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Biofabrication, Characterisation and Antimicrobial Activity of CuO/Ag-based Material

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Abstract

In this work, *Azadirachta indica* leaf extract and the ultrasonic method were applied for the fabrication of a CuO/Ag-based nanocomposite. The CuO/Ag was characterised using different analytical methods such as FTIR, SEM, EDX, and XRD. The well diffusion method was used to evaluate the antibacterial activity of non-calcined and calcined CuO/Ag against some hazardous bacterial strains. After the incubation period, remarkable zones of inhibition were observed around the loaded CuO/Ag. The maximum zones of inhibition were found to be 17.9 (± 0.39), 20 (± 0.17), and 14.3 (± 0.31) mm for *E. coli*, *S. aureus*, and *S. enterica*, respectively. Experimental findings indicated that non-calcined CuO/Ag was a more effective antibacterial agent as compared to calcined CuO/Ag.

Keywords: *Azadirachta indica*, Leaf Extract, CuO/Ag, Antibacterial Activity

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INTRODUCTION

Nanotechnology is a discipline of science and technology that focuses on materials with dimensions of less than 100 nm. Nanotechnology has been applied in medicine, energy storage, life sciences, chemical sciences, electronics, and other fields; therefore, industrial sectors are now adopting nanotechnology.[1] There are several types of nanomaterials, comprising carbon-, metal-, organic-, and composite-based nanomaterials.[2] The optical, surface, electrical, and thermal characteristics of metal and metal oxide nanoparticles differ from those of their original bulk materials in a variety of ways.[3] Some typical metal and metal oxide nanoparticles are iron oxides, titania (TiO_2), copper (Cu), zinc oxide (ZnO), copper oxide (CuO), silver (Ag), and gold (Au). All these nanoparticles possess substantial antibacterial effects against both Gram-negative and Gram-positive bacteria.[4-7] The metal-based nanoparticles provide a viable substitute for conventional antibiotics owing to their diverse physicochemical and functional characteristics and their substantial availability.[8,9] Metal and metal oxide nanoparticles have successfully been produced via the use of biological systems and organic materials in green synthesis, in addition to green chemistry-based methodologies. The green synthesis of metal-based nanoparticles is one of the options that relies on green chemistry concepts and makes use of biological systems. This method is low-cost, effective, and safe.[10,11] Copper oxide nanoparticles (CuO NPs) have received much attention due to their applications in different fields of science and engineering. These nanoparticles are p-type and monoclinic in structure. CuO NPs have been potentially utilised in textiles, biomedicine, sensors, catalysis, water remediation, and high-temperature superconductors.[12-14] Silver nanoparticles (Ag NPs) are extensively used in different industries such as healthcare, food, medicine, cosmetics, energy storage, catalysis, space, and chemical industries due to their unique physical and chemical properties. These include biological characteristics, strong electrical conductivity, optical, electrical, and thermal properties.[15,16] Bhushan *et al.*,[17] used nanoparticles of iron oxide and copper oxide to produce hybrid magnetic nanocomposites. With a bactericidal efficacy comparable to gentamycin, the nanomaterials demonstrated strong antibacterial activity against harmful bacteria. These non-toxic hybrid nanocomposites possess the potential to be used as antibiotics to treat diseases caused by pathogenic bacteria that are resistant to several drugs. Fe/Ni oxide nanocomposites have been developed by Bhushan *et al.*,[18] who then assessed how effective they were in destroying harmful strains of bacteria. An investigation was conducted on their structural, physical, and chemical characteristics. With the MTT test, their cytotoxicity was evaluated. The study conducted by Bhushan *et al.*,[19] revealed that Fe/Mn oxide nanocomposites were compatible with the MCF7 and MCF-12A cell lines. These nanocomposites possessed outstanding bactericidal activities against various bacteria, such as *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), and *Salmonella typhi* (*S. typhi*). It is therefore possible that Fe/Mn oxide nanocomposites will prove to be a viable substitute for conventional antibiotics. Applying the disc diffusion method, Sampath *et al.*,[20] reported the ZnO/Ag nanocomposite's antibacterial activity against *Enterococcus hirae* (*E. hirae*) and *E. coli*. ZnO/Ag nanoparticles showed better antibacterial activity compared to pure ZnO nanoparticles and were found to be stronger than kanamycin. Jaiswal *et al.*,[21] studied the biofabrication of Ag NPs using banana corm extract and silver acetate, achieving MIC values 120, 60, 200, and 100 µg/ml against *E. coli*, *S. aureus*, *Aspergillus brasiliensis* (*A. brasiliensis*), and *Rhizopus stolonifer* (*R. stolonifera*). Through mixing graphitic carbon nitride with $CuCl_2 \cdot 2H_2O$ and $NiCl_2 \cdot 6H_2O$, Gajurel *et al.*,[22] developed a synergistic bimetallic nano-catalyst that improved catalytic performance in the manufacture of triazole, tetrazole, and bis-triazole derivatives. *Azadirachta indica* is also known as Neem tree. The southern parts of Asia and Africa are where neem trees are most often grown. Consider that the different parts of the Neem tree (bark, fruits, leaves, flowers, gum, and oils) have a long tradition of use in treatments for diseases including diabetes, cancer, heart disease, and hypertension. Due to the presence of some polyphenolic compounds

in the leaves of Neem, these leaves are used as antibacterial, antifungal, and anti-inflammatory agents.[23,24]

