

RESEARCH ARTICLE

Synthesis, Characterization and Antibacterial Activity of Copper Oxide Nanoparticles

D. Hemalatha^{1*}, B. Shanmugapriya¹**ABSTRACT**

Copper oxide nanoparticles were synthesized by Chemical Precipitation Method using Copper Chloride Dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), Sodium hydroxide (NaOH) as a precipitating agent. The Synthesized Copper Oxide nanoparticles were characterized by X-ray diffraction (XRD), Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX) Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR). The Antibacterial activity of copper Oxide nanoparticles was tested against both gram positive and negative bacteria. In XRD, the crystal size and dislocation density of Copper Oxide nanoparticles were calculated, Element's purity was determined by EDX spectra. The SEM image confirms the presence of homogeneous spherical distribution of copper oxide nanoparticles. The nanoparticles shows interactions between copper and oxygen atoms were supported by FTIR studies. Copper Oxide nanoparticles have exhibits good antibacterial activity against Klebsiella pneumonia, Escherichia coli, Staphylococcus, and Bacillus cereus.

Keywords: Copper Oxide, Precipitation method, Antibacterial activity, Nanoparticle.

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1. Introduction

Nanotechnology defined as the science involving designing, synthesis, characterization and application of materials. Nanoparticles are different from bulk materials and isolated molecules.^[1] Nanotechnology materials have wide range of application due to their size. Nanotechnology survey many areas such as electronics, communication, medical device, medicine, cosmetics, agriculture, architectural, textile, food, metallurgy, defense and security, space and many more. In general the size of nanoparticles spans the range between 1 and 60 nm.^[2] A nanostructure is a structure of intermediate size between microscopic and molecular structure.^[3] Various methods have been used for synthesis of nanoparticles including chemical, physical, electrochemical, photochemical and biological techniques. Precipitation is the creation of a solid from a solution, thus process is the onset of nucleation. In this process more interest in industries. Copper oxide is a semiconducting compound and the band gap of copper oxide (1.0 eV to 2.08 eV).^[5] It is useful for solar energy conversion.^[6] Copper oxide nanoparticles due to their unique physical and chemical properties and the low cost

of preparation.^[7] Copper oxide nanoparticles have potential industrial use such as gas sensor, catalytic processes, high temperature, superconductor, solar cell.^[8,9] Copper oxide nanoparticles are active against antimicrobial, and characterized by XRD, FTIR, SEM and EDX.

2. Experimental Procedure

0.1 M of Copper Chloride Dihydrate was dissolved in 100 ml deionized water^[4], 0.7 g of Sodium Hydroxide is also dissolved in 100 ml deionized water using magnetic stirring (400 RPM) for 20 minutes separately. Sodium Hydroxide is added to Copper Chloride Dihydrate drop by drop under continuous stirring (400 RPM) at room temperature until pH reached to 14 (2 hours 30 minutes), the precipitant solution was kept ageing 48 hours. Finally, black precipitate was obtained which is filtered, washed several times with deionized water and ethanol until pH reached 7. After the precipitate was dried in oven at 80°C for 20 hours and grounded to fine powder using agate mortar to obtain Copper Oxide Nanoparticles. The resulting fine powder was calcinated using silicon crucible at 500°C for 4 hours.

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2.1 Preparation of piper betle leaf extract

About 50gm of fresh healthy leaves of piper betle leaf were collected from Erode, Tamil Nadu. They were washed thoroughly with de-ionized water, they are cut into fine pieces and boiled with 100ml de-ionized water until the water changes to golden yellow color and the extract was filtered through filter paper. 100ml of distilled water and 4mm of ferric chloride is stirred using a magnetic stirrer.

3. RESULTS AND DISCUSSIONS

3.1 XRD Analysis:

The powder X-Ray diffraction method was used to confirm the presence of Copper Oxide. XRD peaks confirm that the formation of Copper Oxide Nanoparticles. The characteristics peaks located at $2\theta = 35.53^\circ, 38.76^\circ, 48.83^\circ$. That assigned to diffraction from (111), (-111), (-202) plane. The presence of sharp peaks and straight line shows the synthesized powder containing crystalline in nature. The average crystalline size (D) for Copper Oxide was obtained from peaks using Debye Scherer's formula and also dislocation density, strain is calculated for the high peak is shown figure and tabulated.

