhibited a more potent and greater antibacterial activity in comparison to pure ZnO nanoparticles. A standard microbiological approach was used to conduct the investigation against a few test species, including Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*B. subtilis* and *S. aureus*). Also, Pugazhendhi et al. (2018) reported the antimicrobial potency of Fe doped copper oxide nanoparticles against *Staphylococcus aureus*, *Staphylococcus epidermidis* and a fungus (*Candida albicans*). The result from the study revealed a significant antimicrobial potency of the synthesized nanoparticles against the test organisms (Pugazhendhi et al., 2018).

Extensive research has been done on the effect of introducing transition metals into bare iron oxide nanoparticles as dopants. For instance, Casula et al. (2016) reported that desired morphological or structural features and intrinsic magnetic properties could be obtained by doping (Casula et al., 2016). More so, Venkatesan et al. (2015) also investigated that effect of dopant on iron oxide which had led to enhanced antimicrobial activity of the doped iron oxide nanoparticles (Venkatesan et al., 2015). In our recent studies the investigation of the antimicrobial activities of ZnO Nps and Fe-ZnO Nps (iron-doped zinc oxide nanoparticles) was carried out. The obtained results revealed that the iron doped zinc oxide nanoparticles demonstrated a significant antimicrobial activity than the undoped zinc oxide nanoparticles. (Adesina, 2025a; Adesina, 2025b). The obtained results are consistent with what has been previously reported by Sharma et al. (2015). The experiment results revealed that doping in the nanomaterials is important and is capable of improving the antibacterial activity (Fathima et al., 2019; Sharma et al., 2015; Tasnim et al., 2024).

Numerous investigations have demonstrated that inorganic metal oxide nanoparticles, including CaO, ZnO, MgO, and CuO, have strong antibacterial and antioxidant properties (Zhao et al., 2008). Nanoscale inorganic particles may be well-suited for future applications of their antioxidant qualities in a range of sectors, which include functional food additives, cosmetics, and pharmaceuticals (Chem et al., 2018; Xuemei Ge, 2022). These nanoparticles' antimicrobial properties may be attributed to their capacity to produce reactive oxygen species (ROS) on the oxides' surface, which can cause mechanical and physical harm to microorganisms (Li et al., 2018; Park et al., 2019). Due to the fact that these inorganic oxides are extremely active even at low dosages and include essential minerals for human health, using them as antibacterial agents has several advantages. Additionally, inorganic antibacterial agents have greater heat resistance, greater selectivity, less toxicity, and improved durability (Zhao et al., 2008). The antioxidant potency of copper oxide nanoparticles was reported by

Rehana et al. (2017) using three different assays ((ABTS scavenging assay (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay and the hydrogen peroxide (H₂O₂) scavenging assay). The result from the investigation revealed a significant antioxidant property of the nanoparticles (Rehana et al., 2017).

There hasn't been much focus on the antifungal effect of nanoparticles, and only a few reports have been on the subject. Herein, we report the synthesis, characterization of iron doped copper oxide nanoparticles (Fe-CuO Nps). The results obtained from TEM, XRD and SEM/EDX analysis confirmed the successful synthesis of the nanoparticles. We further investigated the antimicrobial and antioxidant properties of the synthesized nanoparticles. The antimicrobial activity was tested against variety of organisms (bacteria and fungi). The antioxidant potency of Fe-CuO Nps was investigated using DPPH free radical scavenging assay. The antifungal and antibacterial susceptibility test was carried out against ten organisms. The result revealed excellent antioxidant property of the nanoparticles and all the organisms were susceptible to the synthesized copper oxide nanoparticles.

II. Materials And Methods

Chemicals and reagents

Iron (III) chloride hexahydrate (FeCl₃.6H₂O), Copper acetate monohydrate [Cu(CH₃COO)₂.H₂O] glacial acetic acid (CH₃COOH), Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO). All the reagents were of analytical grade and purchased from sigma Aldrich. The reagents were utilized in their original form without any further purification. Reagents were prepared and used according to manufacturer's instruction.

