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FORMULATION, EVALUATION AND COMPARATIVE STUDIES ON ANTIBACTERIAL ACTIVITY OF ZINC OXIDE AND COPPER OXIDE NANOPARTICLES

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ARTICLE INFO

Key words
Zno nanoparticle, Cuo nanoparticle, SEM analysis, Cup-plate method, FTIR studies, antioxidant activity

ABSTRACT

In our present investigation, the zinc oxide (Zno) and copper oxide (Cuo) nanoparticles were synthesized and characterized by U.V. studies, SEM, particle sizer, FTIR studies, zeta potentiality, and antioxidant activity. The SEM images of Zno NP show circular beaded structures. The Cuo nanoparticles seemed to be berry shaped. The Zno and Cuo nanoparticles were further evaluated for antimicrobial activity. The antimicrobial studies were preferably carried out using cup plate method against a standard antibiotic. These studies revealed that Zno and Cuo nanoparticles were able to show antimicrobial activity against both Gram positive and Gram negative bacteria. These studies revealed the usage of Zno and Cuo nanoparticles formulations to control antibiotic resistant bacterial pathogens. The nanoparticles were able to show antimicrobial activity because of their nanosize and disinfectant activity.

INTRODUCTION:

Nanoparticles were found to attract the attention of scientists due to an efficient association amid immense huge substances and minute or glimmer structures. Nanotechnology is before now augmenting the medical paraphernalia, acquaintance, and analysis to physicians in nanomedicine [1, 2, 3]. Nanoparticles possess larger outer surface region per mass compared to other larger particles. Apart from these the antimicrobial activity of metal oxide nanoparticles had attracted the attention of scientists to consider as a supplement in the drug formulations of antibiotic resistant drugs [4, 5]. In our present study, we formulated evaluated and compared the antimicrobial activity of Zno and Cuo nanoparticles on four bacterial strains [6, 7.] These studies pointed out the efficient antibacterial activity of Zno and Cuo nanoparticles due to their minute size.

Materials and methods: The chemicals used in this investigation were obtained from Hychem labs and of 99.9% pure grade chemicals. In this work, Zno NP was prepared by precipitation method[8, 9]. Zinc sulfate (0.1M) and sodium bicarbonate (0.1M) were mixed together by constant stirring and reaction was maintained at 45ºc. This resulted in the formation of a white slurry precipitate. The precipitate was filtered and washed several times with distilled water. Then the precipitate was

dried in the hot air oven to remove traces of moisture. The precipitate was subjected to calcification at 500ºc in the furnace for one hour. The copper oxide NP was prepared by chemical reduction method **(**Kooti & Matouri, 2010).A) 2.3 g of copper sulfate was dissolved in 50ml distilled water.[10,11] B) 17.3 gms of sodium potassium tartarate and 6 gms of sodium hydroxide was dissolved in 50 ml of distilled water. An equal volume of solution A and solution B were taken in a beaker and stirred well. To this 2.5 gms of starch was added and the reaction mixture was stirred vigorously for 10 minutes. The reaction mixture was placed in a water bath for 10minutes at 60ºc. The resultant suspension was centrifuged at 5000 rpm for 10 minutes. The supernatant was decanted and the residue obtained was dried off.

Characterization of Zno NP and Cuo NP: **SCANNING ELECTRON MICROSCOPE (SEM)**: Zno NP and CuoNP synthesized were characterized by SEM. It revealed the shape and size of Zno NP [12, 13] and CuoNP. The SEM images showed that synthesized Zno NP and Cuo NP[14, 15] were relatively uniform in size and shape. The morphology of the Zno NP and Cuo NP were examined by a JEOL JSM-6380 LA SEM.

UV-Visible spectrometry: The metal oxide NP Zno NP [16] and Cuo NP were screened by assessing the UV-Visible spectrum of the NP powders at 24 hrs time invterval. The absorbance was recorded at the spectrum of 190-1100nm using shimadzu UV-1800 spectrophotometer.

FTIR spectroscopy: The metal oxide NP Zno NP and Cuo NP showed absorption of electromagnetic radiation in the frequency range of 400cm^{-1}-4000 cm $^{-1}$ [17,18,19]**.** The various functional groups and structural forms in the molecule showed absorbance at characteristic frequencies. The strength of absorption and incidence of absorption indicated the structural geometry and group structures of the molecule. FTIR spectra were obtained using the FTIR spectrum was recorded on a Shimadzu FTIR-8400S, Prestige-21 spectrophotometer in a KBr matrix.

PARTICLE SIZE ANALYZER and ZETA POTENTIAL: The average diameter and size distribution profile and zeta potential analysis of nanoparticles were determined by Zeta sizer. The zeta potential studies were carried for Zno NP and Cuo NP analyzes the stability of the nanoparticles using zeta sizer. (Malven Zeta sizer). Particle size and Zeta potential of niosomes in the dispersion was determined by using photon correlation spectroscopy (PCS) using Malvern zeta sizer at a fixed angle of 90 at 25C using water as a dispersant for size determination and Zeta potential measurement. The laser diffraction system along with a numerous dispersion technique was used to verify the particle size of the Zno NP and Cuo NP powders. The particle size distribution of the Zno NP and Cuo NP samples were analyzed by their dispersion in water by horn type ultrasonic. The computer controlled particle size analyzer Malvern zeta sizer was used to retrieve the particles size distribution.