3.2 FTIR analysis

The FTIR spectrum of the synthesized copper oxide nanoparticles were prepared by precipitation method.

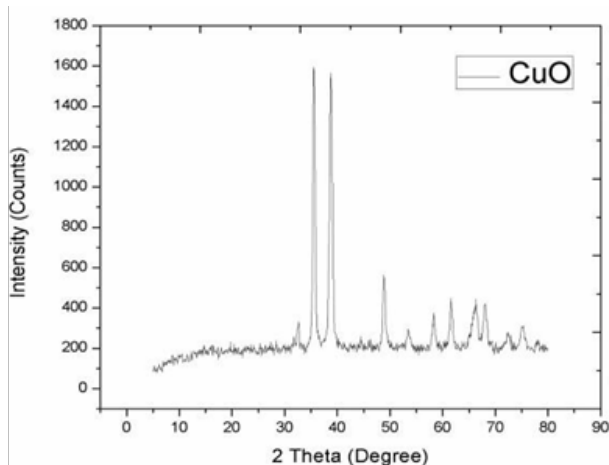


Fig. 1: XRD Spectra of Copper Oxide nanoparticles

FTIR was employed to identify functional group of the material. The FTIR spectra of CuO nanoparticles recorded in the range 400 to 4000 cm^{-1} . Nanoparticles, reported the characteristic peaks of the IR spectra of Copper Oxide in the range 400-700 cm^{-1} . Two peaks at 597.93 cm^{-1} and 514.99 cm^{-1} in the FTIR spectra reported the presence of copper oxide symmetric stretching mode is confirmed.^[9] Therefore, the metal-oxygen frequencies observed for Copper Oxide Nanoparticles are in close agreement with that of literature values. For presence of C-H stretching vibration confirms the alkane compound class at 2889.34 cm^{-1} .^[10] The presence of C=O strong stretching (conjugated aldehyde) confirmed at 1703.14 cm^{-1} , the presence of N-O strong stretching in nitro compound confirmed at 1525.69 cm^{-1} .^[10] The presence of medium O-H bending in carboxylic acid at 1427.32 cm^{-1} and the presence of C-O strong in aromatic ester confirmed at 1309.69 cm^{-1} .^[10]

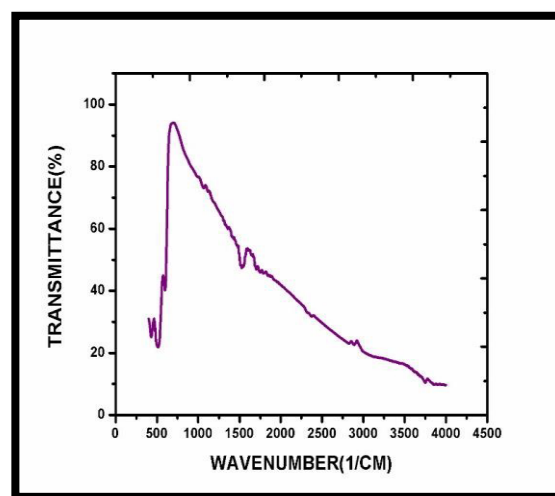


Fig. 2: Fourier Transform Infrared Spectroscopy Spectrum

3.3 SEM analysis

Scanning Electron Microscopy was employed to analyze the morphology and the growth features of the prepared nanoparticles. The SEM images of Copper Oxide nanoparticles prepared by precipitation method. The copper oxide particles with homogeneous spherical distribution particles shape.^[11]

Table 1: XRD Spectra of Copper Oxide nanoparticles

2θ (deg)	FWHM (deg)	FWHM (rad) 10^{-3}	Crystalline size D (10^{-9})m	Average crystalline size D (10^{-9})m	Dislocation Density δ (10^{-3})m	Micro strain ϵ (10^{-3})m
35.5382	0.52360	9.13391	16.6491	15.6413	3.60760	2.17453
38.7602	0.62080	10.8295	14.1757		4.976350	2.55394
48.8346	0.56630	9.87878	16.09929		3.858216	2.24880