MATERIALS AND METHODS

Azadirachta indica leaves, double distilled water (DDW), ethanol, copper acetate [$Cu(CH_3COO)_2$], sodium hydroxide (NaOH), silver nitrate $AgNO_3$, and Mueller Hinton agar (MHA) were used in the present work.

Preparation of extract

After being washed with distilled water, *Azadirachta indica* leaves were allowed to air dry for three to four days. Crushed dry leaves (1 g) were added to 100 ml of DDW. This content was stirred for 25 min and then boiled. 2 ml of 10% ethanol was added to this mixture and shaken for 15 min. The prepared leaf extract was preserved for further study.

Synthesis of CuO/Ag

2 ml of the extract were combined with 100 mL of a 0.1 M $Cu(CH_3COO)_2$ solution. After 35 minutes of shaking, a few drops of 0.1 M NaOH were added. The mixture was stirred for a further 25 min after precipitation. Centrifugation was used to separate the CuO NP precipitate, which was then rinsed and dried. In sealed vials, the non-calcined CuO NPs had been preserved. Calcined CuO NPs were obtained by calcining the dry CuO NPs at 400°C for 2 hr. In 100 ml of a 0.1 M $AgNO_3$ solution, 2 ml of leaf extract was also added. To produce both non-calcined and calcined Ag NPs, the process described above was repeated. The dry powder of Ag NPs was calcined at 200°C for 2 hr. 50 mg of non-calcined or calcined Ag NPs and 100 mg of non-calcined or calcined CuO NPs in 100 ml of DDW were mixed. The mixture was stirred for 45 min and then sonicated for 3 hr. After complete drying, CuO/Ag (non-calcined or calcined) was preserved for characterization and antibacterial activity. SEM (scanning electron microscopy), EDX (energy dispersive X-ray), FTIR (Fourier transform infrared), and XRD (X-ray diffraction) techniques were used for the characterization of powdered CuO/Ag.

Antibacterial activity of CuO/Ag

The well diffusion method was used to investigate the antibacterial activity of CuO/Ag against *S. aureus*, *Salmonella enterica* (*S. enterica*), and *E. coli*. 20 ml of liquid MHA was added to sterile petri dishes and allowed to solidify. On the plates, bacterial cultures were spread, and wells were made for the inclusion of CuO/Ag. There were significant zones of inhibition around the CuO/Ag after incubation.

RESULTS AND DISCUSSION

Characterisation of CuO/Ag

Figure 1(a and b) illustrates the FTIR spectra of non-calcined and calcined CuO/Ag. Non-calcined CuO has distinctive peaks at 3523, 2030, 1620, and 1032 cm^{-1} (Figure 1 a). The existence of O-H (str), C=C (allene), O-H (bending), and C-O (asymmetric) bonds is indicated by these peaks. The designated FTIR peaks for non-calcined CuO/Ag are 3455, 1558, 1384, and 531 cm^{-1}, respectively. These peaks are associated with bonds like O-H (str), O-H (bending), C-N (str), Cu-O, Cu-Ag, etc.[25-28] The FTIR peaks of 3452, 1627, and 531 cm^{-1} have been assigned to calcined CuO and show the presence of O-H (str), O-H (bending), and Cu-O bonds. The FTIR peaks of 3457, 1383, and 506 cm^{-1} were also attributed to calcined CuO/Ag. These peaks demonstrate the presence of the O-H (str), C-N (str), and Cu-O or Cu-Ag bonds, respectively.[26-30]

Figure 2 (a and b) presents the XRD patterns of CuO and CuO/Ag. The XRD patterns of non-calcined CuO NPs indicate an amorphous structure and a cluster of peaks due to the presence of different biological constituents of the extract in CuO NPs. A semicrystalline structure of non-calcined CuO/Ag also appears, with peaks that are not related to pure CuO and Ag nanoparticles. For calcined CuO NPs, the XRD peaks have been assigned at 2 θ = 32.5° (110), 35.7° (002), 38.8° (111), 48.9° (202), 53.5° (020), 61.6° (113), 66.3° (311), 68.3° (113), 72.3° (311), and 75.2° (004). These peaks are related to JCPDS card No. 00-041-0254.[21-23] The XRD peaks of CuO/Ag are assigned as 2 ט = 32.6° (110), 35.6° (111), 38.2° (111), 44.4° (200), 48.9° (202), 58.2° (020), 61.6° (113), 64.5°