Preparation of Iron-doped copper oxide nanoparticles (Fe-CuO Nps)

Iron-doped copper oxide nanoparticles (Fe-CuO Nps) were produced using a conventional co-precipitation method, as described by (Rani et al., 2016) with slight modifications. This method involved combining an aqueous solution of ferric iron and copper acetate. Specifically, a mixture consisting copper acetate (0.3994 g), ferric chloride FeCl₃.6H₂O (4 g), glacial acetic acid (2 mL), sodium hydroxide (8 g) and deionized water (200 mL) was prepared. Simultaneously, copper acetate (0.3994 g) was dissolved in 200 mL of deionized water. The solution containing copper acetate and deionized water was placed on a stirrer. Afterwards, 2 mL of glacial acetic acid was added to enhance complete dissolution of the copper acetate. Ferric chloride was added to the solution and allowed to stir for 20mins. Sodium hydroxide (8 g) was weighed and dissolved in 100 mL of deionized water. The solution was slowly added drop by drop to form precipitate within the mixture and allowed to stir for another 10 min. The solution was allowed to stand for 15 min after which it was filtered in order to separate the filtrate from the residue. The filtrate was then washed three times with deionized water; the filtrate was later subjected to calcination in the furnace for 3 hours. Thus, the iron-doped copper oxide nanoparticles were successfully obtained after this process.

Characterization of Iron-doped copper oxide nanoparticles (Fe-CuO Nps)

The synthesized iron-doped copper oxide nanoparticles (Fe-CuO Nps) were characterized by using some analytical techniques which include X-ray Diffraction (XRD) Analysis, Scanning Electron Microscopy (SEM) Analysis and Transmission electron microscopy (TEM). **X-ray diffraction (XRD)** analysis was performed on a Bruker D8 Discover diffractometer, equipped with a Lynx Eye detector, under Cu-K_α radiation ($\lambda = 1.50405 \text{ \AA}$). Data were collected in the range $2\theta = 10^\circ$ to 70° , scanning at 1° min^{-1} with a filter time-constant of 2.5 s per step and a slit width of 6.0 nm. The samples were placed on a zero-background silicon wafer slide. **Transmission electron microscopy (TEM):** The images were acquired using JEOL 2100F equipment and the copper grid coated (using drop-dry) with materials to be investigated. **Energy dispersive X-ray (EDX)** analysis spectra were obtained using an X-ray microanalysis system added as a module on the Nova NanoSEM 200.

Preparation of media

To create the nutrient agar medium, an estimated quantity of nutrient agar powder (28 g) was measured and combined with one liter of distilled water. The mixture was thoroughly mixed by boiling until all the ingredients were completely dissolved. Next, the solution was subjected to autoclaving at a temperature of 121°C for 15 min to ensure proper dissolution. Afterward, the mixture was left to cool to approximately 45°C and poured into petri dishes. These plates were left undisturbed until they solidified before the process of inoculation took place.

Antimicrobial susceptibility test

Test Organisms

Ten different clinical isolates were collected from out-patients ward of the Federal Medical Centre, Owo, Ondo State, Nigeria. The morphological and biochemical characteristics of the organisms were

determined and compared with those of Bergey's manual of systematic bacteriology 9th edition. The isolates used are *Klebsiella ornithinolytica*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Enterococcus faecalis*, *Candida albicans*, and *Geotrichum candidum*.

Determination of antimicrobial activity

Antimicrobial activity of the synthesized Fe-CuO Nps was determined against each bacterial culture (test organism) with inoculum size of 1.5×10^8 CFU/mL using agar diffusion methods. Each organism was seeded on already solidified Petri plates of Mueller-Hinton agar (MHA). A sterile 6 mm cork borer was used to make 3 wells on already solidified agar, each of the wells was filled with either 100 μ L metal complex dissolved in 30% DMSO, amoxicillin (50 μ g/mL, positive control), or water (30% DMSO, negative control). The plates were allowed to stand for about 2 h to allow absorption of the metal complex and ligands dissolved in 30% DMSO into the medium after which they were incubated at 37°C for 24 h for microbial growth. Macro-broth dilution technique as modified by Ajibade et al. (2012) was used in this research for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Those recorded as MIC were the lowest concentrations of the tested metal complex dissolved in 30% DMSO that inhibited each tested organism. Serial dilutions of the metal complex dissolved in 30% DMSO were carried out to give a concentration of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL. A 2 mL of each diluted concentration (antibacterial agent) was added to 18 mL of pre-sterilized molten MHA mixed properly and allowed to set. After which the standardized inoculum (1.5×10^8 CFU/mL) was seeded on the plates, the plates were incubated at 37°C for 24 h, and results were observed and recorded.