BIOASSAY OF Zno NP and Cuo NP:

Antibacterial activity of the Zno and Cuo NP: Antibacterial activity of the NP was measured using agar diffusion method cup plate method/ cylinder plate method [20, 21, 22, 23]. The cup plate method is based on the diffusion of an antibiotic from a cavity through the solidified agar layer of a petridish. The growth of inoculated microbe is inhibited entirely in a circular zone around a cavity containing a solution of the antibiotics based on the concentration. Prepare a stock solution of the nanoparticle forumulation as 2000µg/1ml (2mg/1ml). Prepare dilutions of the antibiotic of known concentration of the standard. Sterilize the Muller-Hinton agar medium in an autoclave at 121˚c at 15lbs pressure for 15 minutes. Add 1ml suspension of the standard test organism to

Muller Hinton medium and mix thoroughly while maintaining a temperature at 50˚c. Pour the above mixture into petridish to form a layer of about 3mm thickness. Allow the medium to solidify. Now cut the reservoirs/cup with a sharp tool such as cork borer. Remove the cylindrical plugs with a scalpel or sharp forceps. Mark the cups as per dilutions and add in each cup the respective dilutions of the antibiotic and the nanoparticle formulation. Keep the plate carefully in the refrigerator for diffusion of the samples for 20 minutes. Wipe the condensed water carefully from the lid of the petridish, with the sterile cotton plugs. Incubate the petridish at 37˚c for 18-24 hrs. Record the size of the zone of inhibition against each cavity and the size is measured in mm with the help of scale or using antibiotic zone reader. The test organisms used for the microbiological assay were Escherichia, Klebsiella, Staphylococcus, and Bacillus.

Calculation: L=3a+2b+c-e/5**,** H=3c+2d+c-a/5

Where L=the calculated zone diameter for the lowest concentration of the standard curve response line., H=the calculated zone diameter for the highest concentration of the standard curve response line. C= average zone diameter of reference readings.

a,b,d,e = correct average value for the other standard solutions.

DPPH ANTIOXIDANT ACTIVITY: The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) [24] was used to determine the free radical-scavenging (antioxidant) activity of the methanolic Extracts. The samples are kept at incubation for 20 min and readings were recorded at 517 nm. Percent inhibition of antioxidant activity was calculated by using the following formula and readings of the test sample are compared with that of ascorbic acid (Vitamin C) (Positive control).

% inhibition of DPPH =

(Control OD – Test OD)/Control OD) X 100

Results and discussion: The SEM images of the Zno NP and Cuo NP confirmed the shape of NP to be spherical in shape to oval. The SEM image of Zno NP [Fig.1] at the morphology of spherical shaped and that of the Cuo NP is berry shaped.[Fig.2]. Infrared studies were used for the characterization of purity and nature of the metal oxide NP. The FTIR spectrum of Cuo NP was used to understand their surface features [Fig.3]. The FTIR spectrum of Cuo NP was recorded between 400 and 4000 cm^{-1}. The bands in the 400 cm^{-1}-1000 cm-1 are considered to be in the finger print region with complex vibrations. Hence this region is used rarely used for the identification of particular functional groups. The Cuo NP showed bands at 480.29 cm^{-1} was attributed to the CU-O stretching vibration along the direction. The mode at 1047.38$cm^{-1,}$ 1159.26 $cm^{-1}$was assigned C-O stretching. The broad band stretching between 2852.81 and 1629.90cm^{-1} was attributed to O-H stretching and bending modes of water. A mode at 2924.18cm^{-1} and 3715.02 cm^{-1} assigned to C-H stretching and amide stretching (N-H) stretching. The Zno NP characterized in the range of 400-4000 cm^{-1} by FTIR studies [Fig.4]. These studies interpreted the FTIR peak for Zno NP at 434 cm^{-1} which accept with the literature. The peaks at 1383.01cm^{-1} indicate the C=O bonds O-H bending vibrations respectively which reduces gradually after the sample was backed at high temperature. The peak at 1629.09cm^{-1} was used for interpretation of O-H bending vibrations. The peak in the range of 1464-1518 cm^{-1} indicates the C-H bending vibrations. The peak at 1741.78 cm-^{1} was considered for interpretation of C=O. The peak at 2852.81 cm-^{1} indicated the C-H stretching. The peak at 2924.18 cm^{-1} was considered as C-H bending vibrations. The absorption peak at 3452.0 cm^{-1} indicated O-H stretching and deformation.

Serial number	Bacterial strain	Zone of inhibition in mm		
		Control(streptomycin)		
		10µg/ml)	(20 µg/ml)	(30 µg/ml)
1	Escherichia coli MTCC2692	2.7	2.6	2.6
2	Klebsiella pneumonia MTCC4030	2.3	2.2	2.5
3	Staphylococcus aureusMTCC 902	2.4	2.4	2.4
4	Bacillus subtilis MTCC 441	1.9	2.4	2.3

