Formulation, Evaluation, and Comparative Studies on Antibacterial Activity of Zno and CuO Nanoparticles

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ABSTRACT

In the 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 showed circular beaded structures. The Cuo nanoparticles seemed to be berry shaped. These NPs were further evaluated for antimicrobial activity. The antimicrobial studies were preferably conceded by using cup plate method against a standard antibiotic. These studies revealed that Zno and Cuo NP were able to show antimicrobial activity against both Gram-positive and Gram-negative bacteria, and hence can be used to control antibiotic-resistant bacterial pathogens. The nanoparticles were able to show antimicrobial activity because of their nanosize and disinfectant activity.

Key Words: Zno Nanoparticle, Cuo Nanoparticle, SEM Analysis, Cup-Plate Method, FTIR Studies, Antioxidant Activity.

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INTRODUCTION

Nanoparticles were found to attract the attention of scientists due to an efficient association amid immense huge substances and minute or glimmer structures. Nanoparticles possess larger outer surface region per mass compared to the other larger particles. Apart from these, the cidal activity of the metal oxide NP had attracted the attention of scientists to consider it as a supplement in the drug formulations of antibiotic-resistant drugs [1, 2]. In previous studies, the Zno and Cuo NP cidal activity on four bacterial strains has been formulated, evaluated, and compared [3, 4]. These studies pointed out the efficient bactericidal activity of Zno and Cuo NP due to their minute size.

MATERIALS AND METHODS:

The chemicals used in this investigation were obtained from Hychem labs and 99.9% pure grade chemicals. The Zno NP was prepared by the precipitation method [5, 6]. Zinc sulfate (0.1M) and sodium bicarbonate (0.1M) were mixed together by constant stirring, and the reaction was maintained at 45ºc. This resulted in the formation of a white slurry precipitate. The precipitate was purified by filtration, and it was washed a number of times with distilled water. Then, the filtrate was dried in the hot air oven to remove the traces of moisture. The precipitate was subjected to the calcification at 500ºc in the furnace for one hour.

The copper oxide NP was prepared by chemical reduction method. [7]

A. 2.3 g of copper sulfate was dissolved in 50ml distilled water. [8, 9]
B. 17.3 gms of sodium potassium tartrate and 6 gms of sodium hydroxide was dissolved in 50 ml of distilled water. Equal volumes of solution A and solution B were taken in a beaker, and stirred well. Then, 2.5

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gms of starch was added, and the reaction mixture was mixed properly for 10 minutes. The reaction mixture was placed in a water bath for 10minutes at 60ºc. The resultant reaction mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant was poured, and the obtained residue was dried off.

Characterization of Zno NP and Cuo NP:

Scanning Electron Microscope (SEM):

The synthesized Zno NP and CuoNP were characterized by SEM. It revealed the shape and size of Zno NP [10, 11] and CuoNP. The SEM images showed that the synthesized Zno NP and Cuo NP [12, 13] 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 [14] and CuO NP were screened by assessing the UV-Visible spectrum of the NP powders at 24 hrs time interval. 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 the absorption of electromagnetic radiation in the frequency range of 400cm^{-1}-4000 cm^{-1} [14-16]**.** The different functional groups and structural forms in the molecule showed the absorbance at the characteristic frequencies. The strength and incidence of absorption indicated the structural geometry and group structures of the molecule. FTIR spectra were obtained using the FTIR spectrum which 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 out for Zno NP and Cuo NP to analyze the stability of the nanoparticles using a zeta sizer (ZetaSizer Nano). The particle size and Zeta potential of niosomes in the dispersion were 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 of 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:

The antibacterial activity of the NP was measured using agar diffusion method including cup plate method/ cylinder plate method [17-20]. The cup plate method was based on the diffusion of an antibiotic from a cavity through the solidified agar layer of a Petri dish. The growth of inoculated microbe was inhibited entirely in a circular zone around the hollow space containing a solution of the antibiotics based on the concentration. A stock solution of the nanoparticle formulation was prepared as 2000µg/1ml (2mg/1ml). The dilutions of the antibiotic of known concentration of the standard were prepared. The Muller-Hinton agar medium was sterilized in an autoclave at 121°c at 15lbs pressure for 15 minutes. 1ml suspension of the standard test organism was added to Muller Hinton medium and mixed thoroughly while maintaining the temperature at 50°c. The obtained mixture was poured into petri-dish to form a layer of about 3mm thickness. Then, the medium was allowed to solidify, and the reservoirs/cup was cut with a sharp tool such as cork borer. After that, the cylindrical plugs were removed with a scalpel or sharp forceps. The cups were marked as per dilutions, and the respective dilutions of the antibiotic and the nanoparticle formulation were added in each cup. The plate was kept carefully in the refrigerator for the diffusion of the samples for 20 minutes. The condensed water was wiped carefully from the lid of the petri-dish, with the sterile cotton plugs. The petri-dish was incubated at 37°c for 18-24 hrs. The size of the zone of inhibition was recorded against each cavity, and the size was 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 the reference readings.

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

DPPH ANTIOXIDANT ACTIVITY:

The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) [21, 22] was used to verify the free radical-scavenging (antioxidant) activity of the methanolic Extracts. The samples were kept at the incubation for 20 min, and the readings were recorded at 517 nm. The percent inhibition of the antioxidant activity was calculated by using the

following formula, and the readings of the test sample were 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 the shape of oval. The SEM image of Zno NP [Fig. 1] at the morphology of spherical shaped and that of the Cuo NP was 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} were considered to be in the fingerprint region with the complex vibrations. Hence, this region was rarely used for the identification of particular functional groups. The Cuo NP showed that the bands at 480.29 cm^{-1} was attributed to the CU-O stretching vibration along the direction. The modes of 1047.38cm^{-1}, 1159.26 cm^{-1}were assigned to C-O stretching. The broadband stretching between 2852.81 and 1629.90cm^{-1} was attributed to O-H stretching and bending modes of water. The modes at 2924.18cm^{-1} and 3715.02 cm^{-1} were assigned to C-H stretching and amide stretching of (N-H) stretching.

