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Research Paper

Exploring the antimicrobial and antioxidant propensity of copper oxide nanoparticles against some selected clinical microbes

Abiola Olanike Adesina
Department of Chemical Sciences, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria
Correspondence e-mail: abiola.adesina@aaua.edu.ng

Abstract
Recent developments in various interventions, aimed solely at the reduction of the increasing danger of multi-drug resistance (MDR), this have warranted the search for new compounds with antimicrobial activity which could serve as cheap alternative to antibiotics production. Amongst these, metal oxide nanoparticles have been reported as relevant materials. This study investigated the synthesis and characterization of copper oxide nanoparticles (CuO Nps against some clinical microorganisms). The structural and morphological properties of the nanoparticles were thoroughly examined using advanced techniques such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and X-ray Diffraction (XRD). These methods provided comprehensive information of the nanoparticles' particle size, shape, and crystalline structure. Also, the antimicrobial and the antioxidant properties of the nanoparticles were tested against a range of microorganisms. Ten microorganisms [Klebsiella ornithinolytica, Pseudomonas aeruginosa, Enterobacter aerogenes, Aeromonas hydrophila, Acinetobacter baumannii, Staphylococcus aureus, Streptococcus faecalis, Enterococcus faecalis (bacteria), Candida albicans, Geotrichum candidum (fungi)] were employed. The antimicrobial efficacy was evaluated by determining the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for each microorganism. The results revealed that all the test organisms were susceptible to the nanoparticles, which is an indication of the strong antimicrobial potential. The CuO Nps had the highest zone of inhibition against Klebsiella ornithinolytica (24.00 mm) and 19.00 mm was the least value against Streptococcus faecalis and Candida albicans. Also, the synthesized nanoparticles exhibited significant antioxidant activity. This suggests copper oxide nanoparticles could be effective antimicrobial agents, especially when addressing antibiotic resistance problem, thus, this study warrant further investigation in nanomedicine and antibacterial treatment development.

Keywords: *Copper oxide nanoparticles; Antioxidant activity; Antimicrobial activity, Multidrug resistance*

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I. Introduction

Bacterial infections are the unending source of death and chronic illnesses. Since antibiotics are inexpensive and have powerful effects, they have been a key component of treatment plans for a variety of bacterial infections. Numerous studies have provided concrete evidence that the widespread use of antibiotics leads to the emergence of strains of bacteria and fungi that are resistant to multiple drugs. Due to the misuse of antibiotics, microbial strains known as "super bacteria" and "fungi" have become resistant to all antibiotics (Amin et al., 2021; Hsueh, 2010).

Nanoparticles (NPs) are increasingly being used as an alternative to antibiotics to target bacteria and fungi. It may be beneficial to use nanotechnology to cure microbial illnesses. Nanotechnology has emerged as one of the most important fields of study in the contemporary sciences. The fields of pharmaceutical, food, health, electronics, energy science, cosmetics, space, and environmental sciences are all being profoundly impacted by the tremendous growth of nanotechnology. The advent of nanotechnology in medicine has become prevalent particularly in drug delivery. Pharmaceutical research uses nanoparticles to reduce the toxicity and adverse effects of drugs (Dhakshinya et al., 2021).

In recent times researchers are focusing more on the use of nanoparticles due to their unique

physiochemical properties. These special characteristics have made them valuable in a range of biological disciplines, such as gene transfer, biomolecule detection, clinical diagnostics, and sensing applications (Loo et al., 2018; Pandey et al., 2008)**.** Nanoparticles have garnered significant attention for use as antimicrobial agents in medical therapy. As a result of their small size and large surface area, which improve their capacity to interact with microorganisms, they have strong antibacterial properties and are highly effective antimicrobial agents (Rai et al., 2012)**.**

Several organic and chemical compounds, including penicillin (belong to the β-lactams group) and natural items, have antibacterial properties that either kill or inhibit the growth of bacteria (Peter M. Wright, Ian B. Seiple, 2015). The potency of nanoparticles against these microorganisms has made them more efficient in the treatment of bacterial infections. Among these, metallic and semiconductor nanoparticles have garnered increased attention in recent years (Li et al., 2010). The deposition of nanoparticles (NPs) on the surface of microorganisms and their build-up in the cytoplasm/periplasmic region of bacteria can result in the death of microorganisms. The major mechanism involve could be as a result of generation of reactive oxygen species (ROS) include superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^*), and organic hydro peroxides (OHP) (Zhang et al., 2013). In bacteria, ROS can cause physical disruption by producing ROS from NPs, which can then destroy cellular components including proteins, lipids, peptidoglycan, and DNA (Das et al., 2017). Due to their special qualities, metal oxide nanoparticles (MOx-NPs), including copper oxide, SiO_2, zinc oxide (ZnO), iron oxide (Fe_3O_4) and titanium dioxide nanoparticles (TiO_2-Nps), have become quite popular. An innovative and ground-breaking approach to drug research and development is being used to build a novel antibacterial medication formulation using metal and MOx-NPs based nanoparticles.