Table 1

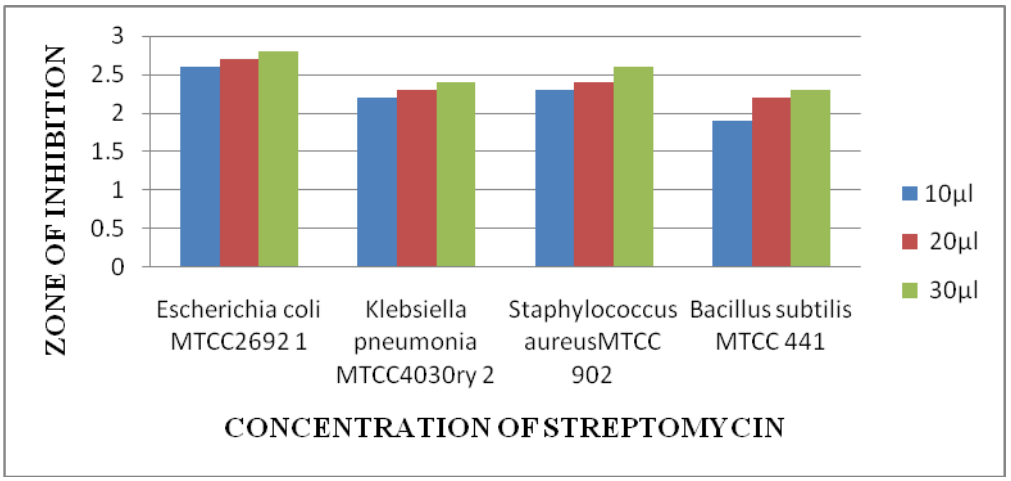


Table 1: Antibacterial activity of Streptomycin against 4 reference strains. The above mentioned graph depicts the antibacterial activity of streptomycin on four reference strains

Serial number	Bacterial strain	Zone of inhibition in mm		
		Zinc Oxide NP		
		10µg/ml	20 µg/ml	30 µg/ml
1	Escherichia coli MTCC2692	1.8	2.0	2.0
2	Klebsiella pneumonia MTCC4030	2.0	2.4	2.5
3	Staphylococcus aureusMTCC 902	1.7	2.0	2.1
4	Bacillus subtilis MTCC 441	2.2	2.4	2.6

Table-2

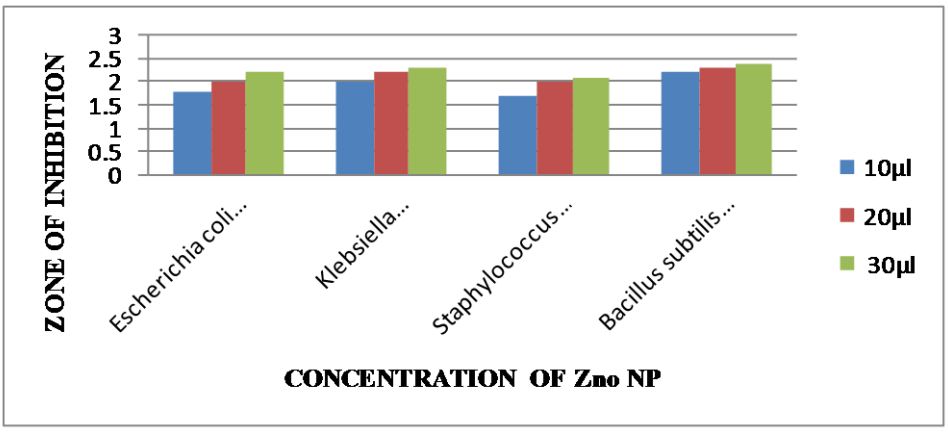


Table 2: Antibacterial activity of Zno NP against 4 reference strains. The above graph depicts the antibacterial activity of Zno NO on four reference strains .

Serial number	Bacterial strain	Zone of inhibition in mm		
		Copper Oxide NP		
		10µg/ml	20 µg/ml	30 µg/ml
1	Escherichia coli MTCC2692	3.0	3.2	3.3
2	Klebsiella pneumonia MTCC4030	2.6	3.0	3.2
3	Staphylococcus aureusMTCC 902	2.7	3.4	3.5
4	Bacillus subtilis MTCC 441	2.2	2.3	2.3

Table 3:

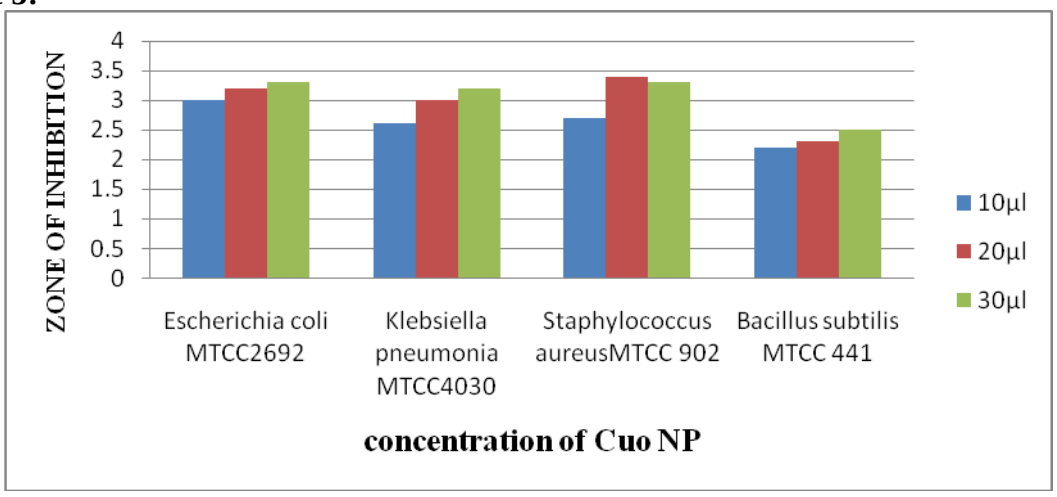


Table 3: Antibacterial activity of Cuo NP against 4 reference strains. The above mentioned graph depicts the antibacterial activity of Cuo NP on four reference strains