The Zno NP was 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 have been accepted in the literature. The peaks at 1383.01cm^{-1} indicated the C=O bonds O-H bending vibrations respectively which gradually reduced after the sample was backed at the high temperature. The peak at 1629.09cm^{-1} was used for the interpretation of O-H bending vibrations. The peak in the range of 1464-1518 cm^{-1} indicated the C-H bending vibrations. The peak at 1741.78 cm^{-1} was considered for the 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.

Zeta Potential

Zeta potential analytical studies revealed that 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 absorbance

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

Antimicrobial Activity:

The antimicrobial studies carried out by the cup plate method/cylinder plate method using Zno and Cuo NP revealed the antimicrobial activity of NP comparing to the 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, 4; Graphs 1, 2, 3, 4]

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 were 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 disclosed that the antioxidant activity of Zno NP was greater than Cuo NP in comparison with the standard ascorbic acid as interpreted by the graph. [Fig. 14, 15, 16].

CONCLUSION:

The present study proved that the metal nanoparticles possessed antibacterial near or greater than the drug formulations. The Cuo NP possessed a remarkable antibacterial activity whereas the Zno oxide NP possessed a good antioxidant activity. These results proved that nanoparticle formulations can be used to treat antibacterial resistant pathogens.

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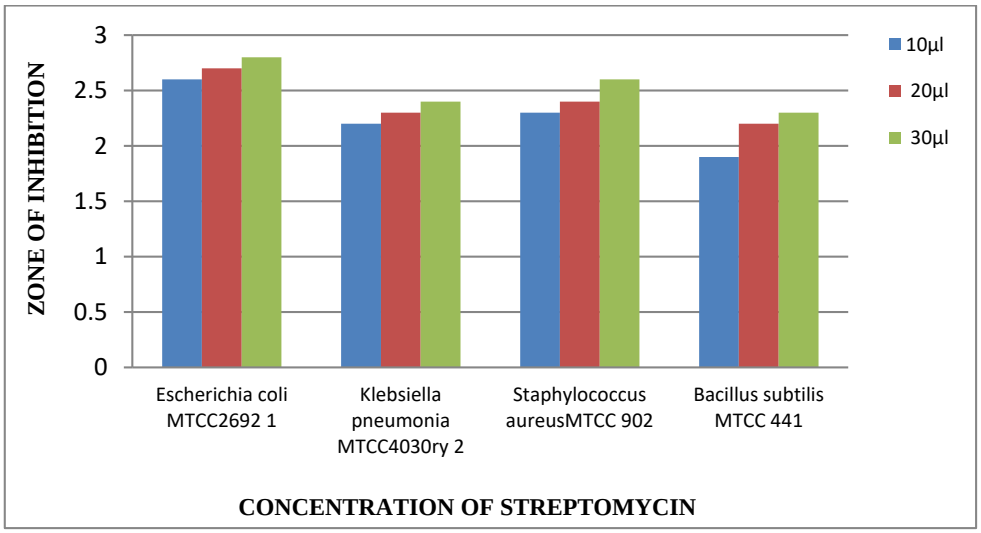
Table 1: Antibacterial activity of Streptomycin against 4 reference strains

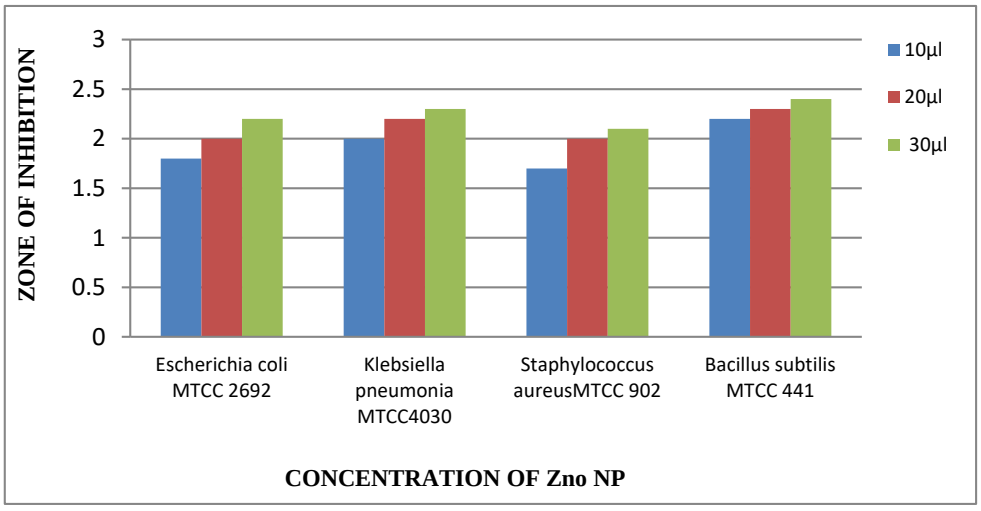
Serial number	Bacterial strain	Zone of inhibition in mm		
		Control(streptomycin)		
		(10 µg/ml)	(20 µg/ml)	(30 µg/ml)
1	Escherichia coli MTCC2692	2.7	2.6	2.6
2	Klebsiella pneumonia MTCC4030	2.3	2.2	2.5
3	Staphylococcus aureusMTCC 902	2.4	2.4	2.4
4	Bacillus subtilis MTCC 441	1.9	2.4	2.3