Copper oxide, a p-type semiconductor having a band gap of about 1.7 eV (Debbichi and Pierson, 2012; Kollu, 2014) has received significant attention as it is widely applicable in various fields like gas sensors (Naika et al., 2015), solar energy transformation high-Tc superconductors, sensors (Kim et al., 2008; Umar et al., 2009), catalytic (Yang et al., 2010), optical, electrical (Naika et al., 2015), giant magnet resistance materials (Zheng et al., 2000).

In addition, copper and copper-based compounds have effective biocidal qualities, which are typically utilized in pesticidal formulations (Borkow and Gabbay, 2009) and several health-related applications. It can also serve as an antimicrobial, anti-biotic, and anti-fungal agent when added to coatings, plastics, textiles, etc. (Sharma et al., 2015).

CuO and other metal oxide nanoparticles have gained attention due to their biocidal qualities and may be used as an efficient component of a wide range of biomedical applications, including drug administration, cellular delivery, disease treatment, and biomedical imaging (D. Vaidehi, V. Bhuvaneshwari, 2018). In the pharmaceutical industry, CuO-NPs are employed as heterogeneous catalysts for drug transport, antioxidants, anticancer, and therapeutic agents (Knetsch and Koole, 2011). Socks and wounds are treated with CuO-NPs to provide them biocidal qualities. Additionally, CuO-NPs provide a lot of promise for commercial applications such as solar cells, high-temperature superconductors, gas sensors, and catalytic processes (Knetsch and Koole, 2011). Due to the fact that copper oxide nanoparticles have high surface areas and extremely unique crystal shapes, they are extremely valuable antibacterial particles (Amin et al., 2021). The shelf life of copper oxide nanoparticles is excessively lengthy in comparison to other organic antibacterial agents, and these nanoparticles are strong and extremely stable.

Oxidants are known to be a class of factors that contribute to aging and a number of diseases (Jan Gruber, Sebastian Schaffer, 2008; Lin et al., 2021; Martins-marques et al., 2021; Shen et al., 2022). Reduction-oxidation reactions or electrical excitation are the two main natural oxidative processes that generate reactive oxygen species (ROS). Research indicated that damage from reactive oxygen species (ROS) may be linked to aging. The free radical theory of aging was the most well recognized hypothesis of aging in recent years, and it focused on mitochondria as both a source and a target of ROS (Jan Gruber, Sebastian Schaffer, 2008; Madkour, 2021; Robb et al., 2014). Recent studies have mostly concentrated on ways to lessen these oxidant effects. Inorganic particles at the nanoscale may be well-suited for future use of their antioxidant properties in a variety of industries, including pharmaceuticals, cosmetics, and functional food additives (Chem et al., 2018; Xuemei Ge, 2022).

Several studies have shown that inorganic metal oxide nanoparticles such as CaO, ZnO, MgO CuO exhibited strong antibacterial and antioxidant activity. The antimicrobial property of these nanoparticles could be ascribed to their ability to generate reactive oxygen species (ROS) on the surface of the oxides (Zhao et al., 2008). The generation of ROS is capable of causing both physical and mechanical damages to the microorganisms (Li et al., 2018; Park et al., 2019). The benefit of employing these inorganic oxides as antibacterial agents is that they contain vital minerals for human health and are highly active even at low dosages. Also, inorganic antibacterial compounds exhibit better durability, lower toxicity, higher selectivity, and stronger heat resistance (Zhao et al., 2008). Rehana et al. (2017) reported the antioxidant efficiency of copper oxide nanoparticles. The antioxidant potency of the nanoparticles was investigated 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 scavenging assay). The result from the study revealed excellent antioxidant potency of the nanoparticles (Rehana et al., 2017).