(220), 66.3° (311), 68.8° (113), 72.3° (311), 75.2° (004), and 77.4° (311), respectively. These all peaks are also satisfying JCPDS card No. 00-041-0254 and JCPDS card No. 04-0783. Based on these patterns, both CuO and CuO/Ag (calcined) phases appear to be crystalline and semi-crystalline. The incorporation of Ag into CuO appears to have affected the crystallinity.[31-37]

The morphological features of CuO NPs and CuO/Ag have been observed using SEM analysis. The SEM images of non-calcined and calcined CuO and CuO/Ag are illustrated in figures 3 and 4. The SEM images of non-calcined CuO indicate the irregular morphology of particles (Figure 3 a and b). Highly agglomerated and irregular-shaped morphology appears in SEM

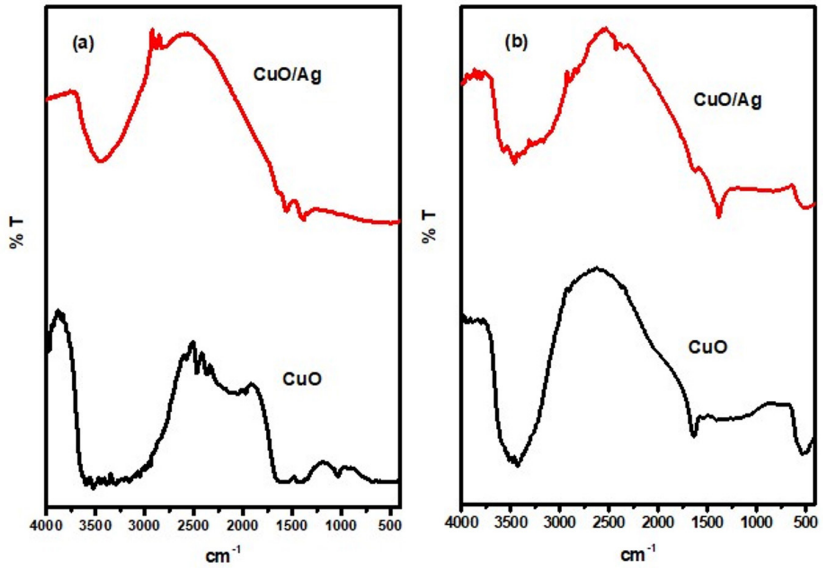

Figure 1. FTIR spectra of (a) non-calcined CuO and CuO/Ag, and (b) calcined CuO and CuO/Ag

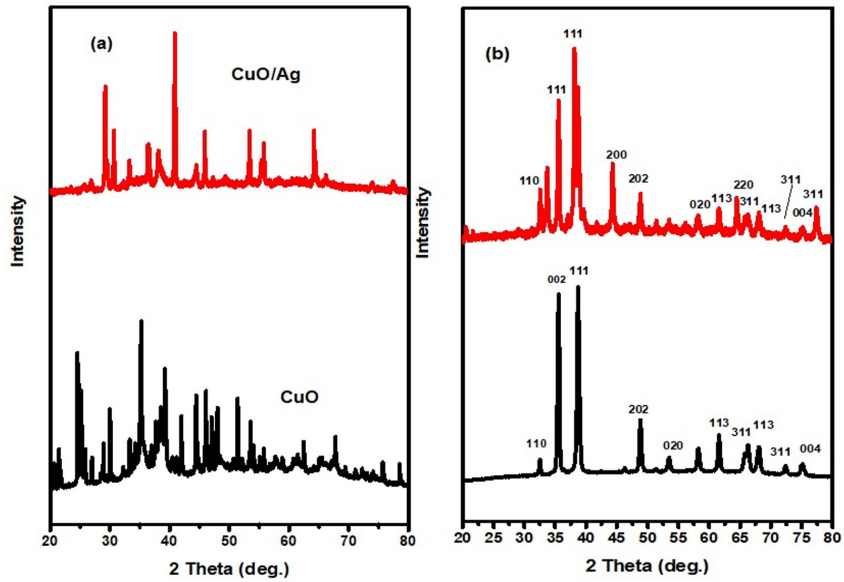

Figure 2. XRD patterns of (a) non-calcined CuO and CuO/Ag, and (b) calcined CuO and CuO/Ag

images of non-calcined CuO/Ag (Figure 3 c and d). After calcination, the morphology of CuO appeared to be regular and sphere-shaped (Figure 4 a and b). The morphology of calcined CuO/Ag has been transformed to be less agglomerated and more regular with the combination of sphere- and semi-sphere-shaped particles (Figure 4 c and d). This material is a combination of Ag and CuO that has been heated to eliminate volatile components. Its overall appearance is less agglomerated, more regular, and a mixture of semi-sphere and sphere-shaped particles, indicating that the material has a range of sizes and orientations.EDX analysis and EDX mapping were used to characterise the elemental composition of the CuO/Ag material. These methods enhance morphological analysis for thorough characterization by offering insights into the material's homogeneity and chemical composition. EDX spectra, composition, and mapping of major elements present in non-calcined and calcined CuO and CuO/Ag are presented in Figures 5 (a and b), 6 and 7, and Tables 1 and 2.