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

Table 2: Antibacterial activity of Zno NP against 4 reference strains

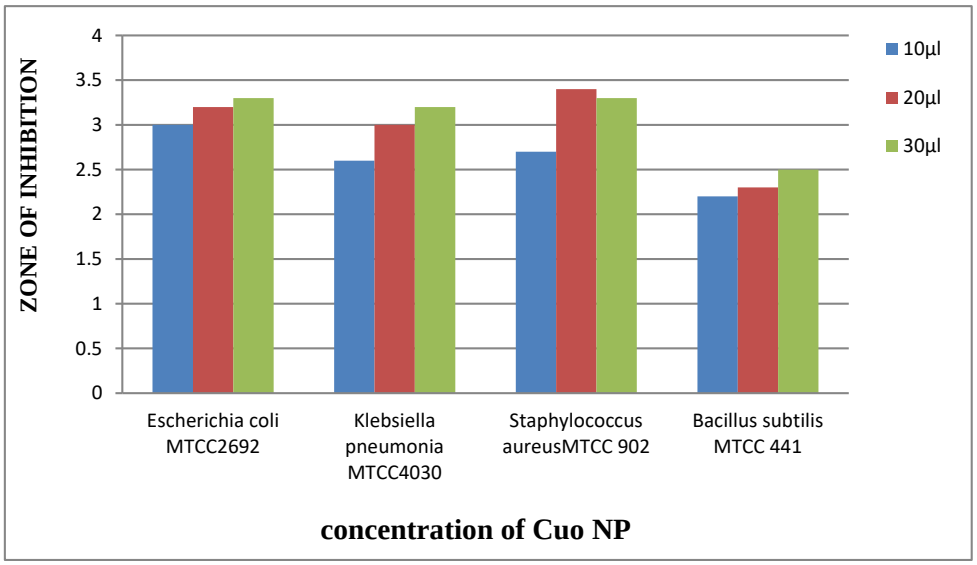
Serial number	Bacterial strain	Zone of inhibition in mm		
		Zinc Oxide NP		
		10 µg/ml	20 µg/ml	30 µg/ml
1	Escherichia coli MTCC2692	1.8	2.0	2.0
2	Klebsiella pneumonia MTCC4030	2.0	2.4	2.5
3	Staphylococcus aureusMTCC 902	1.7	2.0	2.1
4	Bacillus subtilis MTCC 441	2.2	2.4	2.6



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

Table 3: Antibacterial activity of Cuo NP against 4 reference strains

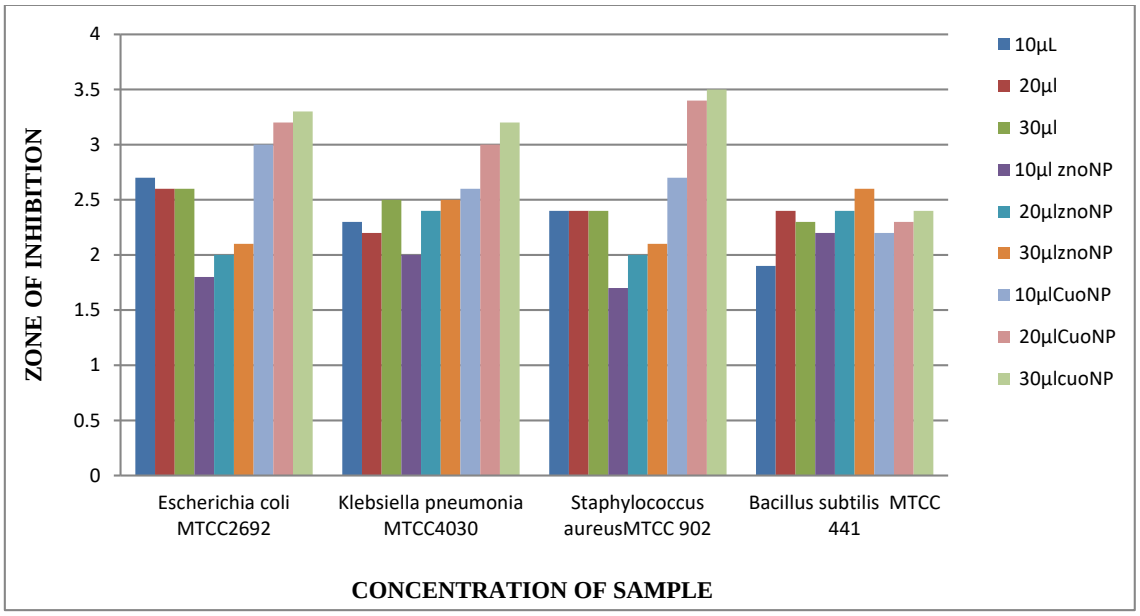
Serial number	Bacterial strain	Zone of inhibition in mm		
		Copper Oxide NP		
		10 µg/ml	20 µg/ml	30 µg/ml
1	Escherichia coli MTCC2692	3.0	3.2	3.3
2	Klebsiella pneumonia MTCC4030	2.6	3.0	3.2
3	Staphylococcus aureusMTCC 902	2.7	3.4	3.5
4	Bacillus subtilis MTCC 441	2.2	2.3	2.3



Graph 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.