Furthermore, in 2021, Dhakshinya et al. investigated the antioxidant property of copper nanoparticles using blue tea extract. The antioxidant assessment was carried out using the DPPH assay. The conclusion from the investigation revealed that the nanoparticles exhibited significant antioxidant property (Dhakshinya et al., 2021).

Gabriel et al 2023, reported the synthesis, antimicrobial and antioxidant activity of copper oxide nanoparticles. The antimicrobial activity was tested against *B. cereus, S. aureus, E. coli,* and *S. typhimurium.* The result showed that the synthesized copper oxide nanoparticles were more susceptible to gram positive organisms. He further carried out the antioxidant activity using the DPPH scavenging assay. Results obtained revealed that the synthesized copper oxide nanoparticles exhibited excellent antioxidant property (Gabriel et al., 2023)

Herein, we report the synthesis, characterization of copper oxide nanoparticles. 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. DPPH free radical scavenging assay were used to investigate the antioxidant potency of the copper oxide nanoparticles. The antimicrobial 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

2.1 Chemicals and reagents

Copper(II) Sulphate ($CuSO_4$), Sodium Hydroxide (NaOH), Dimethyl Sulfoxide (DMSO), 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All the reagents were of analytical grade and purchased from Sigma Aldrich. The reagents were utilized in their original form without any further purification.

2.2 Methodology

2.2.1 Preparation of copper oxide nanoparticles (CuO Nps)

Copper oxide nanoparticles (CuO Nps) were synthesized according to (Rehana et al., 2017) with slight modifications. Copper sulphate (0.02 M) was dissolved in 20 ml of deionized water and was stirred for about 15 mins. A 0.5 M of NaOH were dissolved in 30 ml of deionized water, afterwards, the NaOH solution was poured slowly into the solution of the copper sulphate thereby changing the solution from light blue to dark blue. The solution was stirred for 1 hrs. A black precipitate was formed and the precipitate was collected by filtration. The precipitate was washed severally and dried in the oven at a temperature of 80^0C for 3 hrs.

2.2.2 Characterization of copper oxide nanoparticles (CuO Nps)

The synthesized nanoparticles were characterized using X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy **Dispersive X-ray Spectroscopy (EDX).**

X-ray diffraction (XRD) analysis was performed on a Bruker D8 Discover diffractometer, equipped with a Lynx Eye detector, under Cu-K_α radiation (λ= 1.50405 Å). Data were collected in the range $2\theta = 10^o$ to 70^o, scanning at 1^o 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.

2.3 Antimicrobial Susceptibility Test

2.3.1 Test organisms

Ten different clinical were isolates collected from out-patients ward of the Federal medical centre, Owo whose morphological and biochemical characteristics were confirmed according to Bergeys manual of determinative bacteriology 9^{th} 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.*

2.3.2 Determination of Antibacterial Activity

The cultures were maintained on nutrient broth (bacteria) and sabouraud dextrose broth (fungi). Inoculum size of $1.5x10^8$ cfu/ml or sfu/ml was used to seed already solidified Petri plates of Mueller-Hinton agar (bacteria) and sabouraud dextrose agar (fungi). The antimicrobial activities of the synthesized copper oxide were

determined using agar well diffusion method. 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 and 3.125 mg/ml. For MIC, a sterile 6 mm cork borer was used to make four wells on already solidified and bacterial-seeded agar or fungal seeded agar plates; each well was filled with a concentration of the diluted metal complex and labeled appropriately. The plates were allowed to stand for about 2 hours to allow absorption of the metal complex and ligands dissolved in 30% DMSO into the medium after which they were incubated at 37^oC for 24 hours (bacteria) and at 28^oC for 72 hours (fungi). Macro-broth dilution technique, as modified by Ajibade et al. (2012), was employed in this research for minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). Those recorded as MIC were the lowest concentrationS of the tested metal complex that showed inhibition of tested isolates. Those recorded as MBC were the lowest concentrations of the tested metal complex that showed no visible growth.

2.3.3 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 broth containing 1 ml test organism (inoculum size of 1.5×10^8 cfu/ml or sfu/ml). The killing rate was measured using UV/Visible spectrophotometer at a wave length of 620 nm for 48 hours.