		Zone of inhibition in mm								
		Control (streptomycin)			**Zinc oxide NP**			**Copper oxide NP**		
S.no	**Bacterial Strain**	**10µg**	**20 µg**	**30 µg**	**10µg**	**20µg**	**30 µg**	**10µg**	**20 µg**	**30 µg**
1	Escherichia coli MTCC2692	2.7	2.6	2.6	1.8	2.0	2.0	3.0	3.2	3.3
2	Klebsiella pneumonia MTCC4030	2.3	2.2	2.5	2.0	2.4	2.5	2.6	3.0	3.2
3	Staphylococcus aureus MTCC 902	2.4	2.4	2.4	1.7	2.0	2.1	2.7	3.4	3.5
4	Bacillus subtilis MTCC 441	1.9	2.4	2.3	2.2	2.4	2.6	2.2	2.3	2.3

TABLE 4: Indicating comparison of antibacterial activity of streptomycin, zinc oxide NP, & copper oxide NP

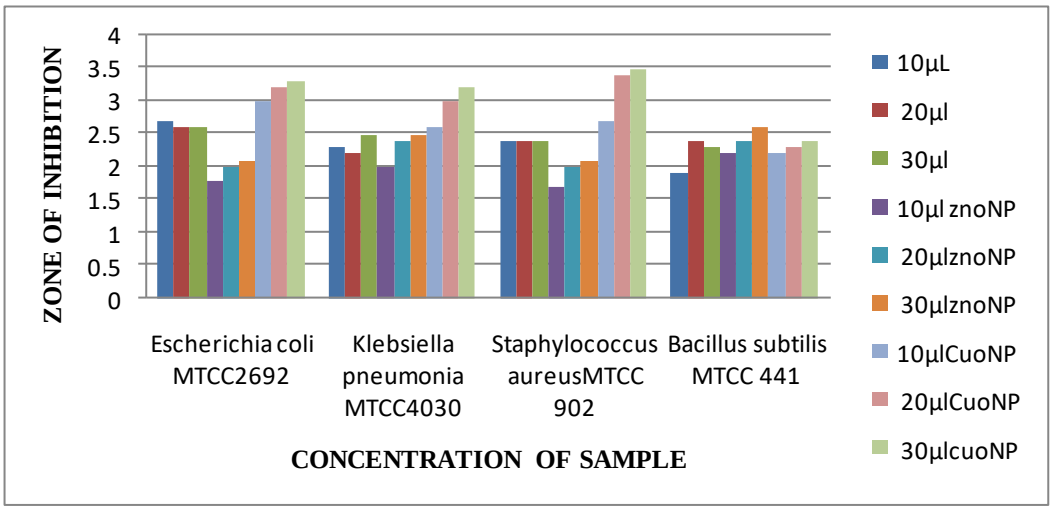


Table-4: Graph indicating comparison of antibacterial activity of streptomyicn, zinc oxide NP, & copper oxide NP

