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# NANOMETAL OXIDES AS ANTIMICROBIAL AGENTS ($Al_2O_3$, CuO, $Fe_3O_4$, and ZnO): COMPARATIVE STUDY

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**ABSTRACT**

The present research work primarily deals with the characterization and antimicrobial efficacy of aluminium oxide, iron oxide, copper oxide and zinc oxide nanoparticles synthesized by a coprecipitation method. The prepared nanoparticles were characterized by XRD (X-Ray Diffraction), FTIR (Fourier Transform Infrared Radiation), UV-Visible spectroscopy and SEM (Scanning Electron Microscope) with EDX (Energy Dispersive X-ray analysis). The antibacterial activity and minimum inhibitory concentration of the nanoparticles were carried out by agar well diffusion method and broth dilution method respectively against gram negative (*Escherichia coli* and *Proteus vulgaris*) and gram positive (*Staphylococcus aureus* and *Streptococcus mutans*) bacteria. The average crystallite size of the metal oxide nanoparticles was found to be 35nm ($Al_2O_3$, IO, ZnO ) and 19nm (CuO) by X-ray diffraction. The antibacterial activity test evidently expressed that gram negative bacteria are much sensitive to metal oxide nanoparticles when compared to gram positive bacteria. The results suggest that the synthesized metal oxide nanoparticles ($Al_2O_3$, CuO, $Fe_3O_4$, and ZnO) are effective antimicrobial agents.

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## INTRODUCTION

Over the years antibiotics have been used to treat infection in both community and hospital environments [1]. Most of the pathogenic bacteria are resistant to at least one of the antibiotics that are generally used to eradicate the infection [2]. The consumption of antibiotics as a regular medication for infectious diseases in due course led to a serious risk of antibiotic resistance. For example, extensive use of methicillin has led to the development of Methicillin Resistant *Staphylococcus aureus* (MRSA) which is still a major issue in hospitals [3]. Microbes are more uncertain to develop resistance against nanoparticles because they attack a broad range of targets which requires the microorganism to simultaneously undergo a series of mutations in order to protect themselves [4]. Therefore, these backlogs directed the scientists to focus on building up of antimicrobial agents to which microorganisms might not develop resistance. Thus came metal oxide nanoparticles into the limelight.

Nanotechnology can be used to modify the material at the nanoscale. The novel properties and low amount of material consumption have attracted global interest across disciplines and industries [5]. The nanostructures are accomplished by improving the physical properties in areas such as antimicrobial properties, water repellence, soil resistance and antistatic properties [6]. Decreasing the particle size can change the physical and structural properties of nanomaterial [7]. Reactive groups on a particle surface are likely to modify its biological activity. Therefore, changes in surface chemistry [8]. The advantages of consuming these inorganic metal oxide nanoparticles as biocidal agents are their superior effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance. Along with these things they offer mineral elements essential to mammalian cells and even trace quantities of them exhibit solid activity [9-11]. Moreover, compared to organic antimicrobial agents, inorganic antimicrobial agents show superior durability, less toxicity, greater selectivity and heat resistance [12]. Different methods are used to synthesize nanoparticles such as co-precipitation [13,14], sol-gel processing [15], high energy ball milling [16,17], and thermal plasma [18] are used for synthesizing nanoparticles. In the present paper, an attempt has been made to compare the antibacterial activity and minimum inhibitory concentration of four nanoparticles ($Al_2O_3$, CuO, $Fe_3O_4$, and ZnO) against gram negative and gram positive bacteria.

## MATERIALS AND METHODS

### Materials

The chemicals used for the synthesis of metal oxide nanoparticles ($Al_2O_3$, CuO, $Fe_3O_4$, and ZnO**)** were purchased from Merck chemicals. The test organisms, *E. coli* (MCC 2412) and *Staphylococcus aureus*(MCC-2408) were procured from MCC, Pune, India.*Proteus vulgaris* (MTCC-426) and *Streptococcus mutans* (MTCC-497) were collected from MTCC, Chandigarh, India. Media required for the cultivation of microorganisms are Nutrient agar (*E. coli* and *Proteus vulgaris*)Trypticase soy yeast extract agar (*Staphylococcus aureus*) and Brain heart infusion agar (*Streptococcus mutans*) were obtained from Hi-Media Pvt Ltd. All the chemicals used in this experiment were analytical grade and used without further purification.

### Synthesis of Metal Oxide Nanoparticles

The metal oxide nanoparticles ($Al_2O_3$, CuO, $Fe_3O_4$, and ZnO) were synthesized by co-precipitation method [13].