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

DPPH radical scavenging assay

The antioxidant activity of the 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 CuO Nps and standard vitamin C was carried out using the stable radical DPPH. 1 ml of different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of CuO Nps was mixed with 1 ml freshly prepared DPPH (1 mM in methanol) solution and thoroughly vortexed. After that, the mixture was left to incubate for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Methanol was used as a blank solution, and DPPH all of the chemicals except the sample was used as a control. Using the following formula, the percentage of inhibition representing the free radical scavenging activity was calculated.

$$\% \text{ of scavenging} = \frac{Pc - Ps}{Pc} \times 100$$

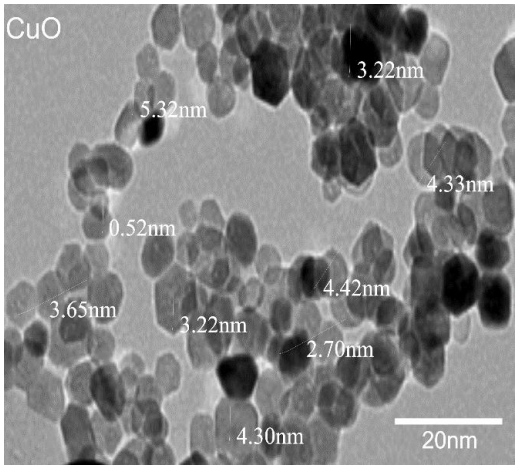
where Pc is the absorbance of control and Ps is the absorption of CuO Nps/vitamin C.

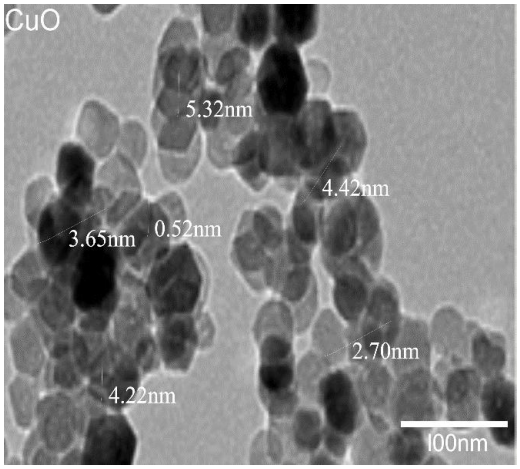
III. Results

3.1 Characterization of copper oxide nanoparticles (CuO Nps)

3.1.1 Transmission electron microscopy (TEM) image of CuO Nps

The TEM image of the synthesized nanoparticles at different magnifications revealed the morphology of the nanoparticles, particle size distribution, the shape of the nanoparticles. From the TEM result, the nanoparticles were spherical in shape. The particle size distribution ranges from 2.70 nm - 5.32 nm with the majority of the nanoparticles between 4.22 - 4.42 nm having the highest distribution. The average particle size diameter was found to be 4.0 ± 0.89 nm (Fig. 1).





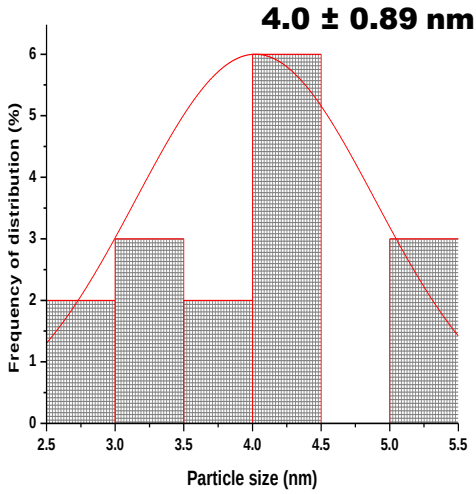


Figure 1: TEM image of the CuO Nps at different magnifications and the corresponding histogram

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

X-ray diffraction was used to confirm the synthesis of copper oxide nanoparticles. XRD diffractogram of synthesized nanoparticles is shown in Fig. peaks were observed in difractogram at *2θ* of 32.5, 35.7, 38.9, 48.9, and 63.7, which were assigned to (110), (111), (111), (200), (220), respectively. These strong peaks indicated the successful synthesis of copper oxide nanoparticles (Fig. 2).