Table 4: Table indicating comparison of the antibacterial activity of streptomycin, zinc oxide NP, & copper oxide NP

		Zone of inhibition in mm								
		Control (streptomycin)			**Zinc oxide NP**			**Copper oxide NP**		
S. No	**Bacterial Strain**	**(10 µg/ml)**	**(20 µg/ ml)**	**(30 µg/ ml)**	**(10 µg/ ml)**	**(20 µg/ ml)**	**(30 µg/ ml)**	**(10 µg/ ml)**	**(20 µg/ ml)**	**(30 µg/ ml)**
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



Graph 4. The comparison between the antibacterial activity of streptomycin, zinc oxide NP, & copper oxide NP

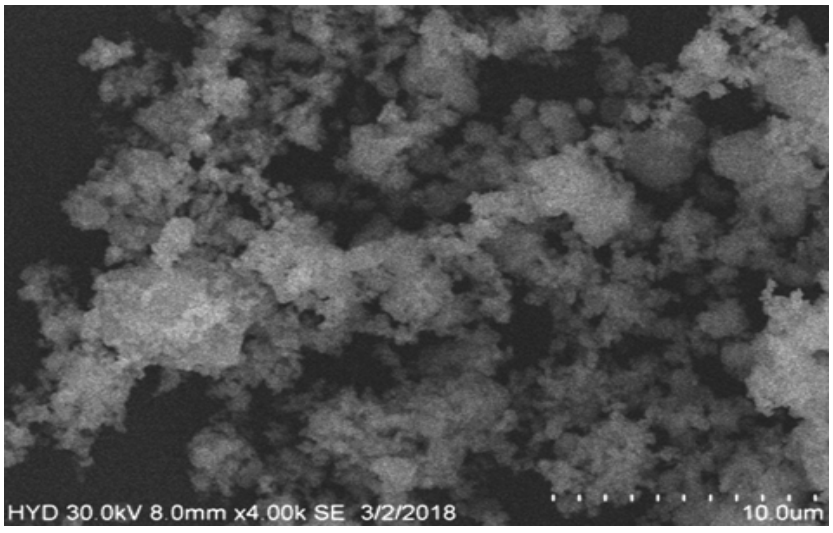


Figure 1: SEM images of Zinc oxide NP

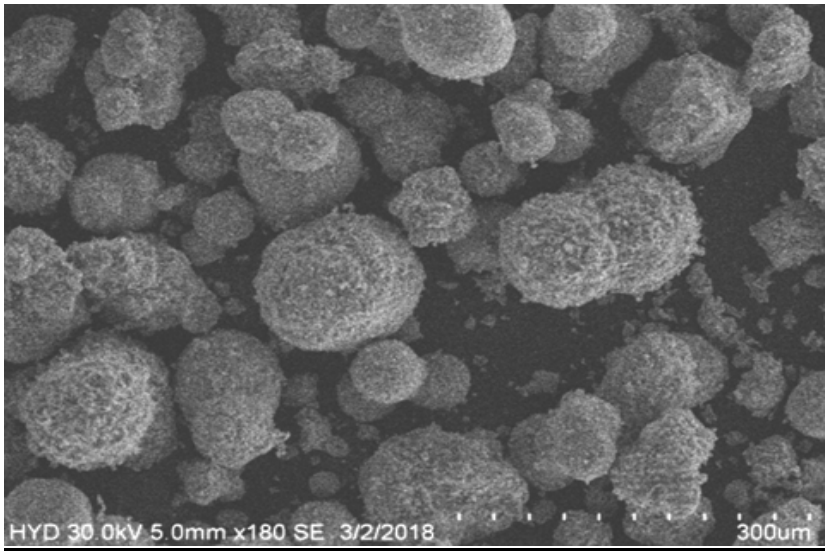


Figure 2: SEM images of Copper oxide NP

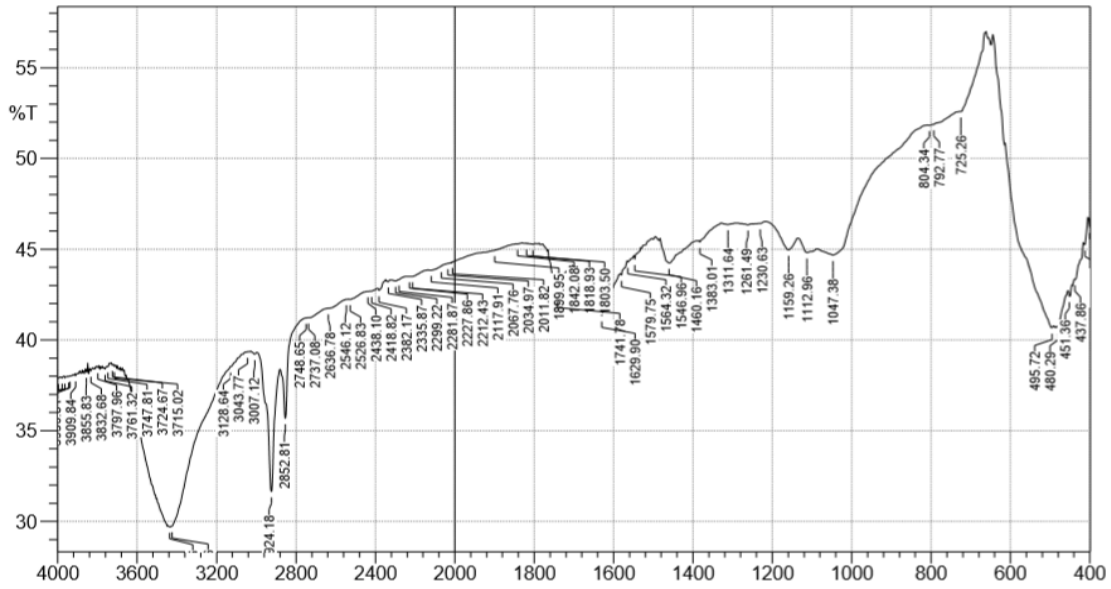


Figure 3: FTIR spectra of Zno NP

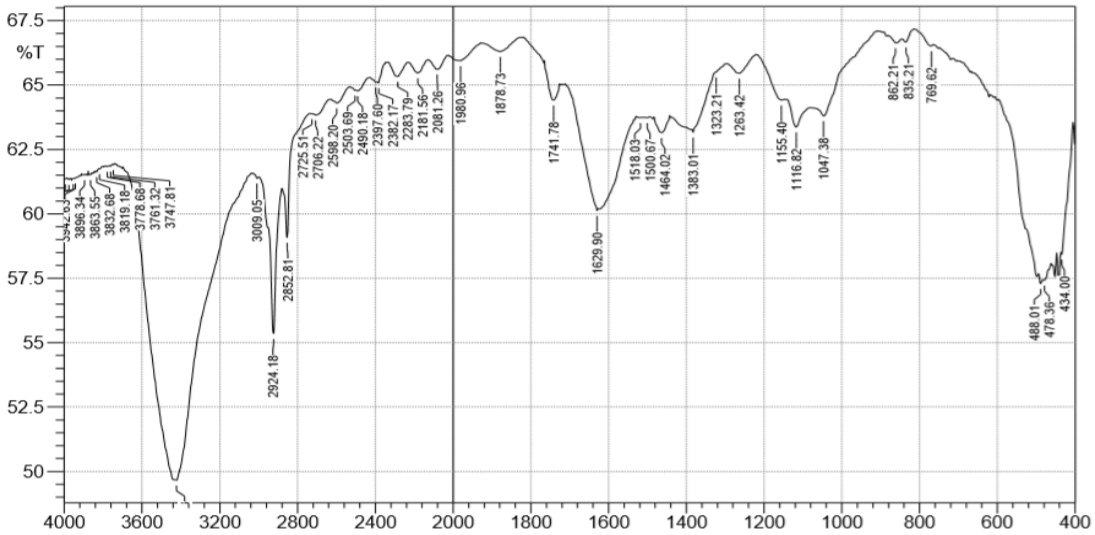


Figure 4: FTIR spectra of Cuo NP

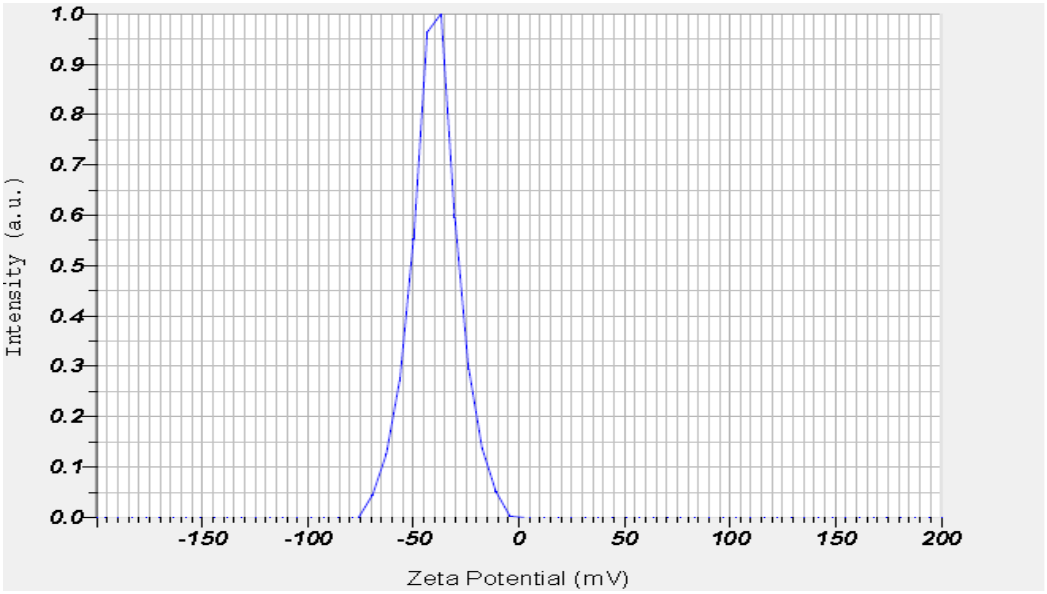


Figure 5: Graph of Zeta potential OF Zno NP

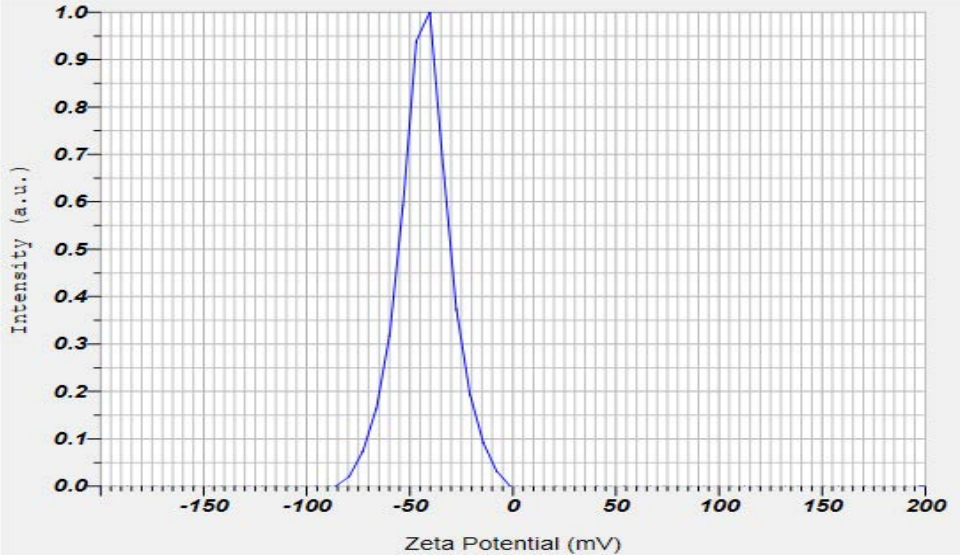


Figure 6: Graph of Zeta potential of Cuo NP

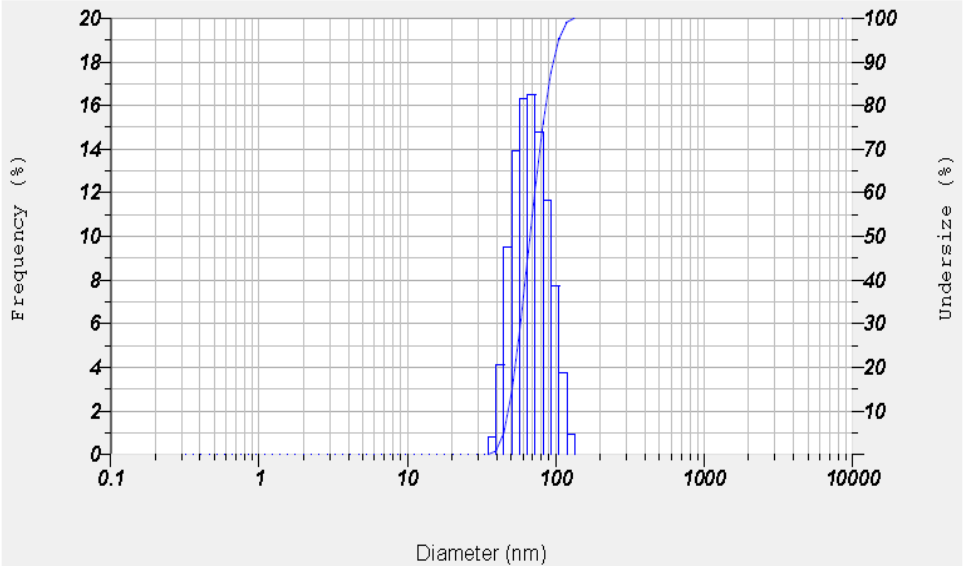


