Determination of Antagonistic Effect of CuO NPs against Bacterial Cultures

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Abstract - **Bacterial cultures are capable of causing infections to the humans. These bacteria can be killed or inhibited by special compounds called antibacterial agents. These compounds are generally not toxic to humans as most of the compounds are obtained from natural sources, such as, b-lactams (like penicillins), cephalosporins. Overuse of traditional antibacterial drugs, resistance may develop by bacterial cells, which ultimately leads to pose greatest health challenges by occurrence of infectious diseases. Therefore, development of non-resistance alternative antibacterial agents for better antibacterial efficacy is mandatory. This paper reports on the synthesis of copper oxide nanoparticles carried out by chemical precipitation method and explored its antibacterial efficacy against hospital borne bacterial infections. Copper oxide nanoparticles were synthesized by solvothermal route. The screening of antimicrobial activity of copper oxide nanoparticles was studied on the bacteria Staphylococcus aureus NCIM 2079 and Bacillus cereus NCIM 5293 by Anti Well Diffusion Assay (AWDA) on nutrient agar (NA) medium. It was evident that the Staphylococcus aureus was more sensitive for CuO NPs compared to Bacillus cereus. The synthesized CuO-NPs showed remarkable antibacterial activity against Bacillus cereus and Staphylococcus aureus. Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of CuO-NPs were determined by calculating concentration dependent colony forming units per milliliter (CFU/mL) on agar plate. MIC and MBC of CuO-NPs against B. cereus, and S. aureus was found to be 10 µg/mL and 100 µg/mL, respectively, for both the cultures proving that CuO-NPs can be used in medicine and food industries.**

Objective: To synthesis of copper oxide nanoparticles carried out by chemical precipitation method and explored its antibacterial efficacy against hospital borne bacterial infections.

Materials and Methods: 1M cupric sulphate was dissolved in 25 mL of distilled water and 50 mL of 2 M NaOH was added drop wise into the precursor solution followed by continuous stirring at 90 ºC for 30 minutes on magnetic stirrer. The brownish black color precipitate was obtained after reaction, which was characterized by UV- Vis Spectroscopy and Scanning Electron Microscopy (SEM) to understand optical and morphological features of materials.

Results: The λmax was found to be at 330 nm and band gap calculated was 3.76 eV. Antibacterial efficacy was determined by anti-well diffusion assay against Staphylococcus aureus NCIM 2079 and Bacillus cereus NCIM 5293. The bacterial cultures were found to be sensitive for CuO NPs at concentration in the range of 10- 1000 µg/mL. It was evident that the Staphylococcus aureus was more sensitive for CuO NPs compared to Bacillus cereus. The synthesized CuO-NPs showed remarkable antibacterial activity against Bacillus cereus and Staphylococcus aureus.

Keywords:* *CuO NPs, chemical reduction method, antibacterial activity, Staphylococcus aureus, Bacillus Cereus

I. INTRODUCTION

Staphylococcus aureus and *Bacillus cereus* are pathogenic bacterial cultures capable of causing infections to the humans [1], [2]. However, such bacteria can be killed or inhibited by special compounds called antibacterial agents. These compounds are generally not toxic to humans as most of the compounds are obtained from natural sources, such as, b-lactams (like penicillins), cephalosporins etc (Von Nussbaum et al., 2006). Broadly, the antibacterial agents can be classified as either bactericidal, which kill bacteria, or bacteriostatic, slowing down bacterial growth. Such antibacterial agents have been used from decades against various pathogenic bacterial cultures to fight infectious diseases. However, overuse of traditional antibacterial drugs, resistance may develop by bacterial cells, which ultimately leads to pose greatest health challenges by occurrence of infectious diseases. Therefore, development of non-resistance alternative antibacterial agents for better antibacterial efficacy is mandatory.

The inorganic materials of size in the range of 1-100 nanometers are considered as nanomaterials (Subedi, 2014), which can used as an alternative non-resistance antibacterial agent. The nanomaterials reported having unique properties like high surface to volume ratio, quantum confinement effect, improved mechanical, chemical, electrical, optical, magnetic, electro-optical, magneto-optical and antimicrobial properties (Subedi, 2014; Varier et al., 2019; Shirsat et al., 2019; Whitesides, 2005). Researchers explored assorted nanomaterials for antimicrobial activity and used as antibacterial (Xiu et al. 2012) and antifungal (Wang et al. 2014) agents. The reported nanomaterials which exhibit antimicrobial properties includes silver nanoparticles (Franci et al. 2015), titanium oxide (Qureshi et al., 2021), magnesium oxide (MgO) (Jin T and He Y, 2011), copper oxide (CuO) (Shirsat et al., 2019), zinc oxide (ZnO) (Pawar et al., 2017).