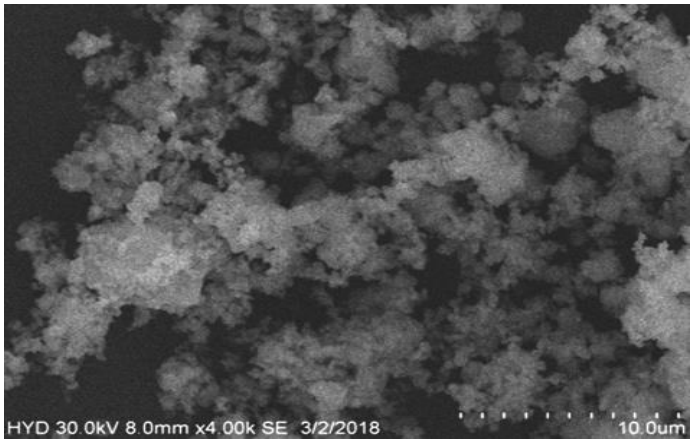


Figure-1SEM images of Zinc oxide NP

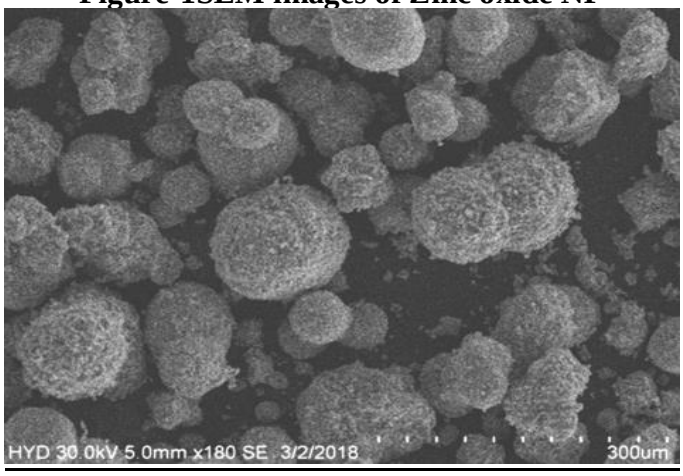


Figure-2 SEM images of Copper oxide NP

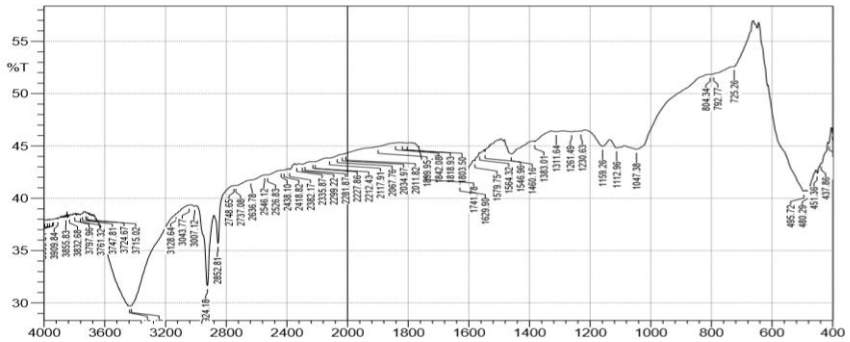


Figure-3 FTIR spectra of Zno NP

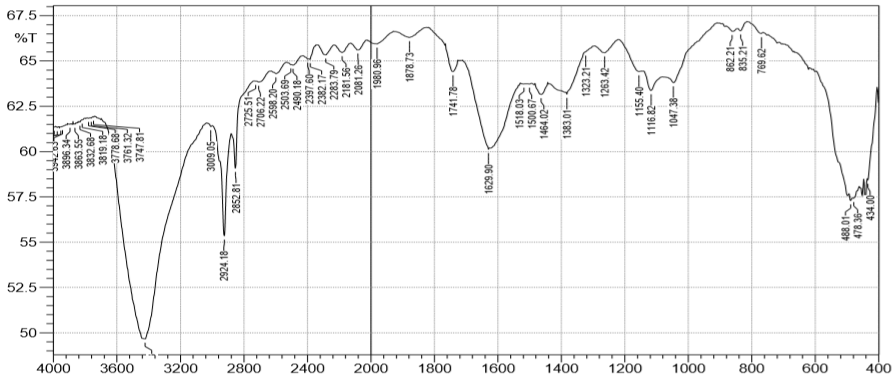


Figure-4 FTIR spectra of Cuo NP

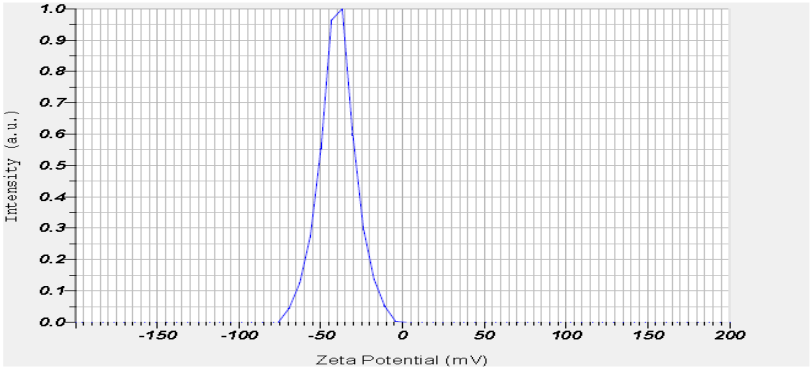


Figure-5 Graph of Zeta potential OF Zno NP

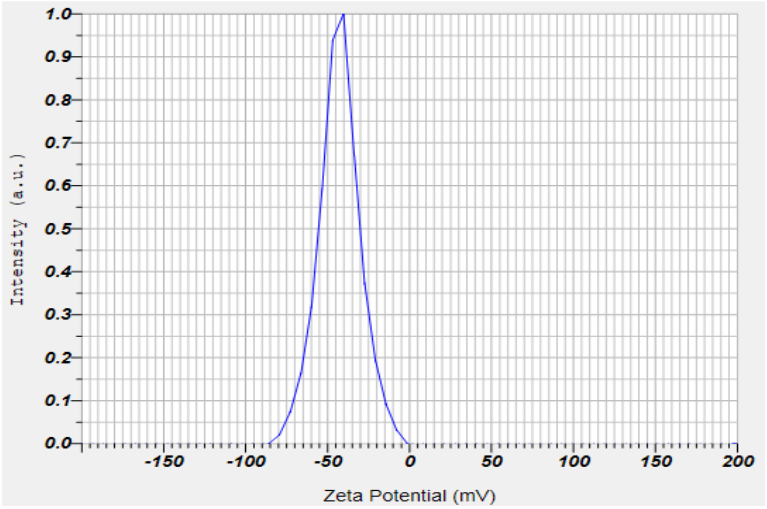


Figure-6 Graph of Zeta potential of Cuo NP

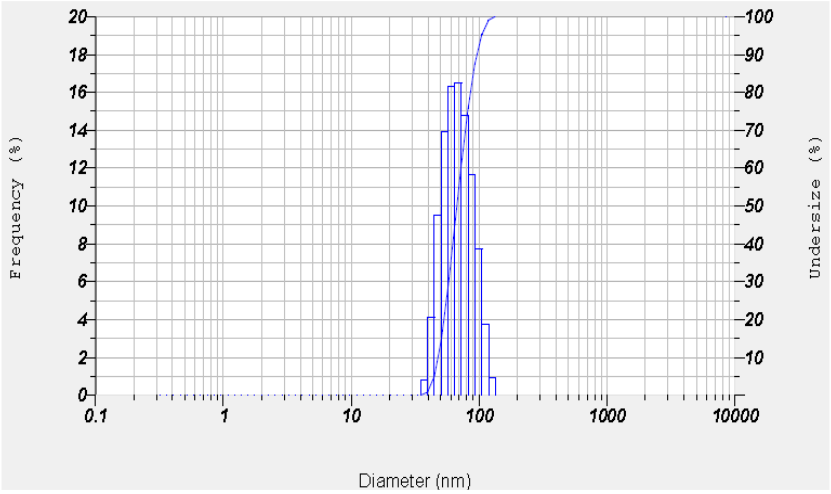


Figure-7 Graph of Zno NP particle size

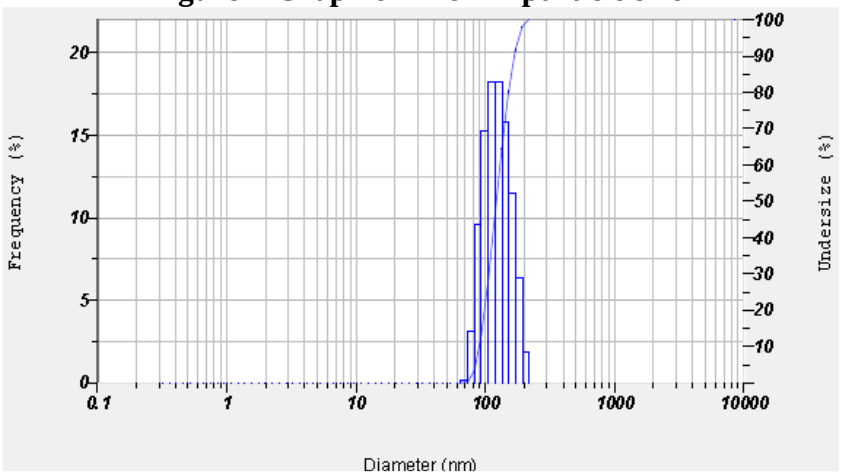