### Characterization of Metal Oxide Nanoparticles

The compounds were characterized for their structure by using X-ray diffraction (XRD-6100 diffractometer, Shimadzu) with Cu $K_\alpha$ radiation (λ= 1.54060 Å). Molecular analysis of the samples was performed by Fourier transform infrared (FT-IR) spectroscopy using IR Affinity-1s (Shimadzu) spectrometer, recorded in the wave number range of 4,000–400 $cm^{-1}$. The absorption spectra of the samples were recorded in the wavelength range of 200-800 nm using a JASCO V 670 UV-Vis spectrometer. Morphological study of the nanoparticles was carried out by scanning electron microscope (SEM) (EVO 18 carlzeiss).

### Antibacterial Activity of Metaloxide Nanoparticles

### Agar Well Diffusion Method

The antibacterial activity of the metal oxide nanoparticles was determined by agar well diffusion method [19,20] against both gram-negative and gram-positive microorganisms. Once the medium was solidified, a suspension of each sample of the bacteria was diluted prior to $10^{-1}$, $10^{-2}$ and $10^{-3}$ (1 ml of 108 cells/ml) and was spread on a solid agar medium in Petri plates (*E. coli* and *Proteus vulgaris*-Nutrient agar medium; *Staphylococcus aureus*-Trypticase soy yeast extract agar medium; *Streptococcus mutans*–Brain heart infusion agar medium). The wells were prepared by using sterile cork borer (6 mm). Each well was filled with different concentrations of nanomaterial ranging from 10-50 mg/ml. The plates were incubated at 37 ° C for 24 h, the zone of inhibition was measured and mentioned in ±SD values.

### Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined by using a broth dilution method [21]. A series of 4 test tubes were taken, add 10ml of media and a loop full of culture to all the test tubes and finally add 2 mg/ml, 4 mg/ml, 6 mg/ml and 8 mg/ml of nanoparticle suspension to each test tube. The test tube without bacterial suspension is considered as control. Keep the test tubes for overnight incubation at 37°C temperature. Read the absorbance at 600nm using a spectrophotometer. MIC is where the absorbance value of sample equals to or near to control.

## RESULTS AND DISCUSSION

### Powder X-ray Diffraction (XRD) Studies

The X-ray diffraction peaks of $Al_2O_3$ and CuO nanoparticles exhibited monoclinic crystal structures whereas IO and ZnO nanoparticles exhibited hexagonal structures respectively. Figure 1 shows the XRD diffraction peaks of all the four nanoparticles and was matched well with the standard JCPDS card numbers 35-0121 ($Al_2O_3$), 85-0987 (IO), 80-0076 (CuO) and 79-2205 (ZnO).

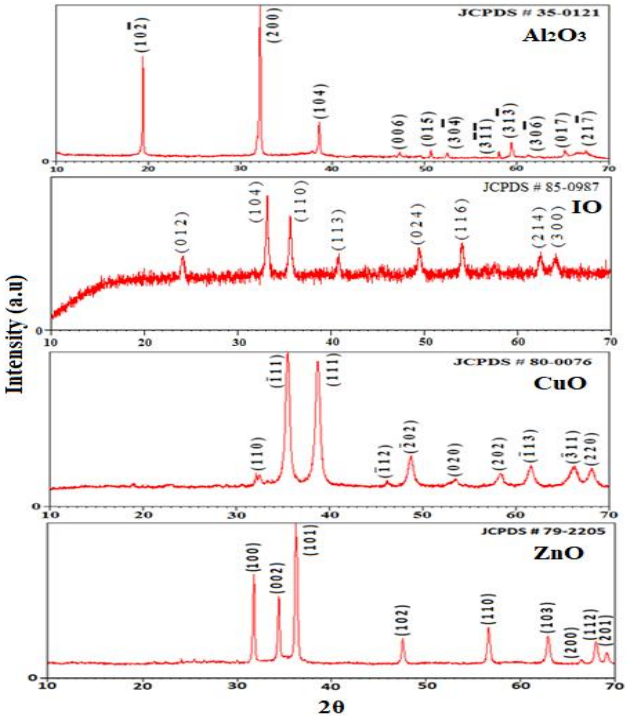


**Fig.No 1: XRD diffraction peaks of metal oxide nanoparticles.**

The average crystallite size of metal oxide nanoparticles was calculated by using Debye Scherrer formula,

$$D = 0.9\lambda / \beta\cos\theta$$

where λ is the wavelength of the X-ray radiation, θ is the diffraction angle and β is the full width half maximum (FWHM) intensity. The average crystallite size of the metal oxide nanoparticles was calculated to be 35nm ($Al_2O_3$, IO, ZnO ) and 19nm (CuO) respectively.