Figure 7: Graph of Zno NP particle size

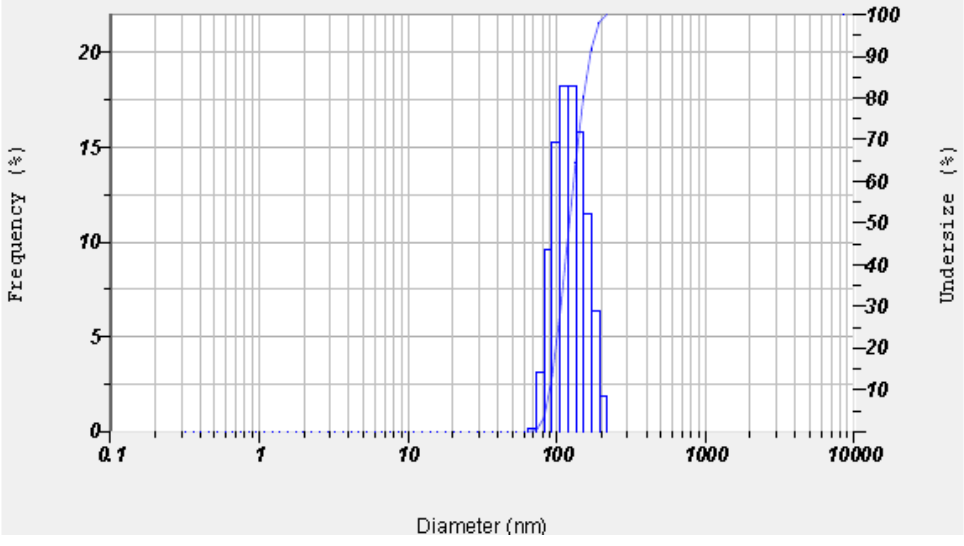


Figure 8: Graph of Cuo NP particle size

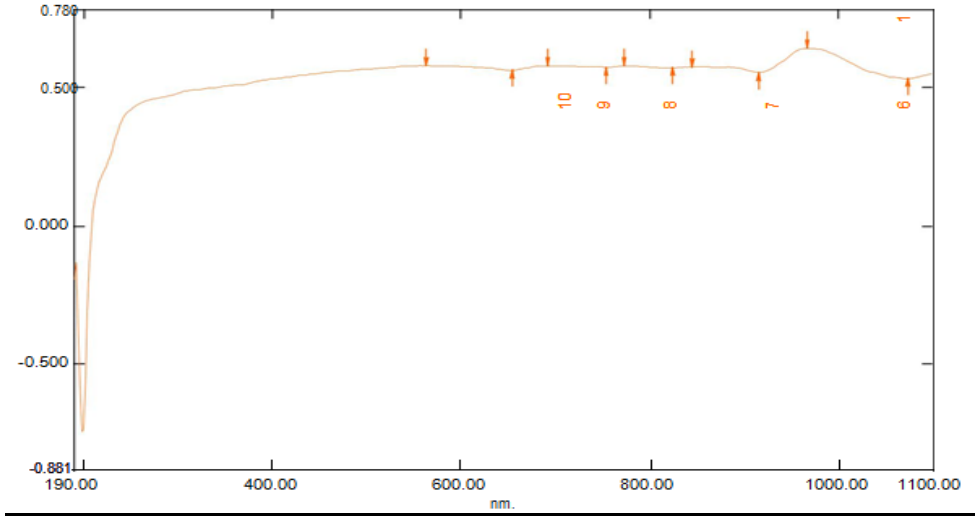


Figure 9: U.V-Visible spectra of Zno NP

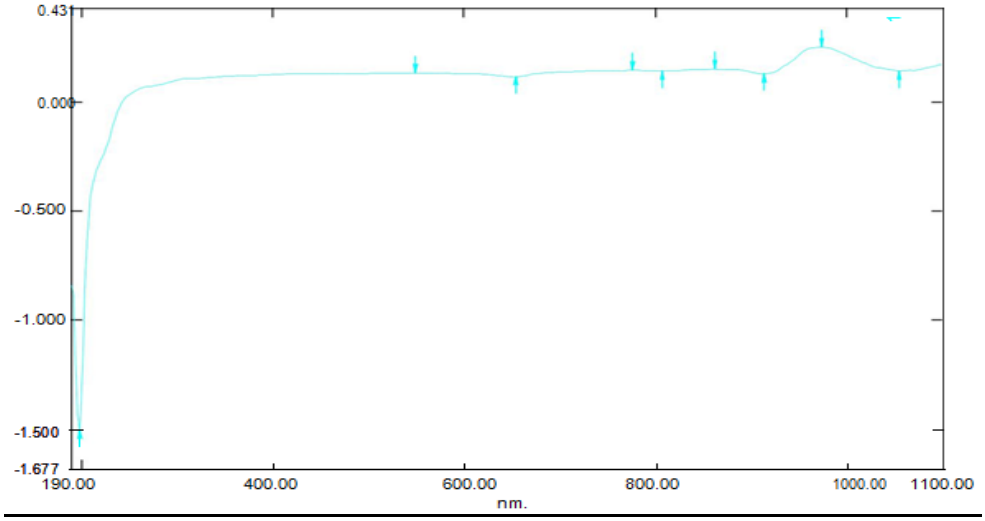
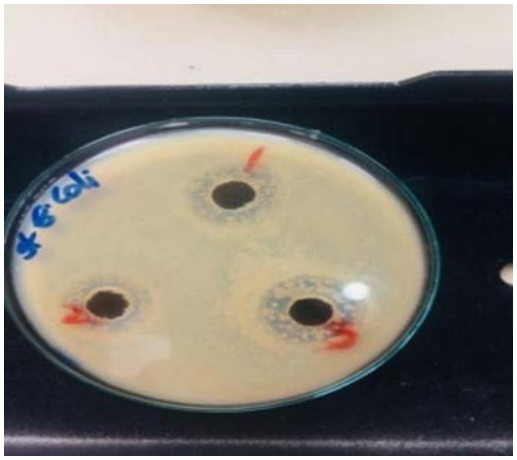


Figure 10: UV-Visible spectra of Cuo NP



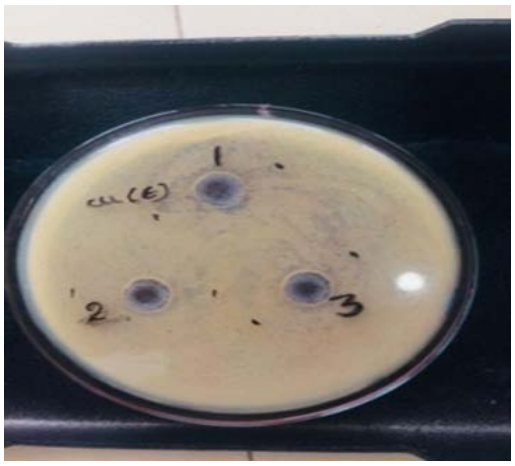
(A) Zone of inhibition of streptomycin on Escherichia coli MTCC2692.

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

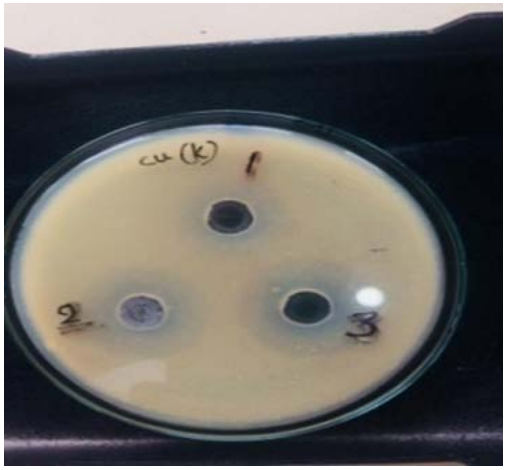