Among all the reported nanomaterials, metal oxide nanoparticles attract considerable interest due to their potential technological applications in the areas of targeted drug delivery, gene therapy, photo imaging, antioxidant activity, antimicrobial activity, cancer therapy and catalysis (Varier et al., 2019). It has been established that the highly reactive metal oxide nanoparticles exhibits bactericidal activity against gram-positive and gram-negative bacteria (Stoimenov et al. 2002). Copper oxide nanoparticles (CuO NPs) has used in a wide range of applications such as high temperature superconductor, solar cells, gas sensors, catalytic applications, optical applications, antimicrobials and medical applications (Rubilar et al., 2013; Shirsat et al., 2019). Copper oxide nanoparticles were synthesized by various methods such as, aqueous precipitation, hydrothermal, sol-gel, chemi-thermal, chemical precipitation, semi-solvothermal (Jain et al., 2019; Pawar et al., 2017; Pawar et al., 2019; Prakash et al., 2017; Shwe and Win, 2013). However, for the large scale production of CuO NPs aqueous precipitation method was found to be good (Tran and Nguyen, 2014), hence, same method was used in present study. CuO NPs are brownish-black color powder and crystalline in nature (Khan et al., 2017). In present study, the antibacterial efficacy of CuO NPs was tested against *Staphylococcus aureus* NCIM 2079 and *Bacillus cereus* NCIM 5293 by anti-well diffusion assay (AWDA) method (Pawar et al., 2012).

II. MATERIALS AND METHODS

A. *Chemicals and Media*

All the bulk chemicals, solvents used in the study were of analytical reagent (AR) grade and procured from Sisco Research Laboratories and Qualigenes, India. The microbiological media components were purchased from Hi-Media Corporation, Mumbai, India.

B. *Synthesis of copper oxide nanoparticles*

In typical experimental procedure, 1M cupric sulphate was dissolved in 25 mL of distilled water by continuous stirring on magnetic stirrer at room temperature for 30 minutes. Similarly, 2 M NaOH was prepared in 50 mL of distilled water and added drop-wise into the cupric sulphate solution with continuous stirring at room temperature. The resultant reaction mixture was further heated at 90 °C for 30 minutes. Followed by heating the precipitate was washed twice by centrifugation at 5000 rpm for 15 minutes with DI water. The final product was dried in hot air oven at 90 °C for 24 hours and then calcination at 250 °C for 4 hours in muffle furnace

$$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2 + Na_2SO_4$$

$$Cu(OH)_2 \rightarrow CuO + H_2O$$

C. *Characterization of copper oxide nanoparticles*

The synthesized material was characterized by UV-Visible spectroscopy and Scanning Electron Microscopy (SEM) to analyze the optical and morphological features.