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectra of $Al_2O_3$, IO, CuO and ZnO nanoparticles were recorded in solid phase using the KBr pellet method in the wave number range of 4000-400$cm^{-1}$ (Fig.2). FTIR spectra of all the four metal oxide nanoparticles ($Al_2O_3$, IO, CuO, and ZnO) exhibited vibration bands in the region of 400-700 $cm^{-1}$ which were assigned to the vibrations of M-O (M=Al, Fe, Cu, and Zn).

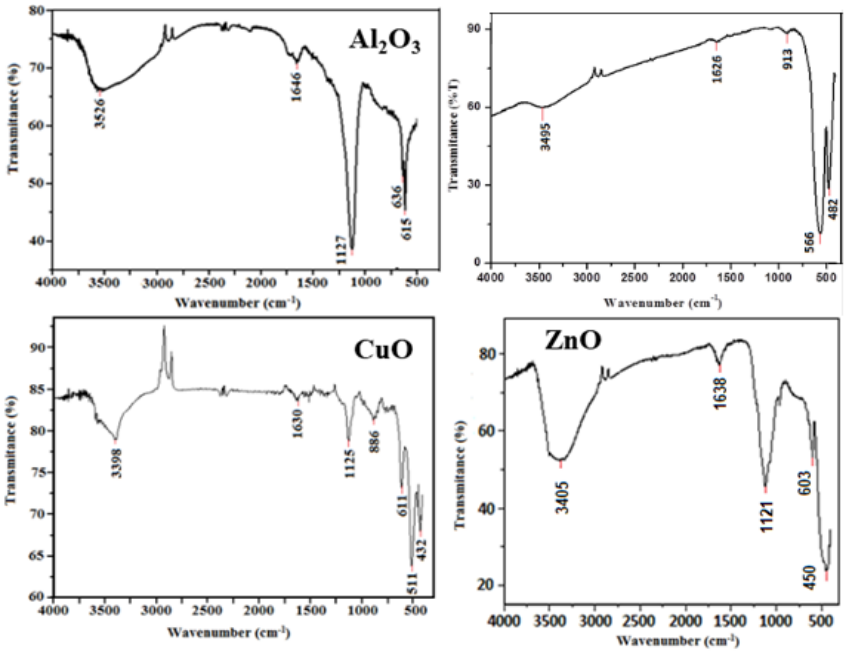


**Fig. No.2: FTIR spectra of metal oxide nanoparticles.**

which confirms the formation of $Al_2O_3$, IO, CuO and ZnO nanoparticles. The remaining peaks around 1650 $cm^{-1}$ and 3450 $cm^{-1}$ were attributed to the bending and stretching vibration of the water molecule.

**UV-Visible Spectroscopy Studies**

UV-Visible spectra of $Al_2O_3$, IO, CuO, and ZnO nanoparticles were recorded in the wavelength range of 200-800nm. The optical absorption bands were observed for $Al_2O_3$ at 340nm, IO at (347, 371, 446, 553 and 685nm) CuO at 402,422nmand ZnO at 383nm which were attributed to the characteristic absorption peaks for that particular metal oxide nanoparticle.