Figure-8 Graph of Cuo NP particle size

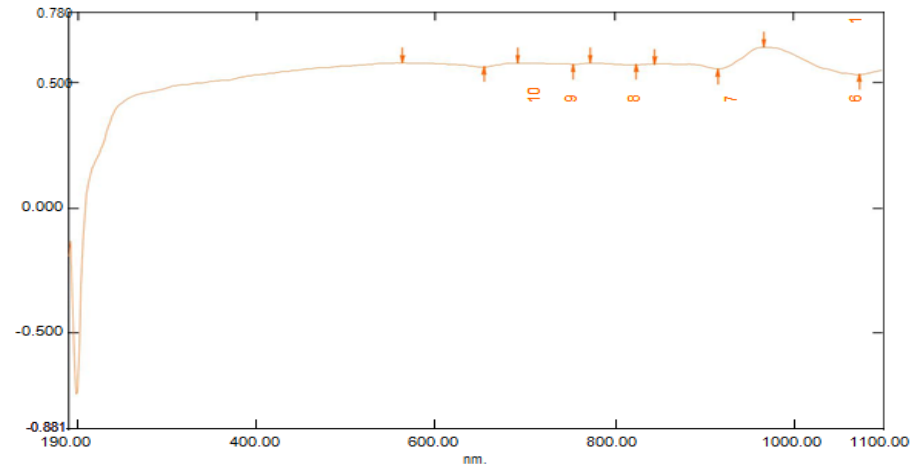


Figure-9 U.V-Visible spectra of Zno NP

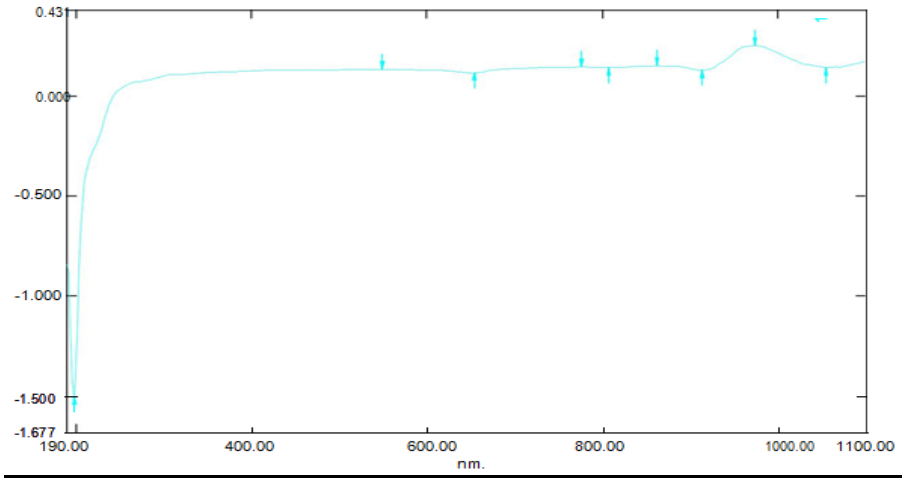
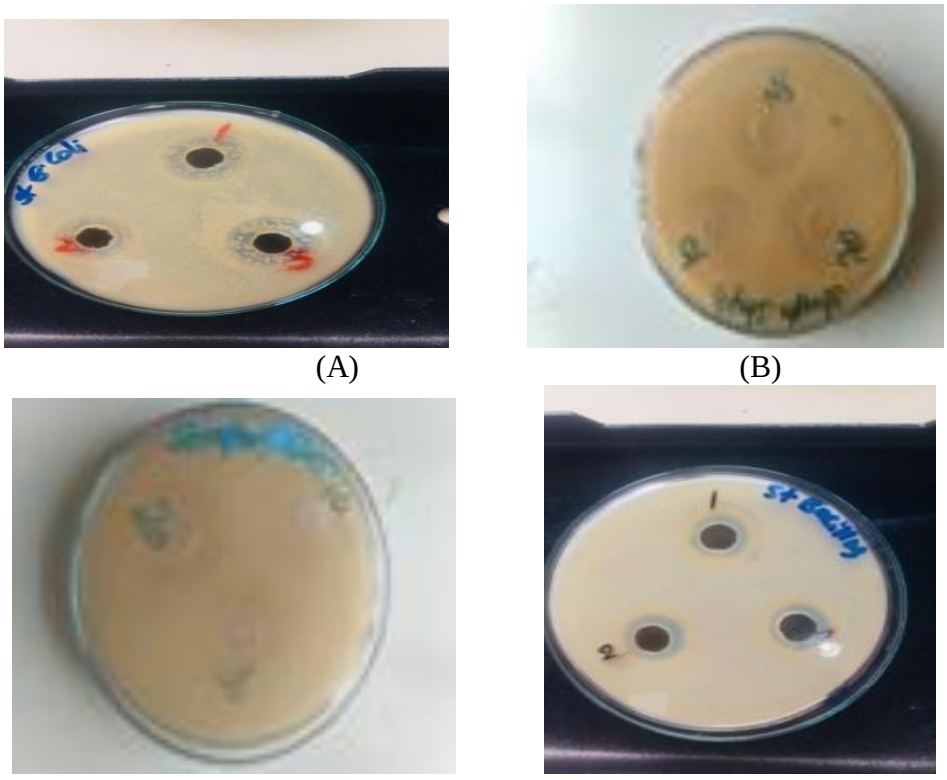


Figure-10 UV-Visible spectra of Cuo NP



(A) (B)

(C) (D)

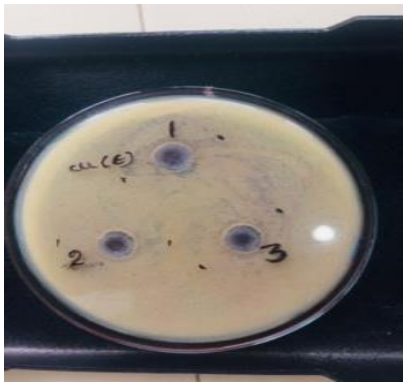
Figure-11 Antibacterial activity of Streptomycin on four reference strains

(A) Zone of inhibition of streptomycin on Escherichia coli MTCC2692.

(B) Zone of inhibition of streptomycin on Staphylococcus aureus MTCC 902.

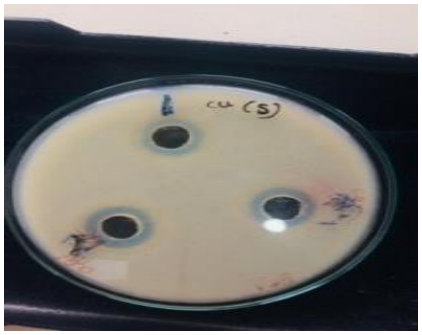