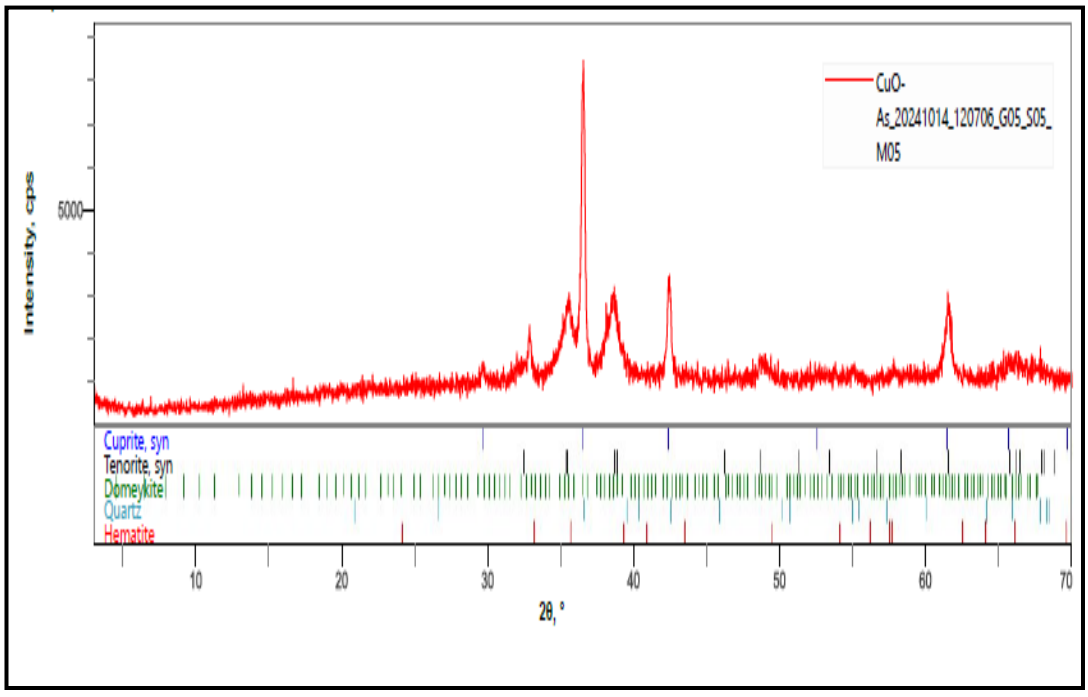


Figure 2: X-ray diffraction (XRD) pattern of CuO Nps

3.1.3. Scanning Electron Microscopic Image (SEM) of 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

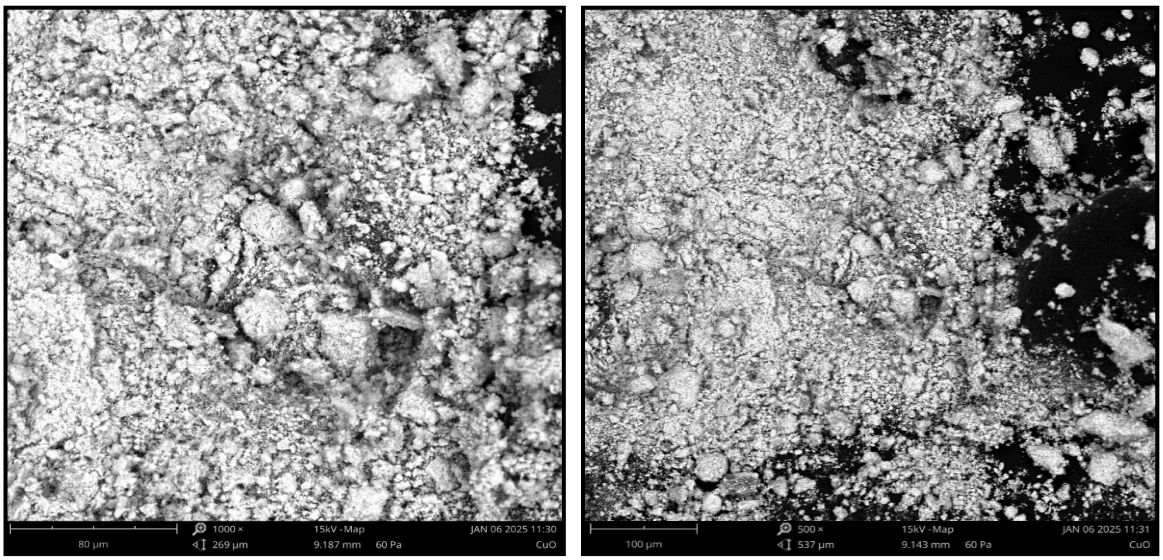


Figure 3: SEM images of CuO Nps at different magnifications

3.2 Antimicrobial susceptibility test (AST)

The results from this study revealed that all the tested organisms (*K. ornithinolytica, P. aeruginosa, E. aerogenes, A. hydrophila, A. baumannii, S. aureus, S. faecalis, E. faecalis, C. albicans,* and *G. candidum*) were susceptible to copper oxide nanoparticles (CuO Nps) (Table 1). The CuO Nps had the highest zone of inhibition against *Klebsiella ornithinolytica* (24.00 mm) and 19.00 mm was the least value against *Streptococcus faecalis* and *Candida albicans*. These findings showed MIC values that ranged between 3.125 and 50 mg/ml for the synthesized nanoparticles. The MIC results (Tables 2) further showed that the synthesized nanoparticles showed activity even at a concentration of 3.125 mg/ml. The minimum bactericidal/fungicidal concentrations results presented also revealed that the synthesized nanoparticles were effective against all the test organisms, even at concentration of 3.125 mg/ml (Table 3).

Table 1: Antimicrobial zones of inhibition (ZOI) using 100mg/ml of copper oxide nanoparticles (CuO Nps) against bacterial isolates

Organism	CuO Nps (mm)
Klebsiella ornithinolytica	24.00
Pseudomonas aeruginosa	21.00
Enterobacter aerogenes	21.00
Aeromonas hydrophila	20.00
Acinetobacter baumannii	21.00
Staphylococcus aureus	20. 00
Streptococcus faecalis	19.00
Enterococcus faecalis	20.00
Candida albicans	19.00
Geotrichum candidum	21.00

Key: Sensitive (s= ≥ 18 mm above, Intermediate (I) = 17 – 11 (mm), Resistance (R) = ≤ 10 mm