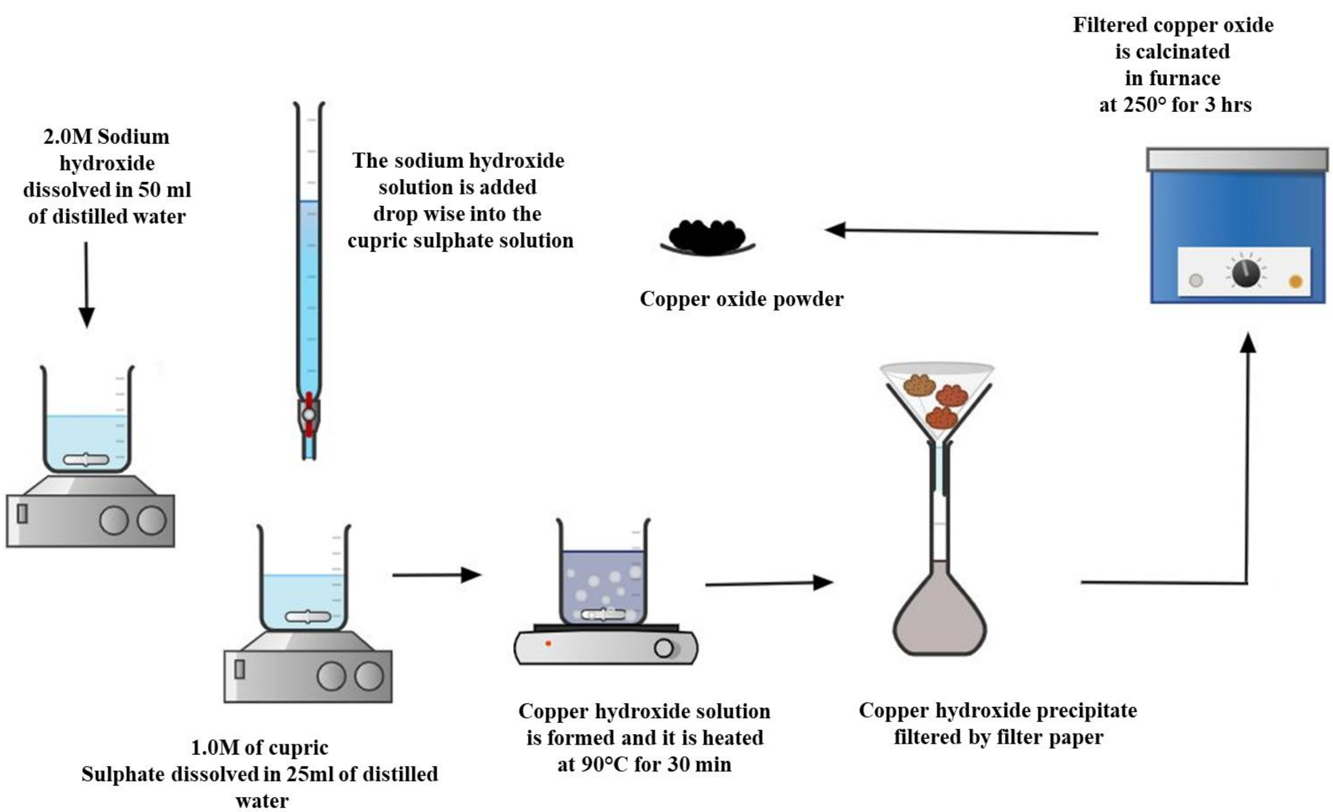

Fig. 1. Schematic of synthesis of CuO-NPs by chemical reduction method

D. Antimicrobial activity of copper oxide nanoparticles

The screening of antimicrobial activity of copper oxide nanoparticles was studied on the bacteria Staphylococcus aureus NCIM 2079 and Bacillus cereus NCIM 5293 by Anti Well Diffusion Assay (AWDA) on nutrient agar (NA) medium. The bacterial inoculum was prepared from the colonies of 24 h old culture on nutrient agar medium. The inoculum was prepared to final concentration of approximately 1 X105 CFU/mL for the selected bacterial cultures. The synthesized CuO-NPs of concentration 100 µg/mL were dispersed in 1 % DMSO by ultra-sonication to make colloidal solution of nanomaterials. On the surface of agar plates, wells of 5 mm in diameter and of 18 µL in capacity were formed by using sterile gel borer. The 15 µL of CuO-NPs suspension was placed in each well and was incubated at 37 °C ± 2 °C for 24 hours and zone of inhibition were recorded to understand antibacterial efficacy of CuO-NPs. Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of CuO-NPs were determined by calculating concentration dependent colony forming units per milliliter (CFU/mL) on agar plate. The MIC and MBC are the lowest concentration of CuO-NPs that inhibit growth and kill more than 3 logs (99.9 %) of bacterial cells, respectively. All the in-vitro antibacterial activities of CuO-NPs were performed in 96-well microtiter plate containing 200 µL of MH broth with CuO-NPs (in the concentration range of 10 µg/mL - 1000 µg/mL) and bacterial cultures (final cell density of 1□10 5 CFU/ mL) and incubated at 37 °C ± 2 °C for 24 hours. Subsequently, growth inhibition of bacterial cultures in each well of microtiter plate was measured by inoculation of 100 µL of respective bacterial suspension on MH agar medium plates and incubated at 37 °C ± 2 °C for 24 hours. The CFU/mL was calculated for all the concentrations compared with 100 % survival for the untreated control plate.