Killing rate dynamics

A 50% dilution of metal complex was used to observe the killing rate of the antimicrobial agent on the test organisms 1 mL of the 50% diluted oil was added to a 9 mL nutrient broth containing 1 mL test organism 18 h old culture (1.5×10^8 CFU/mL). The killing rate was measured using UV/Visible spectrophotometer at a wave length of 600 nm for 48 h.

Antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of the Fe-CuO Nps was investigated using the DPPH method and was carried out with slight modification according to the method reported by Bhakya et al. (2016). The free radical scavenging activity of Fe-CuO Nps was determined using the stable radical DPPH and ascorbic acid was used as standard. A 1 mL of different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) of Fe-CuO Nps was mixed with 1 mL freshly prepared DPPH (1 mM in methanol) solution and vortexed thoroughly. Then, the solution was incubated at room temperature in the dark for 30 min. The absorbance was recorded at 517 nm using UV-Vis spectrophotometer DPPH was used as a control and methanol was used as a blank solution. The free radical scavenging activity was expressed as the percentage of inhibition which was determined using the following formula

$$\text{Percentage (\%)} \text{ of scavenging} = \frac{P_c - P_s}{P_c} \times 100$$

Where P_c is the absorbance of control and P_s is the absorption of Fe-CuO Nps/vitamin C.

III. Results

Characterization of iron-doped copper oxide Nanoparticles (Fe-CuO Nps)

Transmission electron microscopy (TEM) image of Fe-CuO Nps

The TEM image of the synthesized nanoparticles at different magnifications revealed the morphology of the nanoparticles, particle size distribution, and the shape of the nanoparticles. From the TEM result, the nanoparticles were spherical in shape. The particle size distribution ranged from 3.83 nm – 32.83 nm with the majority of the nanoparticles between 15.47 – 17.8 nm having the highest distribution. The average particle size was found to be 17.40 ± 7.9 nm.

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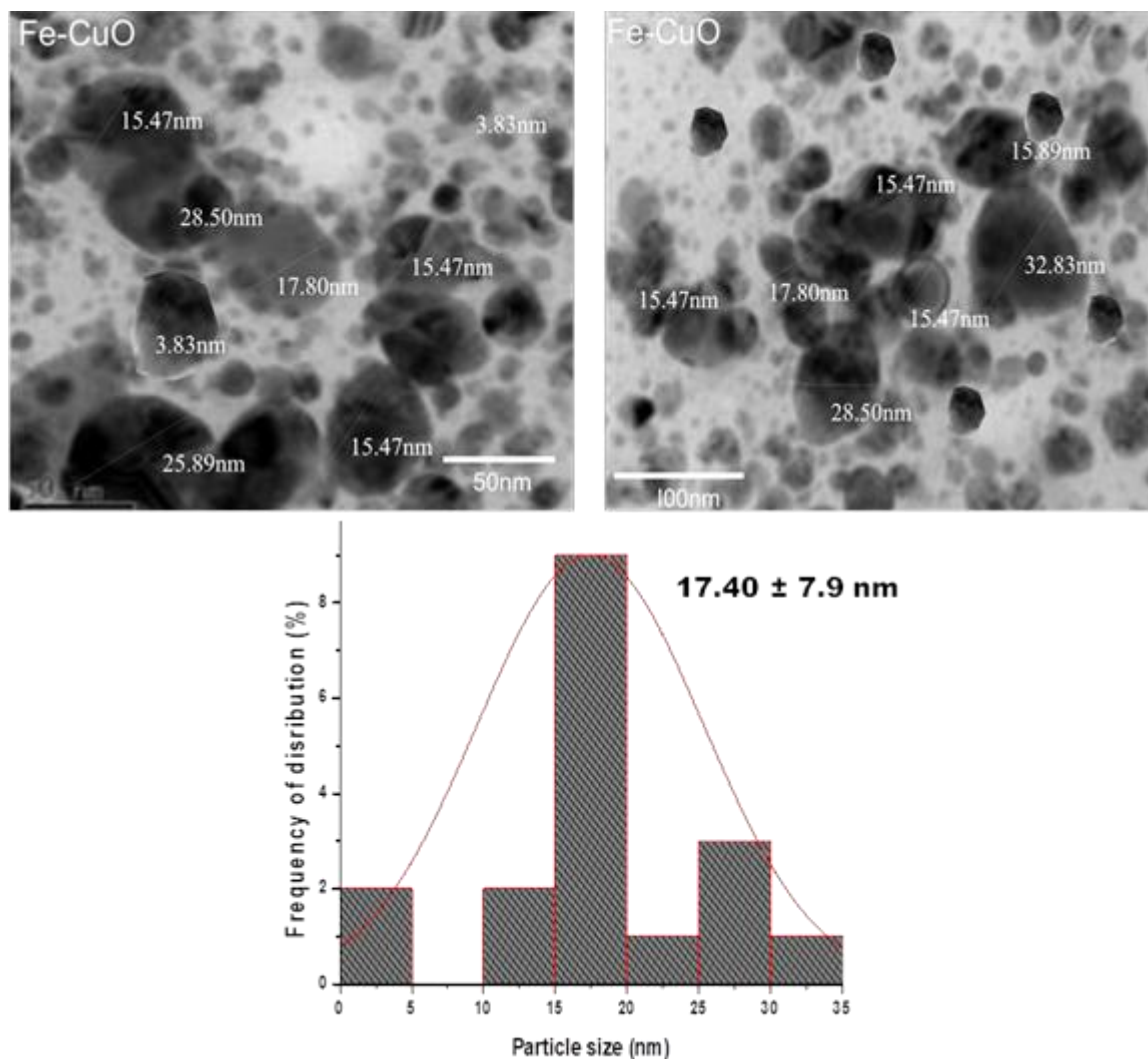