Table 2: Result of Minimum Inhibitory Concentration (MIC) of copper oxide nanoparticles (CuO Nps) against various bacterial isolates

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

Key: + = Growth; - = no growth

Table 3: Result of Minimum Bactericidal Concentration (MBC) of copper oxide nanoparticles (CuO Nps) against various bacterial isolates

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

Key: + = Growth; - = no growth

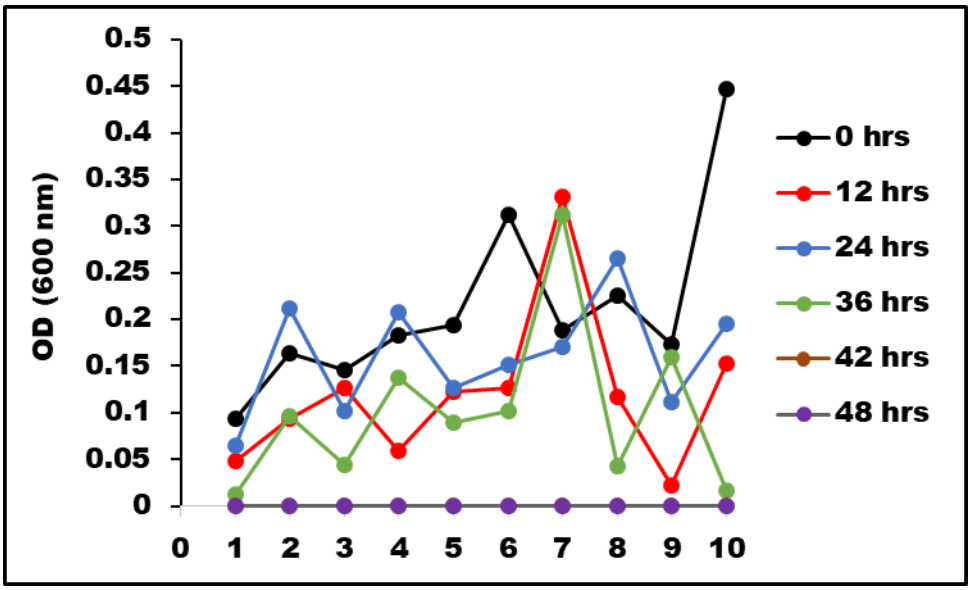


Figure 4: Time-kill plots of 50% dilution of CuO Nps synthesized against some organisms (1: *Klebsiella ornithinolytica,* **2:** *Pseudomonas aeruginosa,* **3:** *Enterobacter aerogenes,* **4:** *Aeromonas hydrophila,* **5:** *Acinetobacter baumannii,* **6:** *Staphylococcus aureus,* **7:** *Streptococcus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

3.3. Antioxidant Activity

The antioxidant activity has been ascribed to a number of mechanisms, including radical scavenging activity, reductive capacity, peroxide breakdown, chain initiation prevention, binding of transition metal ion catalysts, and prevention of continuous hydrogen abstraction (Rehana et al., 2017). Free radicals are extremely unstable chemical entities that damage other molecules by removing electrons from them in order to become stable. They are made up of one or more unpaired electrons. Because they are vital for detoxification, chemical signaling, energy supply, and immunological function, the human body continuously produces these highly reactive, system-based radicals that have the ability to harm short-lived chemical species. The scavenging activity of the synthesized copper oxide nanoparticles was evaluated using the DPPH method.