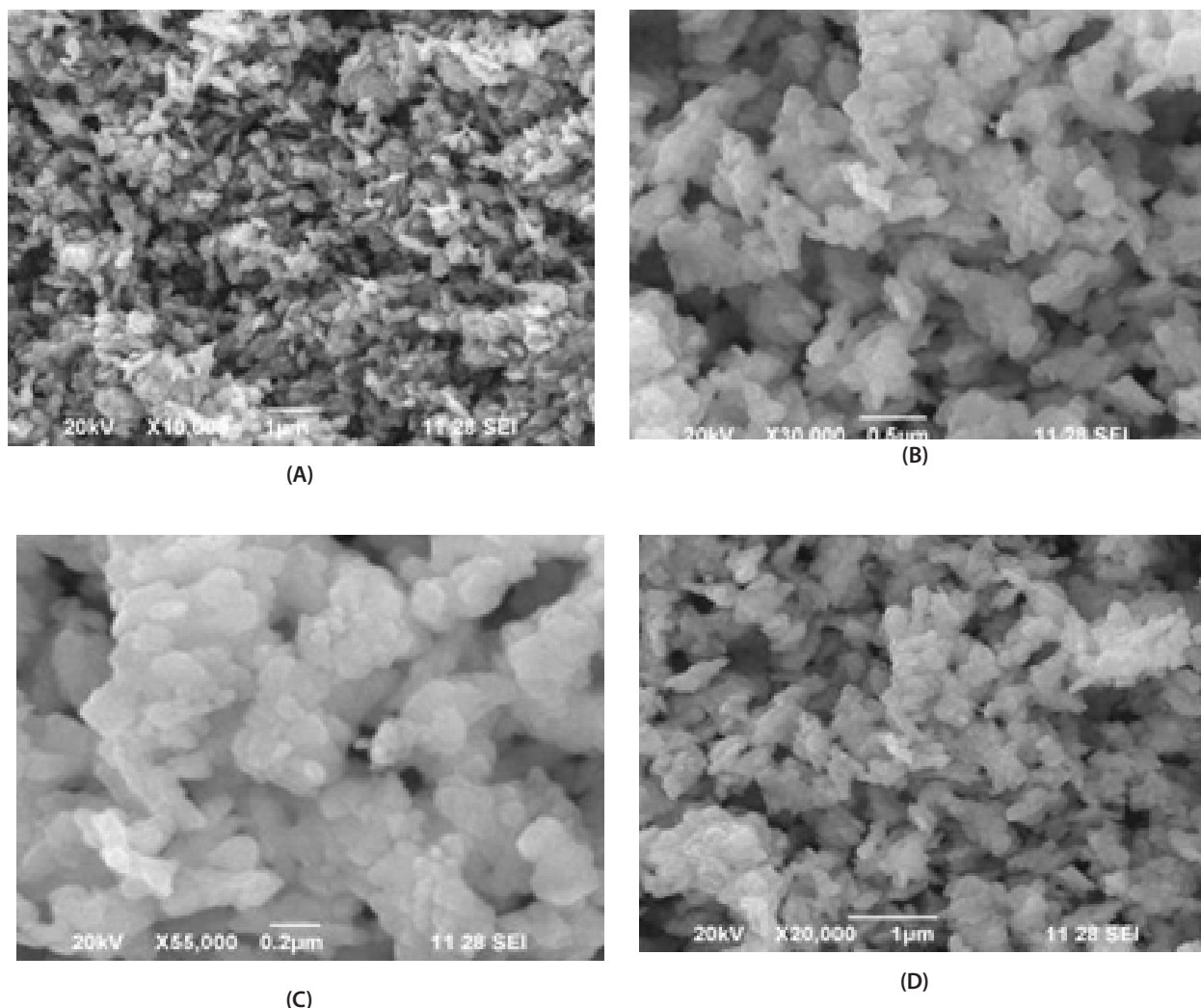


Fig. 3: SEM images of Copper Oxide nanoparticles

3.4 Energy Dispersive X-ray Analysis (EDAX)

The chemical identity and purity of CuO were investigated with Energy Dispersive X-ray Analysis (EDX) and the pattern is