Figure 1: TEM image of the Fe-CuO Nps at different magnifications and the corresponding histogram

X-ray diffraction (XRD) pattern of Fe-CuO Nps

The XRD diffractograms of Fe-CuO Nps, is presented in Figure 2. The diffraction peaks at 2θ corresponds to the expected peaks for iron and copper oxide. Peaks were observed in diffractogram at 2θ equals 35.8, 43.5 (311 and 400) which are assigned to the diffraction peak of iron. Also, peaks found at 35.7 and 53.3 (111 and 202) were assigned to copper oxide.

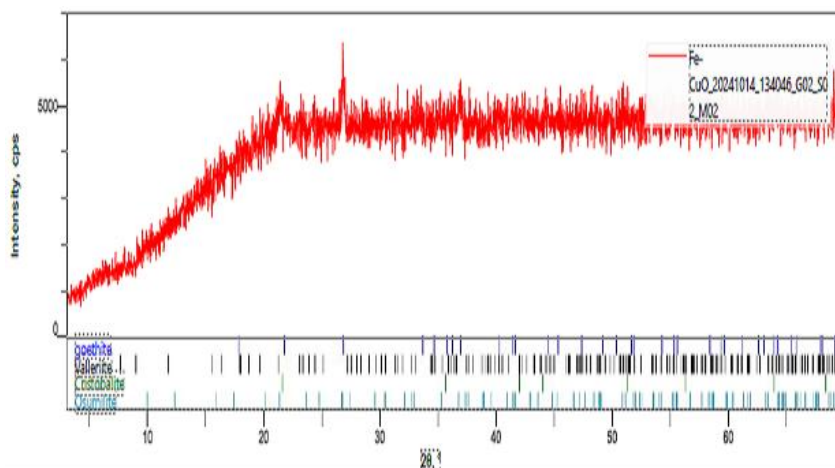


Figure 2: XRD pattern of iron-doped copper oxide nanoparticles (Fe-CuO Nps)

Scanning Electron Microscopic Image (SEM) of Fe- CuO Nps

The SEM image of the synthesized nanoparticles is presented in Figure 4. It shows the morphology of the copper oxide nanoparticles. The nanoparticles had a spherical shape, varied in size, and were not evenly distributed.