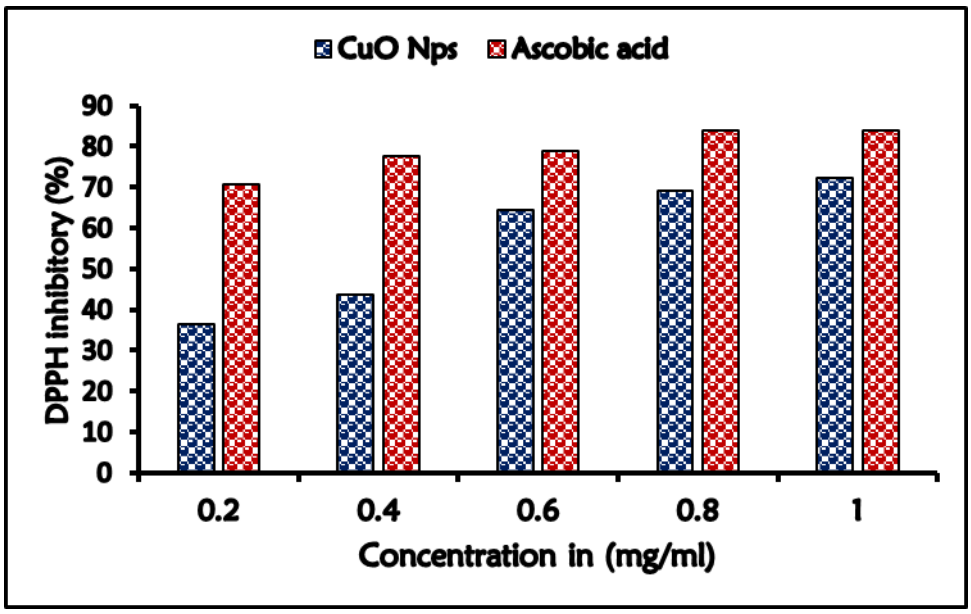


Figure 5: Graph showing the percentage of antioxidant activity of the synthesized CuO Nps compared with the standard ascorbic acid using DPPH assay.

The radical scavenging ability of the synthesized copper oxide nanoparticles was evaluated using DPPH assay. Free radical scavenging activity data at various nanoparticle concentrations were calculated and compared that of the standard antioxidant ascorbic acid (Figure 4). The radical scavenging activity of the synthesized CuO Nps increases with increase in 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 exhibited highest activity at 1.0 mg/ml 72.29 % and that of ascorbic acid was 84.05 % (Figure 5).

IV. Discussion

Metal oxide nanoparticles (MOx Nps) have shown superior antibacterial activity against various pathogenic bacteria (Li *et al* 2018) as well as fungal pathogens. The use of nanoparticles as an alternative to antibiotics has become an area of great interest in research. This study investigated the antimicrobial activity of copper oxide nanoparticles (CuO Nps). The results of the study revealed that copper oxide nanoparticles (CuO Nps) demonstrated excellent and significant antimicrobial activity against all the bacterial isolates. The antimicrobial potency of CuO Nps were tested against ten organisms, *K. ornithinolytica*, *P. aeruginosa*, *E. aerogenes*, *A. hydrophila*, *A. baumannii*, *S. aureus*, *S. faecalis*, *E. faecalis*, *C. albicans*, and *G. candidum*.

The synthesized nanoparticles were used to evaluate the tested organisms' susceptibility. A sample exhibiting ≥ 18 mm inhibition zone is considered significant (Mustopa et al., 2016). Results obtained indicated that the synthesized nanoparticles showed excellent and significant activity against all the tested organisms. The inhibitory zones ranging from (19.00 - 24.00 mm). The obtained result indicated that all the organisms were susceptible to the synthesized CuO Nps. The excellent antimicrobial activity of the nanoparticles against all the tested organisms could be attributed to the fact that the synthesized CuO Nps, could penetrate the cell wall of the organisms. 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 organisms to survive and grow which eventually lead to the death of the organisms.