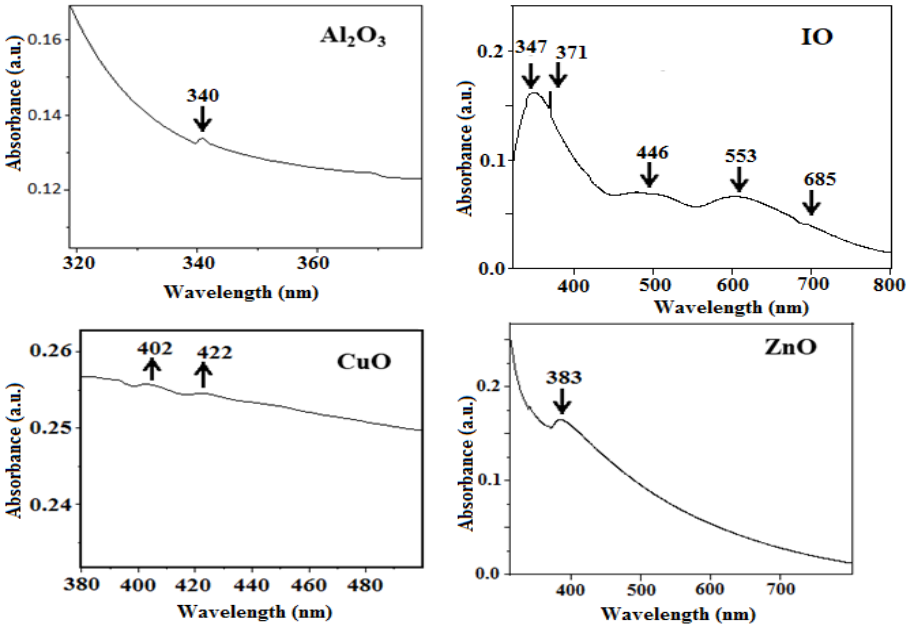


**Fig.No. 3: Optical absorption spectra of metal oxide nanoparticles.**

**Scanning Electron Microscope and EDX Analysis**

Surface morphology of $Al_2O_3$ is an irregular spherical shape, IO shows stone morphology, CuO is in flower shape and ZnO shows spherical morphology (Fig.4). Energy Dispersive X-ray spectroscopy spectrums of synthesized metal oxides have shown the purity of the sample and no other elemental impurity was observed (fig. 5).

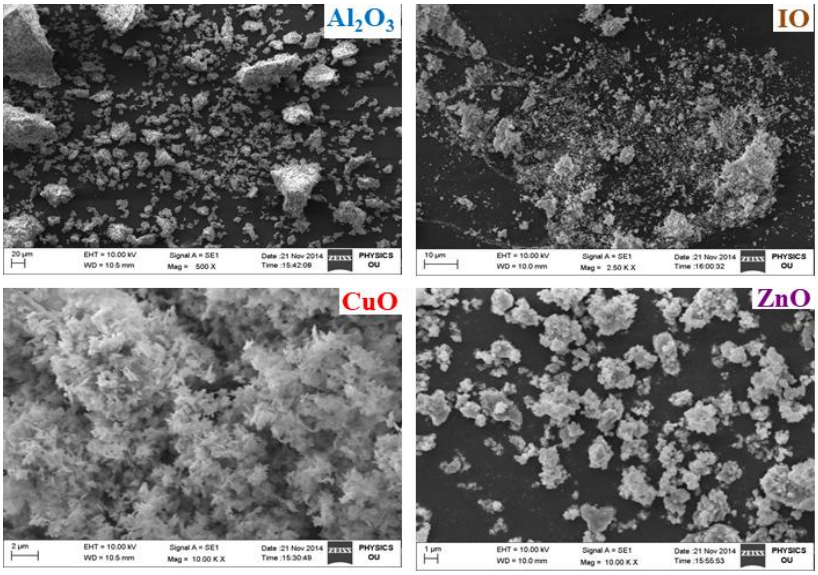


**Fig.No. 4: SEM analysis of metal oxide nanoparticles.**

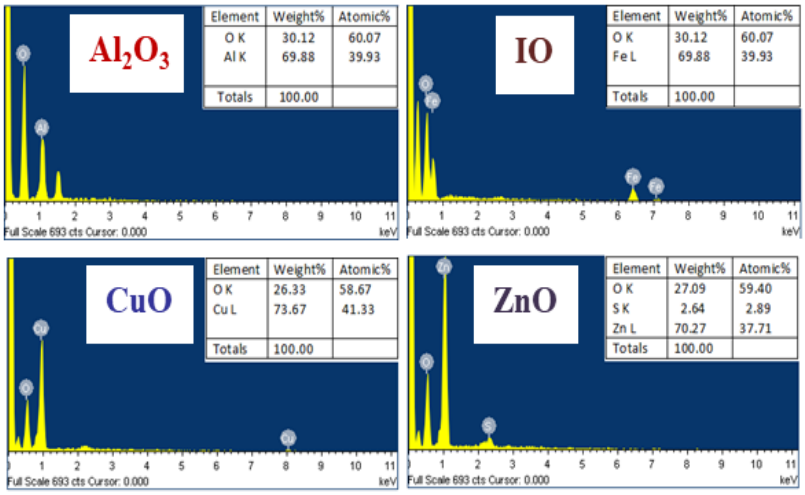


**Fig.No. 5: EDX spectrum of metal oxide nanoparticles.**

**Antibacterial Activity of Metal Oxide Nanoparticles**

The antibacterial activity of all the four metal oxide nanoparticles was examined against gram negative *(E.coli* MCC-2412 and *Proteus vulgaris* MTCC-426) and gram positive (*Staphylococcus aureus* MCC-2408 and *Streptococcus mutans* MTCC-497) bacteria by using agar well diffusion method and ciprofloxacin as a positive control. According to results, the metal oxide nanoparticles such as $Al_2O_3$, IO, CuO and ZnO nanoparticles showed antibacterial activity against both gram negative and gram positive bacteria by using the diameter of inhibition zone which clearly indicated that these nanoparticles are effective antibacterial agents.