(C) Zone of inhibition of streptomycin on Klebsiella pneumonia MTCC 4030.

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

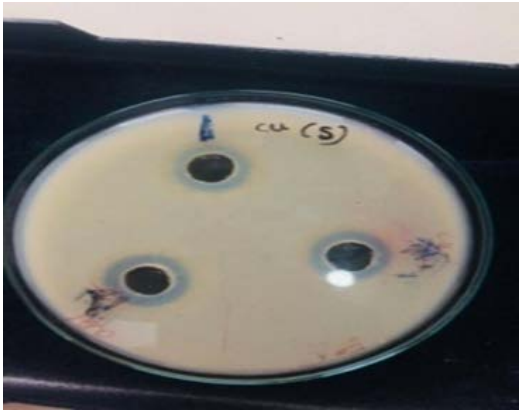
Figure 11: Antibacterial activity of Streptomycin on four reference strains



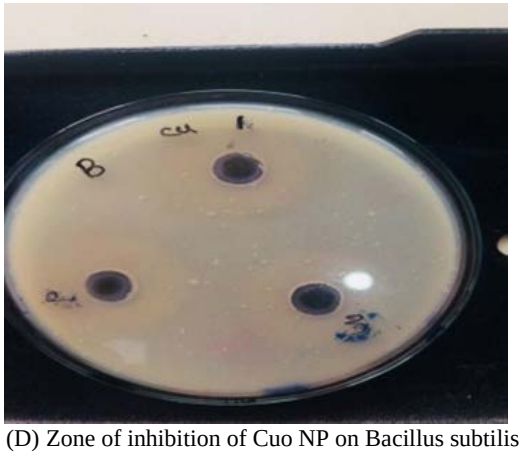
(A) Zone of inhibition of Cuo NP on Escherichia coli MTCC2692.



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

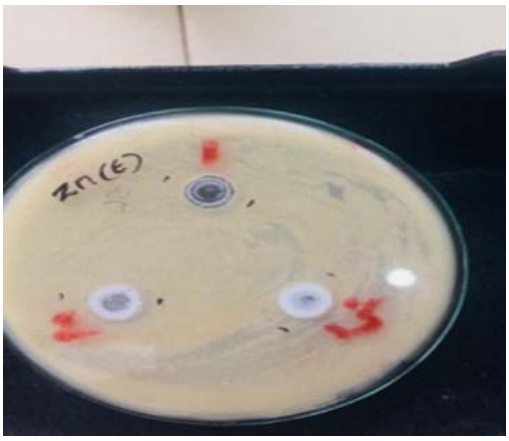


(C) Zone of inhibition of Cuo NP on Klebsiella pneumonia MTCC 4030.