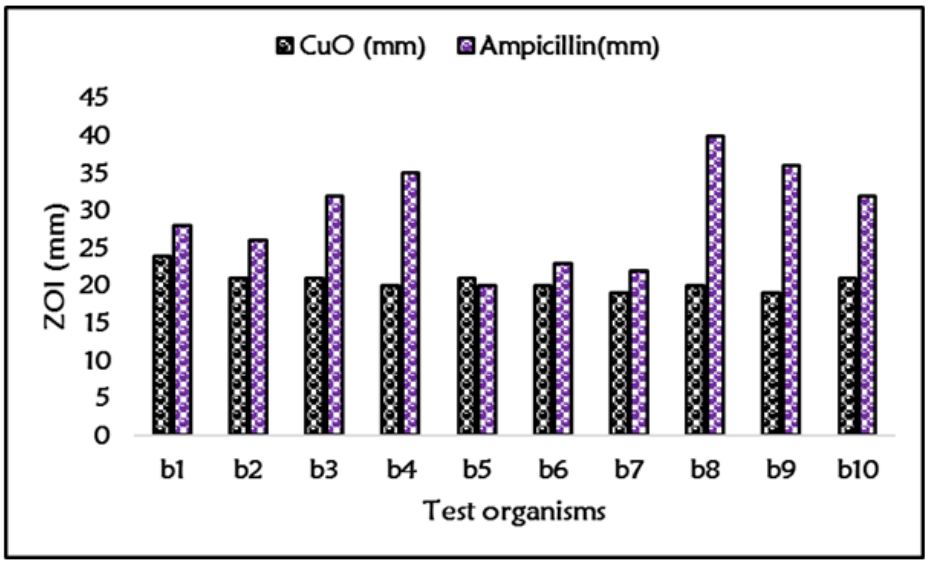


Figure 6: Graphical presentation of antimicrobial activity of CuO Nps against some organisms (b1: *Klebsiella Ornithinolytica,* **b2:** *Pseudomonas aeruginosa,* **b3:** *Enterobacter aerugenes,* **b4:** *Aeromonas hydrophila,* **b5:** *Acenetobacter baumanni,* **b6:** *Staphylococcus aureus,* **b7:** *Streptococcus faecalis,* **b8:** *Enterococcus faecalis,* **b9:** *Candida albican* and **b10:** *Geotrichum candidum***)**

It was revealed from the present study that CuO Nps exhibited significant antimicrobial activity against *K. ornithinolytica, P. aeruginosa, E. aerogenes, A. hydrophila, A. baumannii, S. aureus, S. faecalis, E. faecalis, C. albicans,* and *G. candidum.* According to the MIC results, CuO Nps was the most effective against every organism that was tested. The antimicrobial activity of CuO Nps found in this investigation aligns with earlier findings. The antimicrobial activity of CuO Nps observed in the present study is consistent with the findings of previous studies. For instance, Yudasari et al. (2020) reported that CuO Nps exhibit significant antimicrobial activity against *S. aureus.* Similarly, Neha et al. (2017) demonstrated that CuO Nps exhibited significant antibacterial activity against *S. aureus, Escherichia coli, K. pneumoniae, Salmonella typhi, P. aeruginosa, Bacillus subtilis.* The mechanism of antimicrobial action of CuO Nps has been attributed to their ability to disrupt the bacterial cell membrane, leading to cell death (Arshad et al., 2017). Also, CuO Nps can induce oxidative stress by generating reactive oxygen species (ROS) that damage bacterial DNA and proteins (Mudshinge et al., 2011) thereby causing cell death.

The susceptibility of the tested organisms towards CuO Nps could be attributed to the fact that the synthesized nanoparticles have the ability to penetrate the cell wall of the organisms. The ability of nanoparticles to interact with bacteria causing severe disruption to the cell. The action of the CuO Nps on bacteria causes a membrane attachment, membrane penetration, and subsequent morphological changes thereby leading to cell death.

V. Conclusion

The synthesis, antimicrobial and antioxidant potency of copper oxide nanoparticles revealed that copper oxide nanoparticles could serve as a potential agent in biomedical applications. More so, it could also be employed as alternative to antibiotics in the treatment of bacterial diseases thereby contributing significantly to the on health concept.

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