III. RESULTS

A. Characterization of CuO NPs

Ultraviolet- Visible analysis was done by using analysis of synthesized copper nanoparticle is shown in (**Figure 2)**. UV Visible spectroscopy maximum absorbance in range of λ max at 345 nm. The optical band gap was calculate by Max plank equation (2), Planck constant (h) is 6.626×10-34 Joules sec and velocity of light (c) is 2.99×108 meter/sec and band gap calculated 3,76 eV.

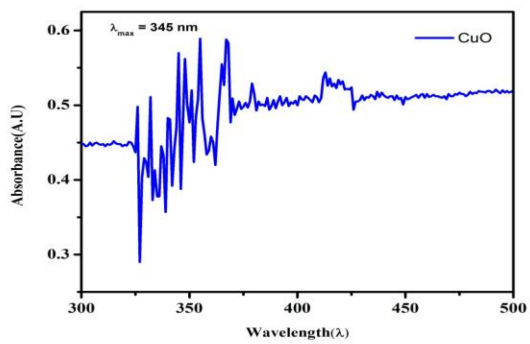

Fig. 2. UV-Visible Spectroscopy

B. Analysis of CuO NPs by UV-Visible Spectroscopy and Scanning Electron Microscopy (SEM)

The SEM images have shown particle agglomeration [3]. Hence, the specific size and shape of CuO nanoparticle is not determined. It is revealed that the agglomerated copper oxide nanoparticles having particle size range of 200 nm1000 nm.