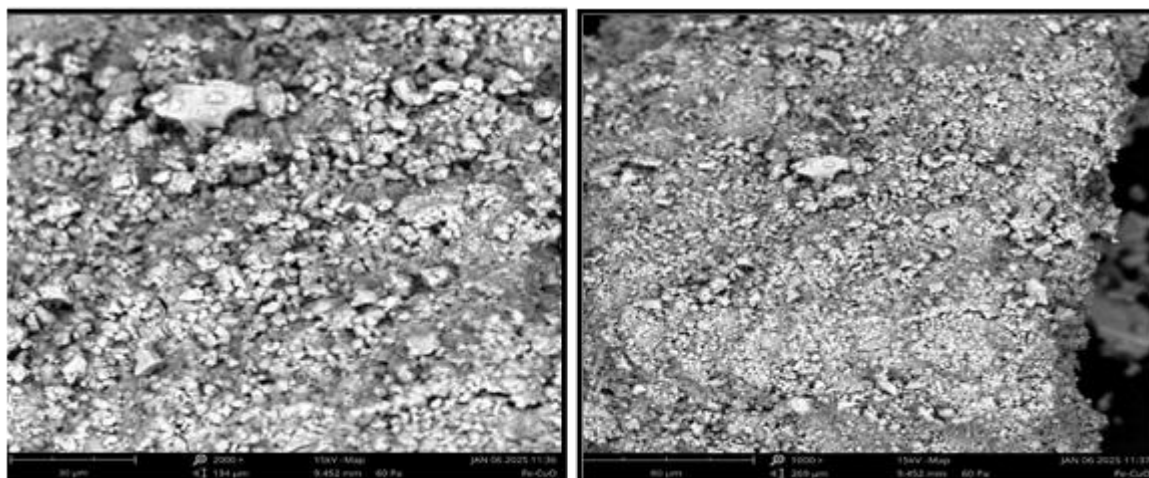


Figure 3: SEM images of Fe-CuO Nps at different magnifications

Antimicrobial susceptibility test (AST)

The AST results revealed that all the tested organisms [*K. ornithinolytica*, *P. aeruginosa*, *E. aerogenes*, *A. hydrophila*, *A. baumannii*, *S. aureus*, *S. faecalis*, *E. faecalis* (bacteria), *C. albicans*, *G. candidum* (fungi)] were susceptible to Fe-CuO Nps. The inhibitory zones ranged between 22.0 and 32.0 mm (Table 1). MIC values ranged between 50 and 3.125 mg/mL for the synthesized nanoparticles. The MIC results (Tables 2) further showed that synthesized nanoparticles were effective against all the ten tested organisms at concentrations ranging from 50 - 3.125 mg/mL. The synthesized nanoparticles showed activity even at a concentration lesser than 3.125 mg/mL which indicated that synthesized iron-doped copper oxide nanoparticles could be further tested for all the test organisms even at lower concentrations. The MBC results presented revealed that the synthesized nanoparticles had MCB value of 3.125 mg/mL, the concentration at which there was no microbial growth.

Table 1: Antimicrobial zones of inhibition (ZOI) using 100 mg/mL of iron-doped copper oxide nanoparticles (Fe-CuO Nps) against test organisms

Organism	ZOI (mm)
<i>Klebsiella ornithinolytica</i>	22.0
<i>Pseudomonas aeruginosa</i>	23.0
<i>Enterobacter aerogenes</i>	20.0
<i>Aeromonas hydrophila</i>	22.0
<i>Acinetobacter baumannii</i>	19.5
<i>Staphylococcus aureus</i>	20.0
<i>Streptococcus faecalis</i>	27.0
<i>Enterococcus faecalis</i>	21.0
<i>Candida albicans</i>	19.0
<i>Geotrichum candidum</i>	32.0

Key: Sensitive (S) = ≥ 18 mm above, Intermediate (I) = 11-17 mm, Resistant (R) = ≤ 10 mm

Table 2: Minimum Inhibitory Concentration (MIC) of iron-doped copper oxide nanoparticles (Fe-CuO Nps) against test organisms

Organism	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL
<i>Klebsiella ornithinolytica</i>	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+
<i>Aeromonas hydrophila</i>	+	+	+	+	+
<i>Acinetobacter baumannii</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+
<i>Streptococcus faecalis</i>	+	+	+	+	+
<i>Enterococcus faecalis</i>	+	+	+	+	+
<i>Candida albicans</i>	+	+	+	+	+

<i>Geotrichum candidum</i>	+	+	+	+	+
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Key: + = Sensitive; - = Resistant

Table 3: Minimum Bactericidal Concentration (MBC) of iron-doped copper oxide nanoparticles (Fe-CuO Nps) against test organisms