(D) Zone of inhibition of Cuo NP on Bacillus subtilis MTCC 441

Figure 12: Images of Antibacterial activity of Cuo NP against 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 13: Images of Antibacterial activity of Zinc oxide nanoparticle on four reference strains.

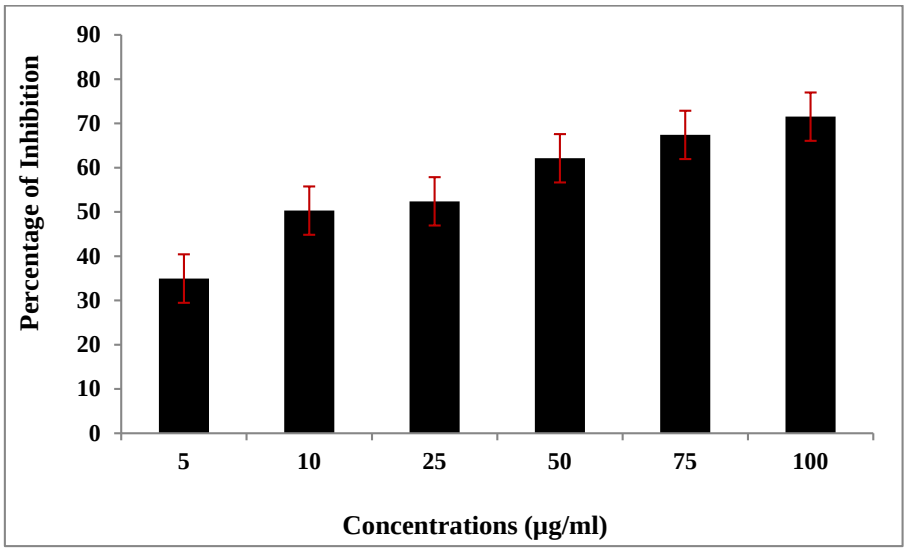


Figure 14: DPPH Antioxidant of Sample Cuo NP

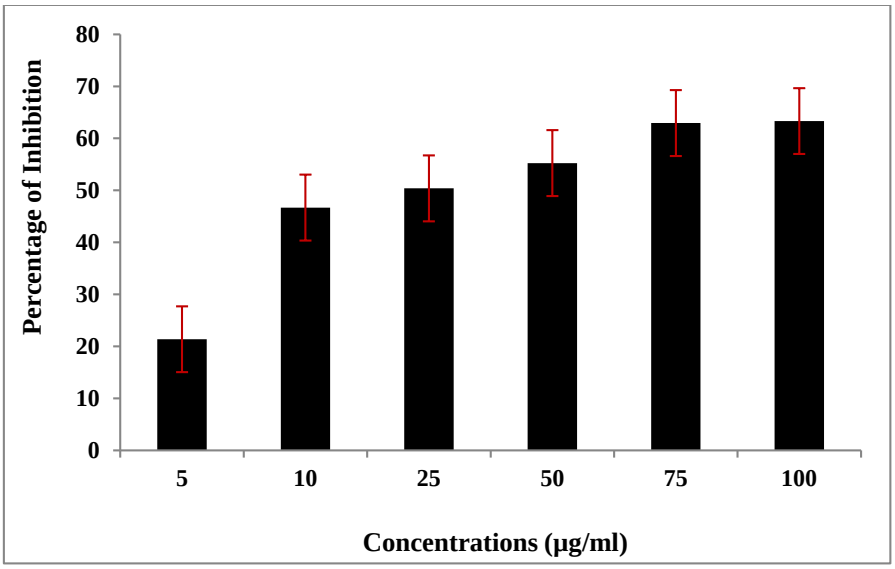


Figure 15: DPPH Antioxidant of Sample Zno NP

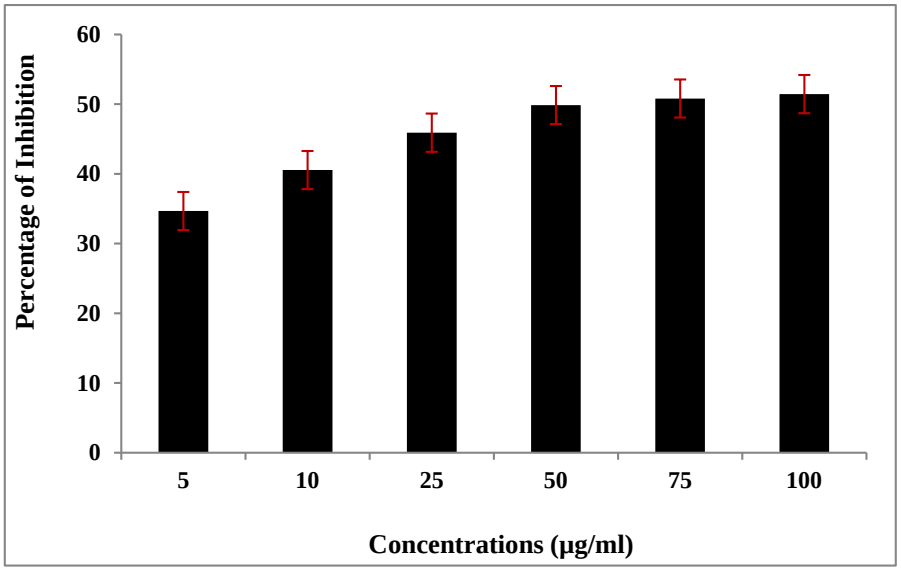


Figure 16: DPPH Antioxidant of Ascorbic Acid