Results have demonstrated that aluminium oxide exhibited the best activity at a maximum concentration with *E.coli* (39±0.35mm) followed by *Streptococcus mutans* (30±0.30mm), *Staphylococcus aureus* (29±0.40mm) and *Proteus vulgaris* (26±0.45mm) [22]. Iron oxide exhibited the best activity with *E.coli* (36±0.40mm) and then followed by S*taphylococcus aureus* (30±0.10mm), *Streptococcus mutans* (27±0.45mm) and *Proteus vulgaris* (20±0.35mm) [23]. Copper oxide has shown the best activity with *Proteus vulgaris* (37±mm). *E.coli* (30±0.30), *Streptococcus mutans* (30±0.10mm) and *Staphylococcus aureus* (23±0.45mm) [24]. Zinc oxide exhibited the best activity against *E.coli* (32±0.20mm) then followed by *Proteus vulgaris* (30±0.45mm), *Staphylococcus aureus* (24±0.35mm) and *Streptococcus mutans* (23±0.30mm) [25] (Tables 1 and 2).

**Table 1: Antibacterial activity of nanoparticles against gram negative bacteria by agar well diffusion method.**

| Sample | *E.coli* | | | | | *Proteus vulgaris* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | concentration mg/ml, Zone of inhibiton (mm) | | | | | concentration mg/ml, Zone of inhibiton (mm) | | | | |
| | **10** | **20** | **30** | **40** | **50** | **10** | **20** | **30** | **40** | **50** |
| $Al_2O_3$ | 9±0.20 | 18±0.25 | 27±0.25 | 31±0.10 | 39±0.35 | 5±0.30 | 10±0.40 | 15±0.45 | 20±0.20 | 26±0.45 |
| IO | 9±0.35 | 17±0.10 | 24±0.30 | 30±0.30 | 36±0.40 | 6±0.40 | 9±0.25 | 12±0.40 | 17±0.25 | 20±0.35 |
| CuO | 6±0.10 | 11±0.20 | 17±0.25 | 24±0.15 | 30±0.30 | 9±0.21 | 17±0.10 | 23±0.30 | 30±0.15 | 37±0.20 |
| ZnO | 7±0.25 | 14±0.30 | 21±0.25 | 28±0.30 | 32±0.20 | 6±0.25 | 12±0.35 | 18±0.45 | 24±0.25 | 30±0.45 |

Number of experiments n=2, mean±SD.

**Table 2: Antibacterial activity of nanoparticles against gram positive bacteria by agar well diffusion method.**

| Sample | *Staphylococcus aureus* | | | | | *Streptococcus mutans* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | concentration (mg/ml), Zone of inhibiton (mm) | | | | | concentration (mg/ml), Zone of inhibiton (mm) | | | | |
| | **10** | **20** | **30** | **40** | **50** | **10** | **20** | **30** | **40** | **50** |
| $Al_2O_3$ | 6±0.15 | 12±0.10 | 18±0.35 | 23±0.25 | 29±0.40 | 8±0.35 | 14±0.35 | 19±0.30 | 25±0.10 | 30±0.30 |
| IO | 6±0.20 | 12±0.45 | 18±0.35 | 22±0.40 | 30±0.10 | 3±0.30 | 10±0.35 | 15±0.05 | 22±0.45 | 27±0.45 |
| CuO | 8±0.25 | 13±0.30 | 18±0.40 | 24±0.30 | 30±0.45 | 5±0.35 | 9±0.25 | 12±0.30 | 17±0.40 | 23±0.10 |
| ZnO | 6±0.35 | 10±0.15 | 14±0.10 | 19±0.15 | 24±0.35 | 4±0.25 | 9±0.35 | 13±0.35 | 18±0.35 | 23±0.30 |

Number of experiments n=2, mean±SD.