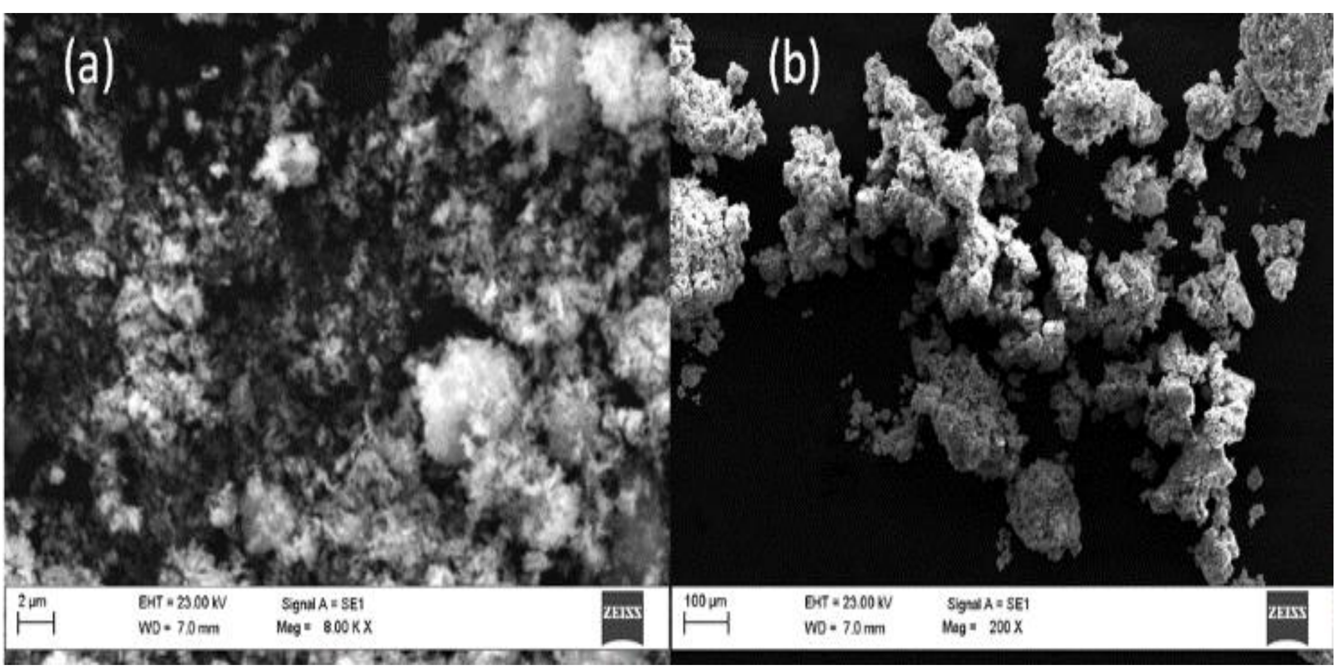

Fig. 3. SEM images of CuO-NPs

C. Antimicrobial activity

In the present study, CuO-NPs synthesized by chemical precipitation method were used to study the antibacterial efficacy against the test bacterial cultures. The antibacterial activity of the dispersed CuO-NPs was confirmed by spotting a zone of inhibition (**Figure. 4**). It has been observed that synthesized material exhibits better antimicrobial activity against *Bacillus cereus* than the *Staphylococcus aureus* at 100 µg/mL and more concentrations of CuO-NPs (**Table 1**). The antimicrobial activity of CuO-NPs was determined by evaluating its MIC and MBC. The antimicrobial effectiveness was determined against the microbial concentrations of 10^5CFU/mL with different concentrations of nanoparticles namely 10, 100 and 1000 µg/ml. It was noticed that, as the concentration of nanoparticles increases, the final microbial concentration decreases. It was noticed that, as the concentration of nanoparticles increases, the microbial growth inhibition increases. MIC and MBC of CuO-NPs against *B. cereus,* and *S. aureus* was found to be 10 µg/mL and 100 µg/mL, respectively, for both the cultures. The corresponding MIC and MBC values are summarized in **Table 1.**

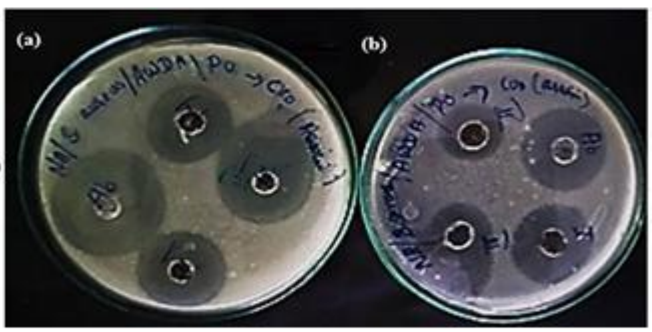

Fig. 4. **Antibacterial Effect of silver nanoparticles by anti-well diffusion assay (a)** *S. aureus* and **(b)** *B. cereus* at different concentration For well number 1 to 4 in each plate, CuO-NPs of 10 µg/mL, 100 µg/mL, 1000 µg/mL concentrations and Ciprofloxacin (10 µg/mL) were added, respectively.

TABLE I. ANTIBACTERIAL EFFICIENCY OF COPPER OXIDE NANOPARTICLES AGAINST BACTERIAL CULTURES

Bacterial cultures	***B. cereus***	***S. aureus***
Concentrations (µg/mL)	**Zone of inhibition (mm²)**	
10*	11	12
100	18	16
1000**	23	21

Note: *MIC and **MBC

D. XRD X-ray diffraction

The X-ray diffraction (XRD) pattern graph 1 of nano-AlOOH shows a broad peak in the reflection angle range for two theta values between 10-15, 25-30, 35-40, 45-50 and 60-70, with the corresponding hkl reflections of (110), (111) ,(111), (112), (202), (020), (202), (113), (022,311), (220), (004) which indicates high crystalline order of the synthesized compound. In addition to that, the XRD pattern shows broadening of the intensity peaks (111,111) and it indicate the ultra fine nature of the crystallite. The crystallite size calculated using Scherrer's formula is about 3.67 nm .

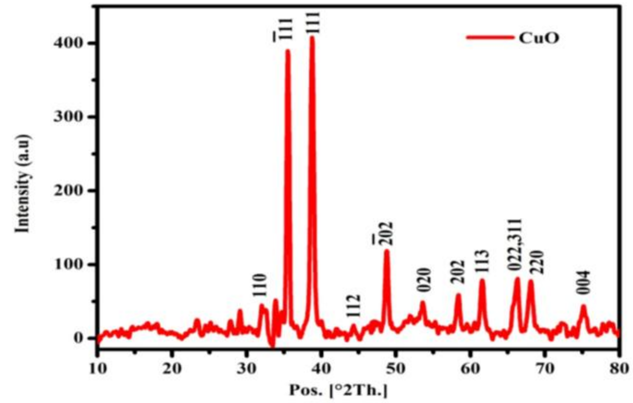

Fig. 5. Shows the XRD spectra of the CuO NPs

E. FTIR-Fourier transform infrared spectroscopy:

The FTIR spectra of copper oxide before and after fluoride Absorption at pH 7 and 4 are presented in this Graph Broad Absorption peaks at 3000-4000 cm^{-1}, and peaks at 1650 cm^{-1} the stretching modes of OH bands related to free water (surface adsorbed water), and the latter were due to the bending mode of H–O–H band. The effect of pH on fluoride adsorption is shown in **Figure 6** The results suggest that solution pH has large impact to fluoride adsorption onto the three adsorbents. With the increase of pH, the adsorption of fluoride onto the adsorbents shows a decreased trend, suggesting acid condition is favourable to adsorption.

