



Biogenic Synthesis of Copper oxide and Zinc oxide Nanoparticles using *Catharanthus roseus* L. flower extract and Evaluation of Its Antioxidant and Antibacterial Properties

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ABSTRACT

Inorganic nano-metal oxides may be efficient replacements for organic antibiotics that are drug-resistant due to their wide range antibacterial activity towards pathogenic and mutagenic bacterial species. In this work, zinc and copper oxide nanoparticles were produced using *Catharanthus roseus* flower extract. It is a feasible alternative to other techniques because the biosynthesized nanomaterials made from plant extract are non-toxic and harmless to the environment. The X-ray diffraction (XRD), scanning electron microscopy (SEM), UV-Vis, and Fourier transform infrared spectrometry (FT-IR) investigations were achieved to confirm CuO and ZnO nanoparticles produced. In the DPPH experiment, the antioxidant properties of CuO and ZnO nanoparticles performed well. *Gram-positive* and *Gram-negative* microorganisms are both effectively combatted by the produced copper and zinc oxide nanoparticles. The findings suggest that CuO nanoparticles were more effective towards microorganisms than ZnO nanoparticles.

Keywords: *Catharanthus roseus*, SEM, XRD, UV-Vis, EDAX, FTIR, DPPH assay and Antibacterial activity.

INTRODUCTION

A continuing quest for novel replacements has resulted from the proliferation of drug-resistant pathogens¹. Among these infections, bacterial pathogens in water pose a serious concern to healthcare system since they are accountable for illnesses like diarrhoea, which account for 2195 new born global deaths worldwide and becomes bigger

every day². It has been shown that nanostructures are toxic to a variety of bacterial pathogens that produce disease³. Inorganic nanoparticles have a well-known widespread bactericidal action, although the mechanism behind this effect is still unclear⁵. It has recently been suggested that reactive oxygen species produced by the discharge of ions in solution are hazardous to microorganisms. Several investigations have discovered that because of its



small size, nanoparticles may enter bacterial cell walls and damage organelles, which results in cell death. In contrast to their organic cousins, inorganic antibiotics may target several infections to battle their resistance via mutation⁶. Metal oxide nanoparticles belong to the inorganic nanoparticles that are most often utilized due to its antibacterial properties⁷. This is frequently owed to the statistic that metal oxides are more affordable than metal nanoparticles like silver and gold have a straightforward synthesis route that can be manipulated to modify the size and form of the nanoparticles⁸. Copper and zinc oxides are appropriate substitutes for antimicrobials obtained from organic materials. Numerous elements, including size, shape, and other elements mostly determined by synthetic means, affect their antibacterial effects⁹. Because of their small size and strong reactivity, metal nanoparticles may quickly infiltrate through bacterial cell walls and connect to proteins and interior organelles, which causes bacterial death^{10,11}.

When tested against various bacterial species, copper oxide and zinc oxide had distinct antibacterial activities¹². Because of their antibiotic properties, they remained employed in packaged foods, surface coatings, and wound healing^{13,14}. CuO nanoparticles have shown therapeutic properties like antioxidant, immunomodulatory, sunscreen, and anticancer effects in addition to the antibacterial activities of these nanomaterials. The effectiveness of copper oxide nanoparticles in the medical area has been demonstrated¹⁵. This study examined the antibacterial efficacy of copper and zinc oxide nanoparticles towards both *Gram-positive* and *Gram-negative* bacteria.

The present study has shown that copper oxide, zinc oxide, and a plant extract have therapeutic promise, especially in terms of their antibacterial property. The *Catharanthus roseus* flower is used to treat eye issues and has been shown to have anti-tumor and therapeutic properties. Alstonine, a component of the root, is used to lower blood pressure. The antibacterial efficacy of copper and zinc oxide nanoparticles towards cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was investigated *In vitro*. Zinc oxide nanoparticles are found in sunscreen. It has been shown that ZnO nanoparticles have antibacterial effects towards common foodborne illnesses¹⁶⁻¹⁸.

EXPERIMENTAL

Catharanthus roseus fresh flowers were gathered from the local area of Tiruchirappalli District, cleaned with deionized water, and boiled with distilled water¹⁹.

Synthesis of Copper oxide nanoparticles

CuCl₂·2H₂O subsequently dissolved in 10 mL of floral extract solution for this plant component, and the combination was let to settle for three hours at room temperature. The reaction mixture was then combined with 1 mL of a 10% NaOH solution. The substance that had precipitated was filtered and dried. In the oven, the raw product was reserved at 150°C for 12 hours. The powder was obtained and calcined at 450°C for six hours²⁰.

Synthesis of Zinc oxide nanoparticles

Anhydrous ZnCl₂ (0.1g) was dissolved in deionized water and combined with a 10 mL *Catharanthus roseus* floral extracts for 3 h at room temperature to create zinc oxide nanoparticles. The mixture was then poured in 1 mL of a 10% NaOH solution, and it was filtered and dried thereafter. The raw product spent 12 h in the oven at 150°C. The obtained product was calcined for six hours at 450°C. Characterization of Copper and Zinc oxide nanoparticles

Employing UV-Visible spectroscopy in the 200 to 800nm range, the copper oxide and zinc oxide nanoparticles were examined. The crystalline structure of the CuO and ZnO Nanoparticles was determined via X-ray diffraction spectroscopic (XRD) analysis. The elements contained in nanoparticles were recognized using energy dispersive X-ray spectroscopy (EDAX) coupled with FE-SEM. Scanning electron microscopy (SEM) was used to examine the nanoparticles' structural details. The Fourier transform infrared spectra of the nanoparticles were documented on an FT-IR spectrometer ranges over 400 and 4000 cm⁻¹.