According to the results obtained, the order of sensitivity of both gram positive and gram negative bacteria against all the four metal oxide nanomaterials is described below. The sensitivity of *E.coli*: $Al_2O_3$ (39±0.35) > IO (36±0.40) > ZnO (32±0.20) > CuO (30±0.30), *P.vulgaris*: CuO was (37±0.20) > ZnO (30±0.45) > $Al_2O_3$ (26 ±0.45) > IO (20±0.35). *S.aureus*: IO (30±0.10) > $Al_2O_3$ (29 ±0.40) > ZnO (24±0.35) > CuO (23±0.45), and *S.mutans:* CuO (30±0.10) > $Al_2O_3$ (30±0.30) > IO (27± 0.45) > ZnO (23±0.30). By the results, it can be concluded that gram negative bacteria are much sensitive to metal oxide nanoparticles when compared to gram positive bacteria [25, 26]. The order of antibacterial activity of all the above mentioned metal oxide nanoparticles against both gram positive and gram negative bacteria was $Al_2O_3$ (31±0.37) > CuO (30±0.26) > IO (28±0.32) > ZnO (27±0.32).

The reason for the variation in the sensitivity of both gram negative and gram positive bacteria might be due to structural and compositional differences of the cell wall [27]. Gram positive bacteria have a thicker peptidoglycan layer when compared to gram negative bacteria. Due to this kind of difference in structure, it is tough for nanoparticles to penetrate into membrane resulting in a reduced bactericidal action [28]. All microbial species and strains are not exhibiting the same sensitivity to metal oxide nanoparticles [29]. The concentration of the nanoparticle plays a significant role in the resolution of antibacterial activity. The surface area of the metal oxide nanoparticles that comes in contact with bacterial cells is directly proportional to the extent of antimicrobial activity recommended by the particle. At biological pH, the charge of bacterial cells was negative due to the additional carboxylic groups present in the lipoproteins on the bacterial surface, which, upon dissociation, makes the cell surface negative [30]. One of the important features of nanoparticles is the large surface area, due to the electrostatic interaction between nanoparticles with bacterial cell membrane they can tightly bind to the surface of the bacterial cells to disrupt the membrane which would lead to the leakage of intracellular components and that kills the bacterial cells.

**Minimum Inhibitory Concentration**

Determination of minimum inhibitory concentration (MIC) is important in diagnostic laboratories because it helps in confirming resistance of a microorganism to the antimicrobial agent and it monitors the activity of new antimicrobial agents. The MIC of each nanoparticle was determined by using the broth dilution method. The MIC of all the four nanoparticles was around 2-8mg/ml against both gram positive (*Staphylococcus aureus* and *Streptococcus mutans*) and gram negative (*E.coli* and *Proteus vulgaris*) bacteria.

*E. coli and Proteus vulgaris* showed MIC at 4 mg/ml and 8 mg/ml, *staphylococcus aureus* showed MIC at 4 mg/ml and *streptococcus mutans* showed MIC at 6 mg/ml for $Al_2O_3$nanopowder. In case of IO, *E. coli and Proteus vulgaris* showed MIC at 2 mg/ml, *staphylococcus aureus* showed MIC at 4 mg/ml and *streptococcus mutans* showed MIC at 8 mg/ml. *E. coli and Proteus vulgaris* showed MIC at 6 mg/ml and 4 mg/ml, *staphylococcus aureus* and *streptococcus mutans* showed MIC at 4 mg/ml for CuO nanopowder.*E. coli and Proteus vulgaris* showed MIC at 6 mg/ml, *Streptococcus mutans* showed MIC at 8 mg/ml *and Staphylococcus aureus* showed MIC at 4 mg/ml for zinc oxide nanopowder (Table 3).

**Table 3: Minimum inhibitory concentration (MIC) values (mg/ml) of metal oxide nanoparticles.**

| Sample | gram negative bacteria MIC (mg/ml) | | gram positive bacteria MIC (mg/ml) | |
|---|---|---|---|---|
| | ***E.coli*** | ***P.vulgaris*** | ***S.aureus*** | ***S.mutans*** |
| $Al_2O_3$ | 4 | 8 | 4 | 6 |
| IO | 2 | 2 | 4 | 8 |
| CuO | 6 | 4 | 4 | 4 |
| ZnO | 6 | 6 | 4 | 8 |

## CONCLUSION

According to the results, it can be concluded that the above four metal oxide nanoparticles are effective antimicrobial agents against both gram negative and gram positive organisms and their antimicrobial activity is increased with increase in concentration.

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## CONFLICT OF INTERESTS

Declared none

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