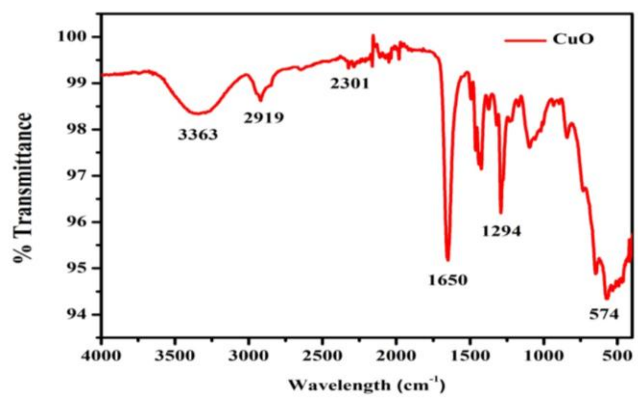

Fig. 6. FTIR Spectra of copper oxide.

IV. DISCUSSION

Synthesis of CuO-NPs was performed successfully using chemical reduction method. The λ_{max} of synthesized CuO-NPs was found to be at 330 nm and the bang gap was calculated to be 3.76 eV. The optical property obtained for CuO-NPs synthesized by hydro-thermal method was found to have λ_{max} at 339 nm (Pawar et al., 2019), which is nearly same obtained through present work. The SEM images of CuO NPs shows cluster of nanostructures having length and width of few microns. However, it is revealed that the agglomerated CuO-NPs having particle size range of 100 nm- 300 nm.

The synthesized CuO-NPs showed remarkable antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*. Similarly, CuO-NPs synthesized by hydro-thermal method were tested against *Bacillus cereus* by the MBRT method and found to be effective antibacterial agent (Pawar et al., 2019). Moreover, few other metal oxide nanoparticles were tested for their antimicrobial efficacy against different bacterial cultures and found to have similar effects. For instance, ZnO-NPs synthesized by semi-solvo thermal method found affective antibacterial agent against *Pseudomonas aeruginosa* (Pawar et al., 2017) *Salmonella typhimurium* and *Bacillus cereus* (Pawar et al., 2017). In present study the antibacterial mechanism of CuO-NPs not been studied, however, it was investigated by many researchers that due to; release of Cu^{2+} ions which kills bacterial cell on interaction (Hsueh et al., 2017), Cu^{2+} ions may rupture the bacterial cell by interacting with negatively charged bacterial cell wall, thereby leading to protein denaturation and cell death (Azam et al., 2012).

It has been observed that synthesized material exhibits better antimicrobial activity against *Staphylococcus aureus* NCIM 2079 and *Bacillus cereus* NCIM 5293. In another study conducted by Azam *et al* it was found that CuO nanoparticles have shown greater antimicrobial activity against *B. subtilis* and *S. aureus*. Therefore, the difference in sensitivity or resistance to both Gram positive and Gram negative bacterial cultures has evidenced due to the differences in their cell wall structure, physiology, metabolism or degree of contact of organisms with nanoparticles (Shirsat et al., 2019). The interactions between the negative charges of microorganisms and the positive charge of nanoparticles produces an electromagnetic attraction between the microbe and effective levels of active nanoparticles. Such interactions lead to oxidation of surface molecules of microbes resulting in their death (Shirsat et al., 2019).

V. CONCLUSION

Copper nanoparticles have been synthesized by chemical reduction technique and tested the same for their antibacterial potential against *Staphylococcus aureus* NCIM 2079 and *Bacillus cereus* NCIM 5293. The λmax was found to be at 330 nm and band gap was 3.76 eV. The study revealed that the agglomerated copper oxide nanoparticles having particle size range of 200 nm1000 nm. MIC and MBC of CuO-NPs against *B. cereus*, and *S. aureus* was found to be 10 μg/mL and 100 μg/mL, respectively, for both the cultures. Therefore, CuO-NPs can be used in medicine and food industries.

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