(C) Zone of inhibition of streptomycin on Klebsiella pneumonia MTCC 4030.

(D) Zone of inhibition of streptomycin on Bacillus subtilis MTCC 441

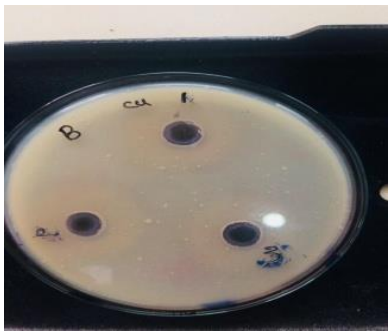


(A)

(B)



(C)



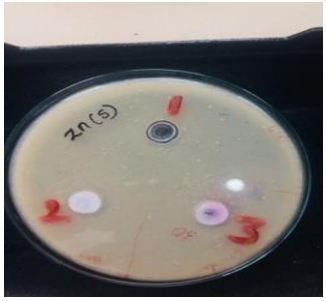
(D)

Figure 12 Images of Antibacterial activity of Cuo NP against four reference strains

A) Zone of inhibition of Cuo NP on Escherichia coli MTCC2692.
B) Zone of inhibition of Cuo NP on Staphylococcus aureus MTCC 902.
C) Zone of inhibition of Cuo NP on Klebsiella pneumonia MTCC 4030.
D) Zone of inhibition of Cuo NP on Bacillus subtilis MTCC 441.