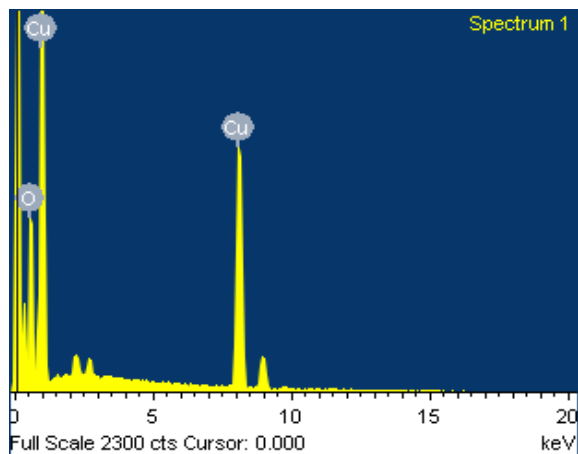


Fig. 4: Energy Dispersive X-ray Analysis of Copper Oxide nanoparticles

shown in figure clearly. EDX analysis was O: 25.59% of weight and Cu: 74.41% of weight. There is no other elements impurity in the EDX spectra. The element analysis of the sample shows that the prepared sample was copper oxide.

3.5 Antibacterial

The given sample was tested for antimicrobial activity by well diffusion method. Liquid Mueller Hinton agar media and the Petri plates were sterilized by autoclaving at 121°C for about 30 minutes at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 18 hrs culture of Gram positive microorganisms such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), Gram negative microorganisms such as *E. coli* (MTCC 1698) and *Klebsiella pneumoniae* (MTCC10309) obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with loading of 100 μl of each sample to the distinct well with

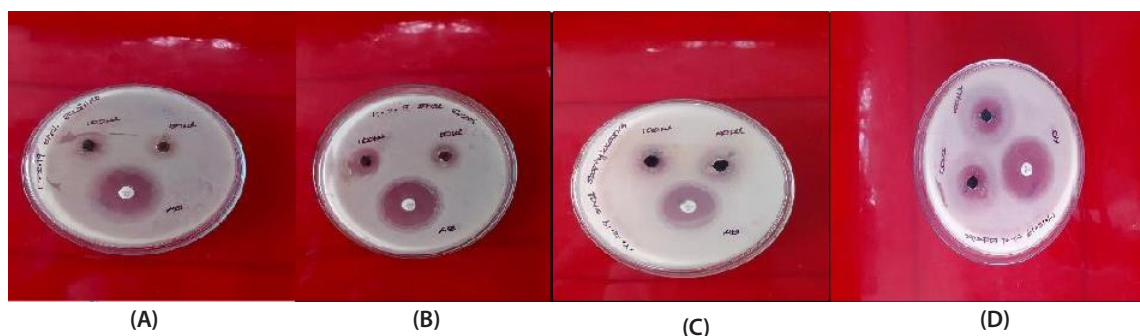


Fig. 5A to D: (A) *Bacillus cereus*, (B) *Escherichia coli*, (C) *Staphylococcus aureus*, (D) *Klebsiella pneumonia*
Photographs of Antibacterial activity of Microorganism

Table 2: Antimicrobial results of Copper Oxide Nanoparticles

S. No	Microorganism	Zone of Inhibition in Diameter (mm)			Std. Antibiotic (vancomycin) 30mcg
		Control 100µl	Copper Oxide		
			50µl	100µl	
1	Bacillus cereus	Nil	12	15	30
2	Staphylococcus aureus	Nil	14	17	22
3	Escherichia coli	Nil	15	18	25
4	Klebsiella pneumonia	Nil	24	28	31