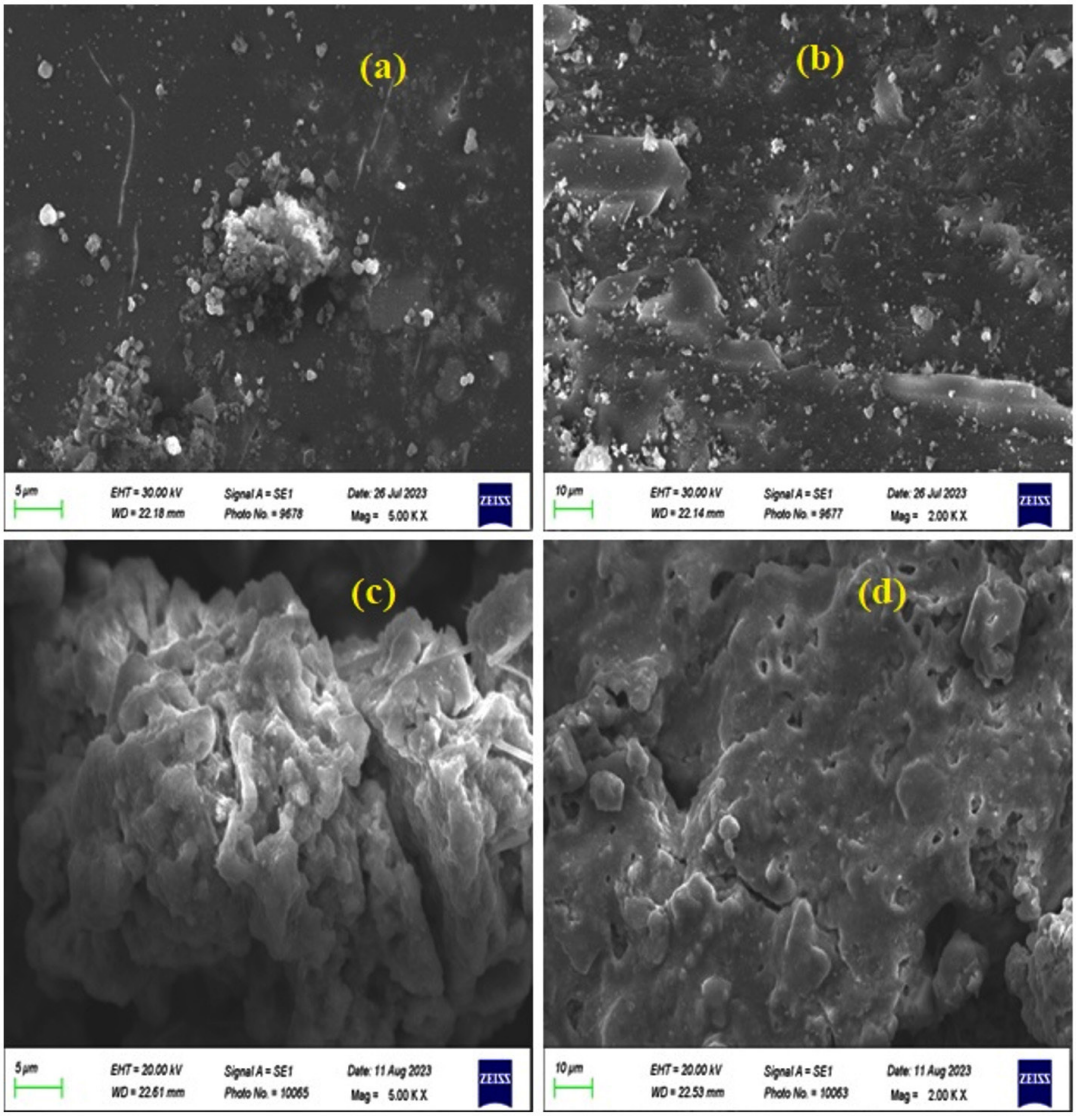

Figure 3. SEM images of (a) & (b) non-calcined CuO, and (c) & (d) non-calcined CuO/Ag