(A)

(B)

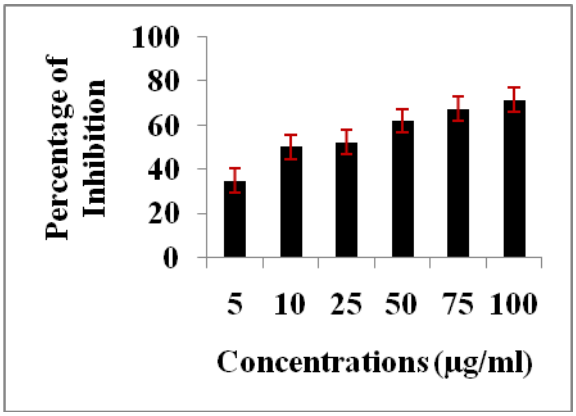


(C)

(D)

Figure 13 Images of Antibacterial activity of Zinc oxide nanoparticle on four reference strains.

A) Zone of inhibition of Zinc oxide NP on Escherichia coli MTCC2692.
B) Zone of inhibition of Zinc oxide NP Staphylococcus aureus MTCC 902.
C) Zone of inhibition of Zinc oxide NP Klebsiella pneumonia MTCC 4030.
D) Zone of inhibition of Zinc oxide NP Bacillus subtilis MTCC 441.



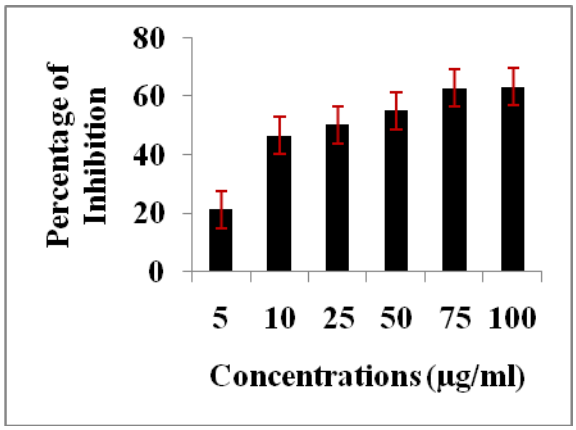


Figure 14: DPPH Antioxidant of Sample Cuo NP
Figure 15: DPPH Antioxidant of Sample Zno NP

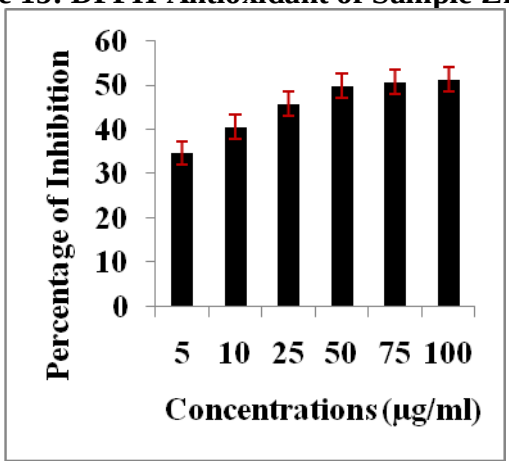


Figure 16: DPPH Antioxidant of Ascorbic Acid

ZETA Potential: Zeta potential analytical studies revealed Zno NP showed zeta potential mean at -39.7mV and that of Cuo NP studies revealed zeta potential mean at -42.8 mV [Fig.5,6].

Particle size Analysis: The particle size analysis reinstated Zno NP **z** average-value 72.3nm and pi of 0.364. Whereas the particle size analysis of Cuo NP exhibited **z** average-value of 125.5 nm and pi of 0.164. [Fig.7, 8].

UV absorabance: The U.V. absorbance studies of Zno NP and Cuo NP interpretation showed maximum absorption at a wavelength of 968 nm with a maximum absorbance value of 0.642 for Zno NP and Cuo NP showed maximum absorption at a wavelength of 974 nm with a maximum absorbance value of 0.255 for Cuo NP [Fig.9, 10].

Antimicrobial Activity: The antimicrobial studies carried by cup plate method/cylinder plate method using Zno and Cuo NP revealed the antimicrobial activity of NP as compared with standard streptomycin [Fig.11]. The Cuo NP showed a greater zone of inhibition i.e., antimicrobial activity than Zno NP.
[Fig.12, 13] and the graph.[Table-1,2,3]

DPPH Assay: The DPPH Assay studies showed the antioxidant activity of Zno NP and Cuo NP compared with ascorbic acid. These studies indicated that the IC50 Value (µg/ml) of Ascorbic acid 18.67±2.78 and IC 50 Value (µg/ml) of Zno NP was found to be 44.15±3.48 and that of IC 50 Value(µg/ml) of Cuo NP was 62.73±3.63. These studies disclose that the antioxidant activity of Zno NP is greater than Cuo NP in comparison with standard ascorbic acid as interpreted by the graph. [Fig.14, 15, 16]

Conclusion: The present study proved that the metal nanoparticles possess antibacterial near or greater to the drug formulations. The Cuo NP possesses remarkable antibacterial activity where as the Zno oxide NP possesses good antioxidant activity. These studies prove that nanoparticle formulations can be used to treat antibacterial resistant pathogens.

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