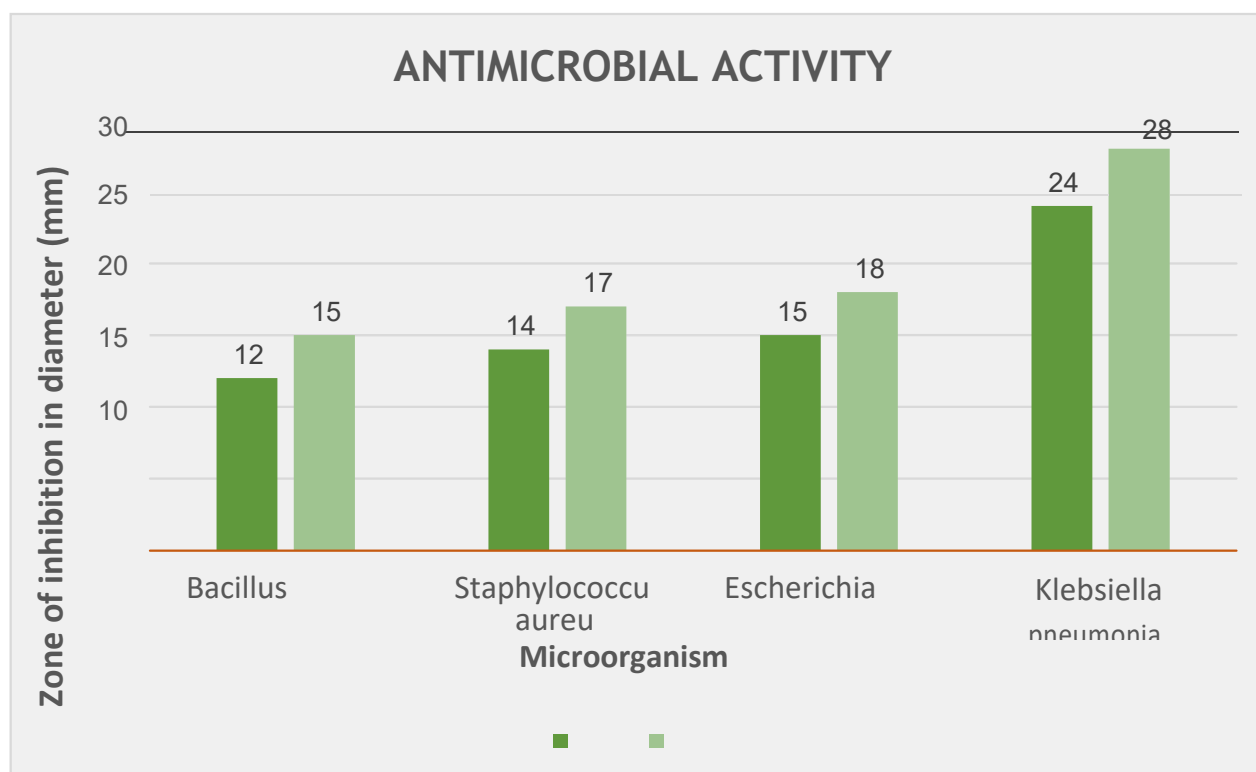


Fig. 6: Comparison graph for Antibacterial activity of Microorganism

sterile distilled water as negative control and vancomycin (30mcg/disc) as positive control. The sample loaded plates were then incubated at 37°C for 24 hours to observe the zone of inhibition.

Gram positive microorganism such as *Bacillus cereus*, *Staphylococcus aureus*, Gram negative microorganism, *E. coli* and *Klebsiella pneumonia* were tested. Copper oxide nanoparticles are more destroyed *Klebsiella pneumonia* when compare to *Staphylococcus*, *Bacillus cereus*, *E. coli*. *Klebsiella pneumonia*. *K. pneumonia* can be treated with antibiotics if the infections are not drug-resistant. Infections by *K. pneumonia* can be difficult to treat because fewer antibiotics are effective against them.

4. CONCLUSION

Copper oxide nanoparticles was synthesized that confirmed with XRD analysis its average crystalline size 15.64 nm, dislocation density, and micro strain were calculated by using Scherer's formula. The SEM image confirms the particles are homogenous spherical distribution of copper oxide nanoparticles. The element qualification ratio of copper oxide nanoparticles were confirms the present of Cu, O by EDX analysis. The FTIR spectra confirmed the presence of Copper Oxide and change the functional group during each reaction, the functional group are C-H, C=O, N-O stretching and O-H bending are present in Copper Oxide nanoparticles. Antibacterial activity against *Klebsiella pneumonia* have been performed using Copper Oxide nanoparticles.

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