Antibacterial activity of CuO/Ag

Metal and metal oxide nanoparticles have multitarget capabilities that make them potent antibacterial agents.[38] These nanoparticles are capable of distinguishing bacterial cells from mammalian cells.[39] The capability of metal and metal oxide nanoparticles as antibacterial agents has been explained by several mechanisms. Cellular integrity damage, reactive oxygen species (ROS) production, and internalisation of the nanoparticles by the bacterial and fungal cells are a few of these.[40] These nanoparticles generally interact with bacterial membranes, resulting in membrane collapse and rupture, which allows bacterial cytoplasm to flow out. The oxidative stress caused by the production of reactive oxygen species (ROS) causes the breakdown of bacterial membranes. In general, ROS consists of oxygen, superoxide anion, perhydroxyl radicals, and the hydroxyl radical. These may oxidise proteins and lipids while also damaging DNA and RNA, which causes bacterial death and decomposition.[41] The antibacterial activity of non-calcined CuO/Ag has been conducted using a dosage of 5 to 50 mg/mL. The minimum inhibitory concentration (MIC) was found to be 5 mg/mL. After the incubation period, the zones of inhibition around the non-calcined CuO/Ag were evaluated as 8 (± 0.23),

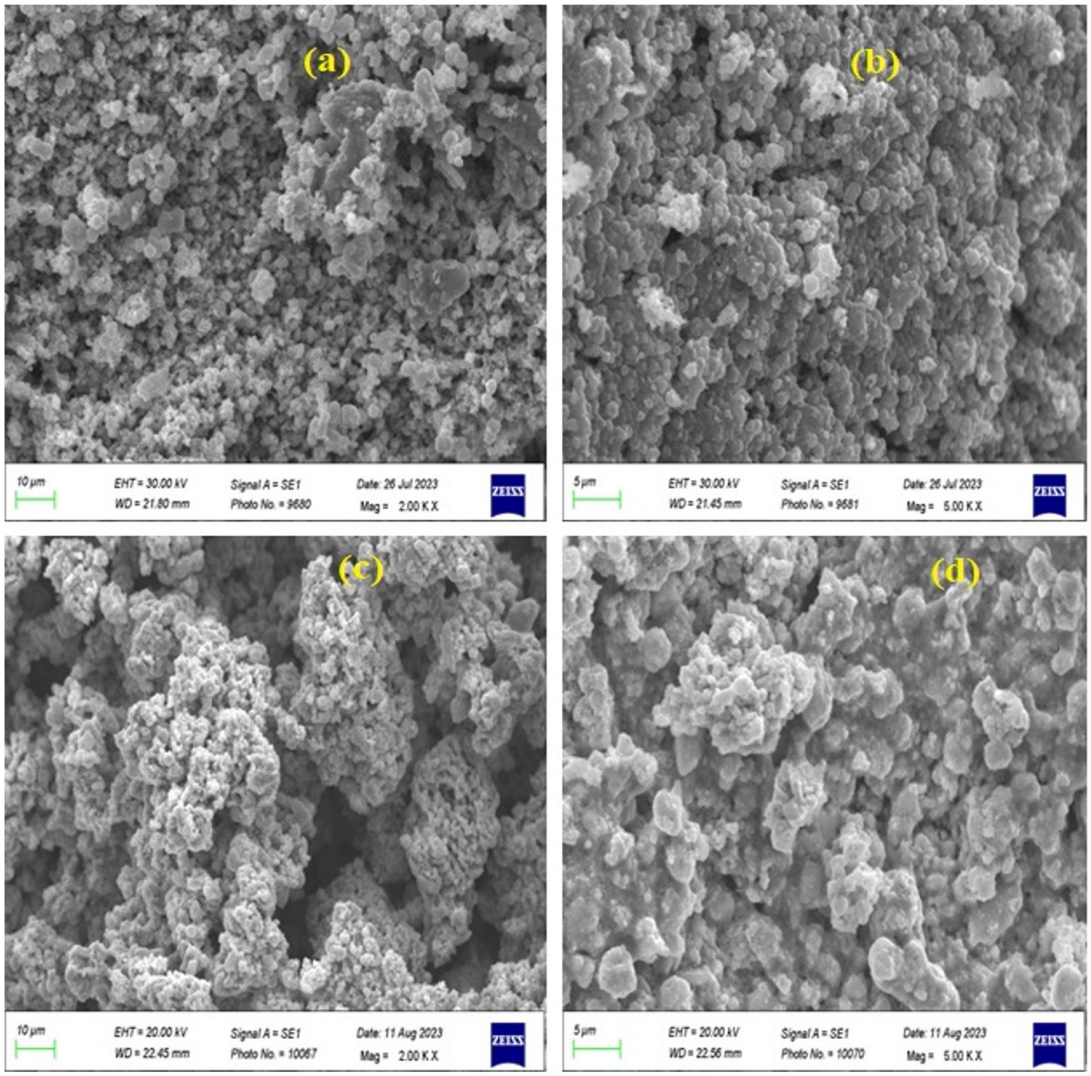

Figure 4. SEM images of (a) & (b) calcined CuO, and (c) & (d) calcined CuO/Ag

9.2 (± 0.31), and 7.6 (± 0.81) mm for *E. coli*, *S. aureus*, and *S. enterica* at an initial dosage of 5 mg/mL. This was increased to 12.6 (± 0.69), 12.9 (± 0.23), and 9.6 (± 0.13) mm at a dosage of 20 mg/L. At 40 mg/mL, the zones of inhibition were found to be 16.7 (± 0.17), 17.8 (± 0.25), and 13 (± 0.51) mm. Then the maximum zones of inhibition were found to be 17.9 (± 0.39), 20 (± 0.17), and 14.3 (± 0.31) mm for *E. coli*, *S. aureus*, and *S. enterica* at 50 mg/mL (Figure 8). The MIC of calcined CuO/Ag was found to be 10 mg/mL. The zones of inhibition for *E. coli, S. aureus*, and