Organism	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL
<i>Klebsiella ornithinolytica</i>	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+
<i>Aeromonas hydrophila</i>	+	+	+	+	+
<i>Acinetobacter baumannii</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+
<i>Streptococcus faecalis</i>	+	+	+	+	+
<i>Enterococcus faecalis</i>	+	+	+	+	+
<i>Candida albicans</i>	+	+	+	+	+
<i>Geotrichum candidum</i>	+	+	+	+	+

Key: + = Growth; - = no growth

Time-kill plot

At 50% Fe-CuO Nps concentration, the decline in viable counts is depended on the organism and duration of exposure. Fifty percent (50%) MIC of Fe-CuO Nps at 48 h decreased the relative viable counts for test organisms, but the effect was negligible for *Klebsiella ornithinolytica*, *Acinetobacter baumannii*, and *Geotrichum candidum* (Figure 4).

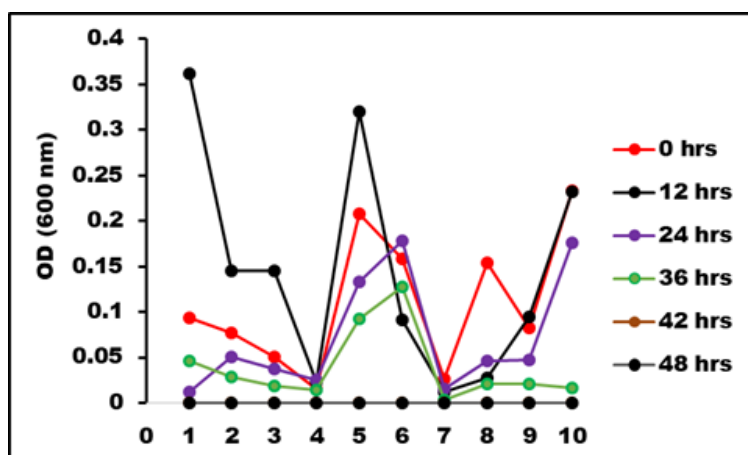


Figure 4: Time-kill plots of 50% dilution of Fe-CuO Nps synthesized against test organisms (1: *Klebsiella ornithinolytica*, 2: *Pseudomonas aeruginosa*, 3: *Enterobacter aerogenes*, 4: *Aeromonas hydrophila*, 5: *Acinetobacter baumannii*, 6: *Staphylococcus aureus*, 7: *Streptococcus faecalis*, 8: *Enterococcus faecalis*, 9: *Candida albicans* and 10: *Geotrichum candidum*).

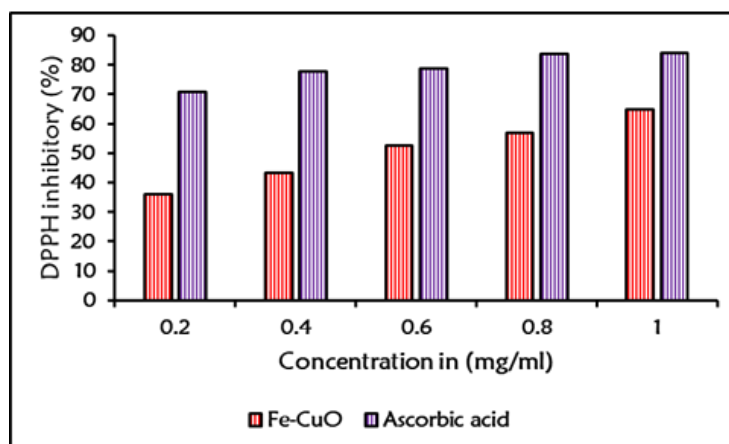


Figure 5: Percentage of antioxidant activity of the synthesized Fe-CuO Nps compared with the standard ascorbic acid using DPPH assay.

Antioxidant Activity

The DPPH assay was employed to evaluate the radical scavenging ability of the synthesized iron-doped copper oxide nanoparticles. Free radical scavenging activity data at various nanoparticle concentrations were calculated and compared that of the standard antioxidant ascorbic acid (Figure 5). The result indicated that the radical scavenging activity of the synthesized Fe-CuO Nps increased with increased concentration of the nanoparticles (0.2 – 1.0 mg/mL). The observed order of percentage of inhibition is as follows (1.0 > 0.8 > 0.6 > 0.4 > 0.2 mg/mL). The nanoparticles demonstrated highest activity at 1.0 mg/mL 65.0 % and that of ascorbic acid was 84.1 % (Figure 5).