Table 1. EDX composition of non-calcined CuO/Ag

Element	Weight %	Atomic %
C K	0.6	1.6
O K	40.3	79.6
Cu K	7.7	3.8
Ag L	51.4	15.1

Table 2. EDX composition of calcined CuO/Ag

Element	Weight %	Atomic %
O K	12.9	40.8
Cu K	57.1	45.2
Ag L	30.0	14.0

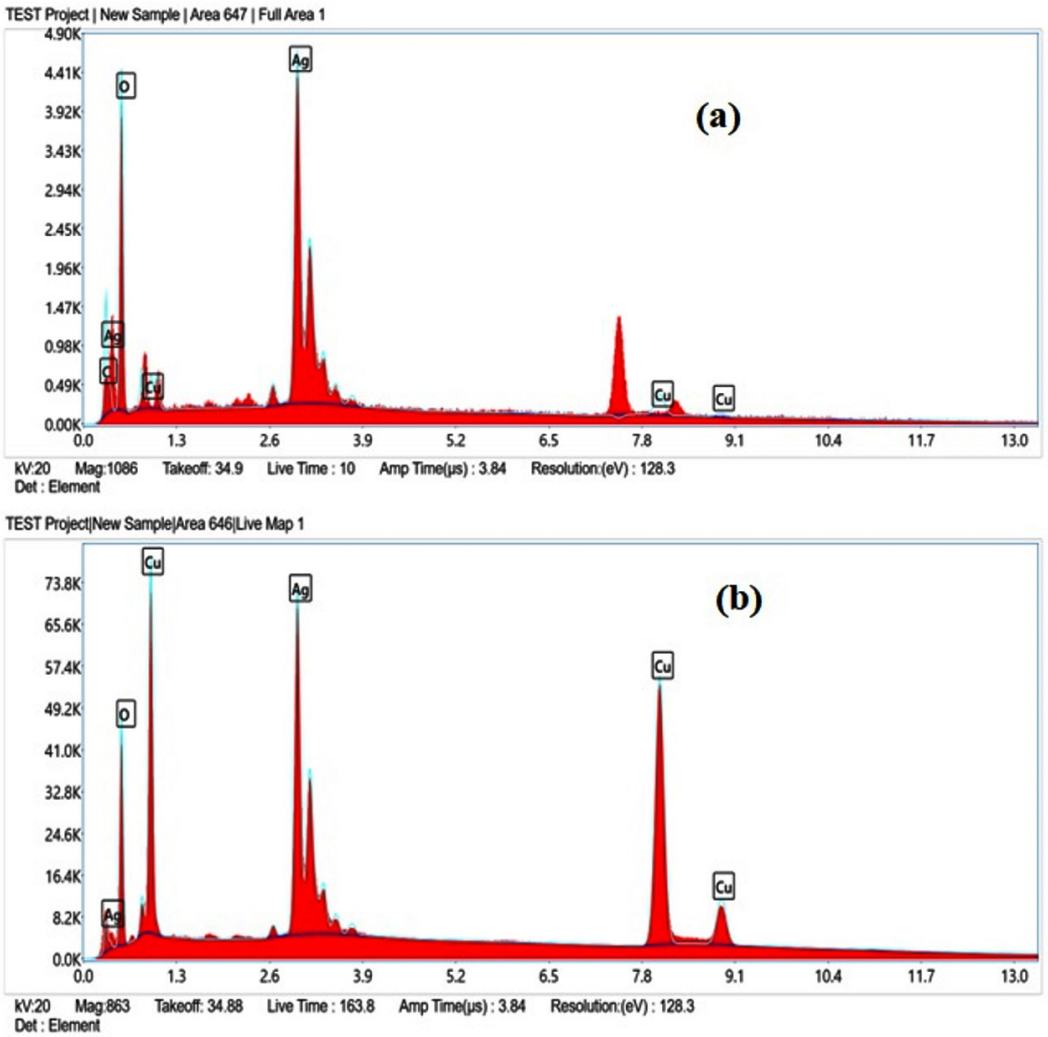

Figure 5. EDX spectra of (a) non-calcined CuO/Ag and (b) calcined CuO/Ag

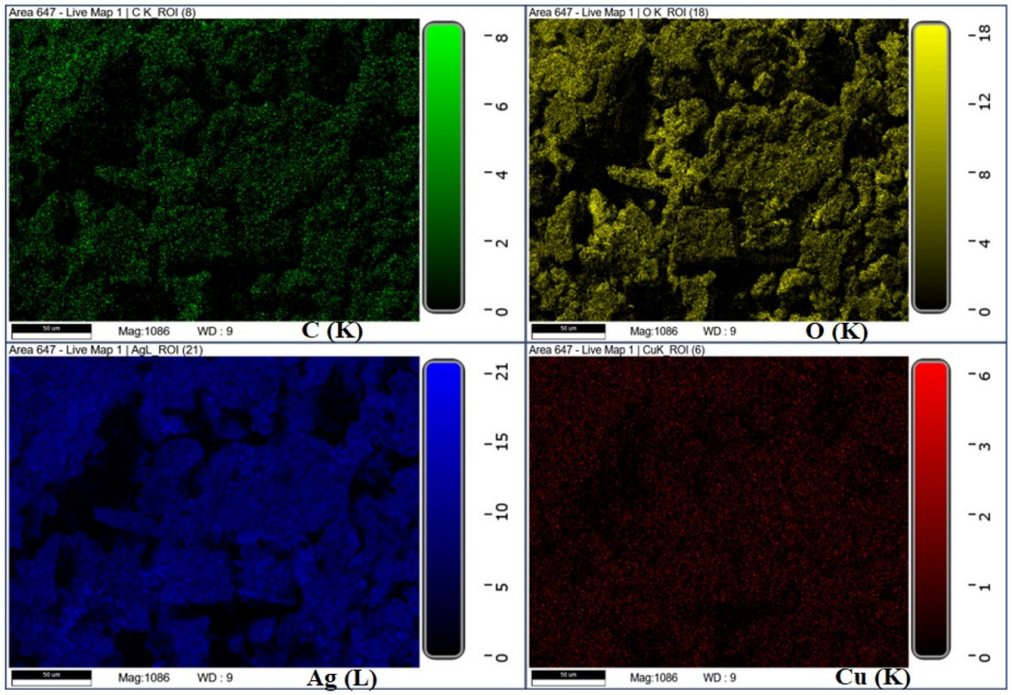

Figure 6. EDX mapping CuO/Ag (non-calcined)

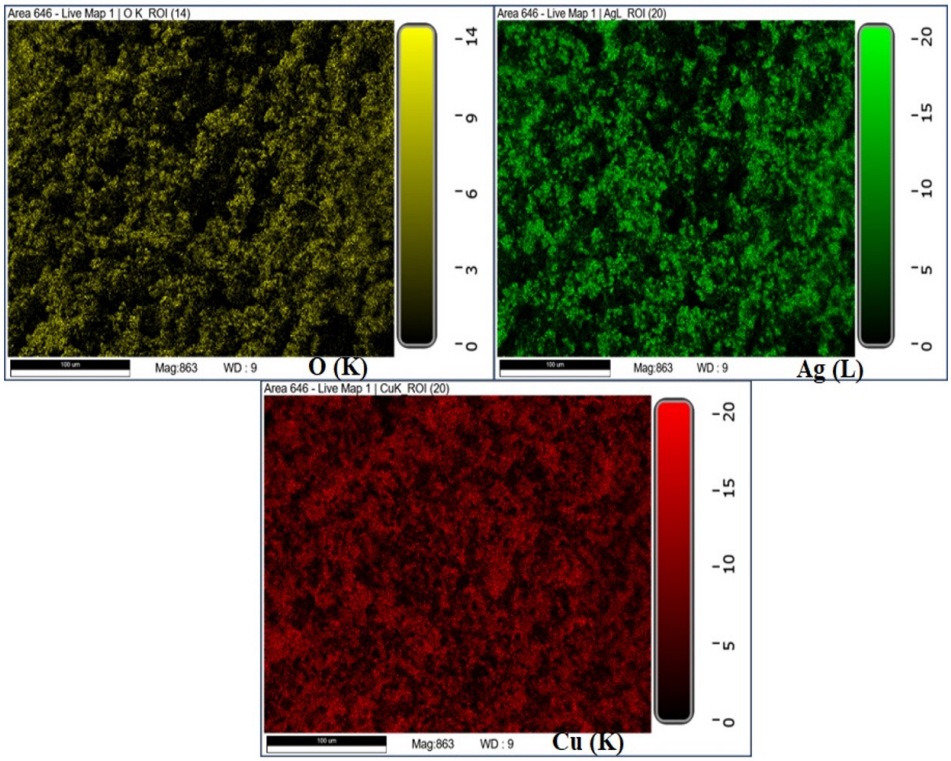

Figure 7. EDX mapping CuO/Ag (calcined)

S. enterica were 8.6 mm (± 0.33), 9.3 mm (± 0.18), and 7.9 mm (± 0.25), respectively, at this dose. These values increased to 12.8 mm (± 0.58), 13.5 mm (± 0.63), and 9.9 mm (± 0.37), respectively, at 25 mg/mL. The zones of inhibition were evaluated as 17.8 (± 0.75), 18.7 (± 0.89), and 13.2 (± 0.65) at 50 mg/mL of calcined CuO/Ag. Maximum zones of inhibition were observed as 19.2 (± 0.25), 20.2 (± 0.53), and 14.3 (± 0.36) mm for *E. coli*, *S. aureus*, and *S. enterica* at 60 mg/mL (Figure 9). The experimental findings indicated that non-calcined CuO/Ag was a better antibacterial

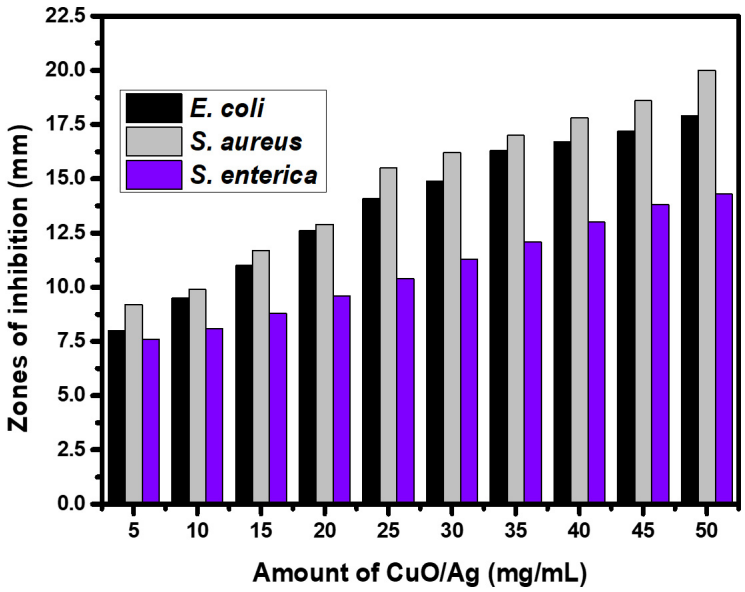

Figure 8. Antibacterial activity of CuO/Ag (non-calcined)

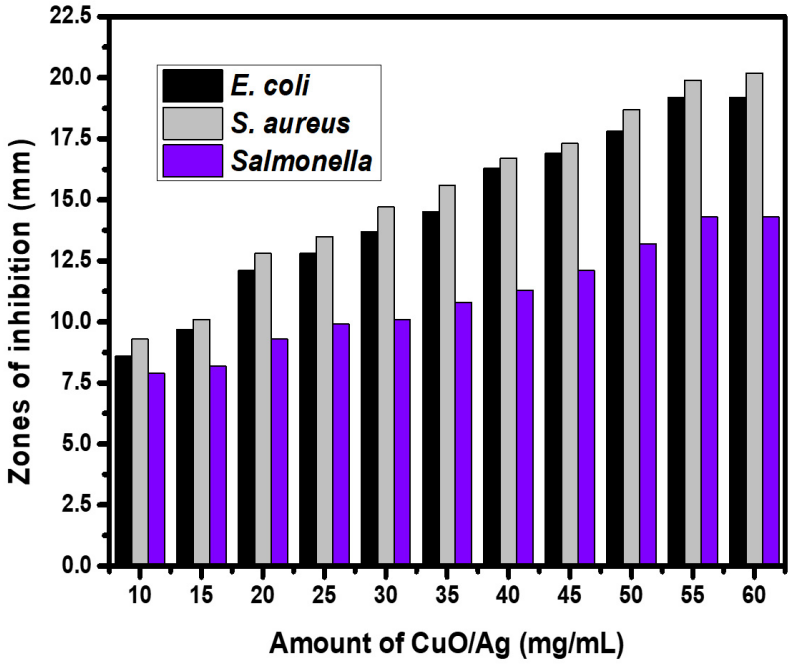

Figure 9. Antibacterial activity of CuO/Ag (calcined)

agent as compared to calcined CuO/Ag. It may be due to the incorporation of biologically active components from the leaf extract into CuO/Ag. After calcination, these components disintegrated and reduced the antibacterial potential of calcined CuO/Ag.[42,43]

CONCLUSION

Azadirachta indica leaf extract and ultrasonication-based synthesis of CuO/Ag were found to be highly efficient, low-cost, and eco-friendly. CuO/Ag has been characterised using different analytical methods. The experimental findings indicated that non-calcined CuO/Ag was a better antibacterial agent as compared to calcined CuO/Ag. At 50 mg/mL of non-calcined CuO/Ag, maximum zones of inhibition around the non-calcined CuO/Ag were found to be 17.9, 20, and 14.3 mm for *E. coli*, *S. aureus*, and *S. enterica* at 50 mg/mL. At the same dosage of calcined CuO/Ag, the zones of inhibition were found to be 17.8, 18.7, and 13.2 mm, respectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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