IV. Discussion

Metal oxide nanoparticles have been reported in several studies as a strong antimicrobial agent against various pathogenic bacteria (Gabriel et al., 2023; Qamar et al., 2020; Tasnim et al., 2024). Researchers are focusing on the use of nanoparticles as an alternative to antibiotics. This study investigated the antimicrobial activity of iron-doped copper oxide nanoparticles (Fe-CuO Nps). The presence of an inhibition zone clearly indicated the antimicrobial potency of the synthesized nanoparticles (Fe-CuO Nps). The synthesized nanoparticles were used to evaluate the tested organism's susceptibility. Samples that exhibited ≥ 18 mm inhibition zone have been considered as sensitive (Mustopa et al., 2016).

The synthesized nanoparticles exhibited the strongest antimicrobial effect with the largest zone of inhibition produced against a fungus, *Geotrichum candidum* (32.0 mm). The result clearly showed that all the organisms were susceptible to the synthesized Fe-CuO Nps. The excellent antimicrobial activity of the nanoparticles against all the tested organisms could be attributed to the fact that the synthesized Fe-CuO Nps, could penetrate the cell wall of the organisms. Fe-CuO Nps may generate reactive oxygen species (ROS) in bacterial cells resulting from strong affinity of the nanoparticles for the bacterial cell membrane (Li et al 2018). This may significantly reduce the ability of bacteria to survive and grow which eventually lead to the death of the organisms.

The antimicrobial activity of Fe-CuO Nps observed in the present study is consistent with the findings of previous studies. For instance, Yudasari et al. (2020) reported that Fe-CuO Nps exhibited significant antimicrobial activity against *S. aureus*. Similarly, Neha et al. (2017) demonstrated that Fe-CuO Nps exhibited significant antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, *S. typhi*, *P. aeruginosa*, and *Bacillus subtilis*. The ability to disrupt the bacterial cell membrane has been the major mechanism of antimicrobial action attributed to Fe-CuO Nps thereby leading to leading to cell death (Arshad et al., 2017). Also, Fe-CuO Nps has the ability to induce oxidative stress by generating ROS that damage bacterial DNA and proteins (Mudshinge et al., 2011) thereby causing cell death.

The free radical scavenging activity of the nanoparticles was investigated using DPPH assay. From all indications, the antioxidant potency of Fe-CuO Nps, revealed a significant antioxidant activity. Numerous mechanisms have been identified as responsible for the antioxidant activity, including reductive capacity, radical scavenging activity, peroxide breakdown, chain initiation prevention, binding of transition metal ion catalysts, and prevention of ongoing hydrogen abstraction (Rehana et al., 2017). Free radicals are a class of chemical entities that have one or more unpaired electrons. They are extremely unstable and damage other molecules by removing electrons from them in order to become stable. Since the human body needs these radicals for detoxification, chemical signaling, energy supply, and immunological function, they are constantly created inside the system and are extremely reactive, potentially harming short-lived chemical species. Because of their significant biocidal effects against a variety of test bacteria, nanoparticles are regarded as potent antimicrobial agents (Venault et al., 2021). This might be because of the higher surface area and positive surface density, which enable better interaction with the cell membranes of negatively charged organisms. Additionally, the increased cell permeability and penetration of Fe-CuO Nps, which kill these organisms, may also be the cause (Venault et al., 2021).

V. Conclusion

The findings from the characterization of the synthesized nanoparticles using TEM, XRD, SEM was an indication of the successful synthesis of the Fe-CuO Nps. Results obtained revealed that all the ten organisms were susceptible to the synthesized nanoparticles. This implies that iron doped copper oxide nanoparticles are promising lower cost alternatives that could inhibit the growth of certain organisms and can serve as a means of overcoming the challenges of multidrug resistance to antibiotics. Also, the nanoparticles demonstrated significant antioxidant property. This study is an eye opener to the potential application of Fe-CuO Nps as antimicrobial and antioxidant agents. Therefore, they could be explored in pharmaceuticals and biomedical industries.

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