Antioxidant activity by DPPH method

Activity that scavenges free radical for the synthesized nanoparticles were tested by DPPH method. The samples were prepared in 20, 40, 60 and 80 µg/mL concentration and combined with DPPH for 30 min of incubation in dark and absorbance was recorded at 517nm.

% of antiradical activity = $(A-B) / A \times 100$

Where,

A = control absorbance; B = Sample absorbance

Antibacterial activity determination

The antimicrobial property of CuO and ZnO nanoparticle were analyzed through disc diffusion method against *Staphylococcus aureus* B23, and *Pseudomonas aeruginosa* 424. The inhibition zone was determined for microorganism that are Gram-positive and Gram-negative and compared with that of standard chloramphenicol. Whatmann No.1 sterile channel paper plates (6 mm width) were impregnated with required grouping of fluid and ethanolic extricates and put on the immunized agar. All of the plates underwent 24-h hatching at 37°C. Restraint zones were estimated and contrasted with the standard. Assessment of antibacterial movement

was estimated through the breadth of the zones of restraint against the tried strains of microbes.

RESULTS AND DISCUSSION

The flower of *Catharanthus roseus* was collected near Trichy and described using the Flora of the Madras presidency, and the fresh flower extract exhibits significant role in the synthesized of CuO and ZnO nanoparticles.

The fluorescence analysis of the *Catharanthus roseus* flower powder

In day light and ultraviolet light, the fluorescence activity of drug powder with the following chemicals was observed, which was found to offer different shades of color. The presence of alkaloids and flavones is indicated by the brown and red while the presence of sterols is indicated by the green fluorescence.

Table 1: The fluorescence analysis of the *Catharanthus roseus* flower powder

Sr.No	Treatment	<i>Catharanthus roseus</i>			
		24 Hours		48 Hours	
		UV Light	Day Light	UV Light	Day Light
1	Powdered drug	Green	Green	Green	Green
2	Powdered drug+Hexane	Green	Pale Green	Pale Green	Light Yellow
3	Powdered drug+Benzene	Pale Green	Pale Green	Pale Green	Pale Green
4	Powdered drug+Chloroform	Pale Green	Pale Green	Pale Green	Pale Green
5	Powdered drug+Ethyl acetate	Pale Green	Pale Green	Pale Green	Pale Green
6	Powdered drug+Alcohol	Pale Green	Pale Green	Pale Green	Pale Green
7	Powdered drug+Acetone	Green	Pale Green	Green	Green
8	Powdered drug+50% H ₂ SO ₄	Black	Dark Brown	Black	Dark Brown
9	Powdered drug+1 N HCl	Pale Green	Light Brown	White	Light Brown
10	Powdered drug+Aq. 1N NaOH	Red	Dark Red	Green	Red
11	Powdered drug+Alc. 1N NaOH	Green	Pale Green	Green	Pale Green
12	Powdered drug+H ₂ O	Red	Red	Green	Dark Orange

Table 2: Preliminary phytochemical screening of drug powder and various extracts of *Catharanthus roseus*

Sr.No	Phyto constituents	Results					
		Plant Powder	Hexane Extract	Chloroform Extract	Methanolic Extract	Ethanolic Extract	Aqueous Extract
1	Alkaloid	+	+	+	+	+	-
2	Tannin	+	-	-	+	+	+
3	Quinones	+	+	-	-	-	-
4	Flavones	+	-	-	+	+	+
5	Terpene	+	+	+	+	+	-
6	Coumarin	-	-	+	-	+	+
7	Sterol	+	+	+	+	+	-
8	Lignin	+	+	+	+	-	-
9	Saponin	+	-	+	+	+	-
10	Carbohydrate	+	+	+	+	+	+
11	Phenol	+	+	+	+	+	+

Quantitative analysis of phytochemicals

From the table major secondary metabolites such as phenol, flavanoids and terpenes are present. Compare to other two, plant have terpenes in high amount (Table 3).

Table 3: Major metabolites-Quantitative analysis

S. No	Secondary Metabolites	Amount (mg/g)
1	Phenol	1.24
2	Flavanoids	2.40
3	Terpenes in plant powder	31.66
4	Terpenes in Hexane extract	70.00

Optical characterization

Color variation in the mixture was used to visually track the reduction of Cu^{2+} ions to CuO Nanoparticles by *Catharanthus roseus* flower extract. It was found that the color of the solution

gradually transformed from pale green to sky blue. Similarly, Zn^{2+} ions is reduced to ZnO Nanoparticles. The steady color shift in the reaction mixture from light green to pale yellow.

UV-Visible spectroscopy

In CuO Nanoparticles showed absorption peak at 390nm specifies the distinction Surface Plasma Resonance band for copper oxide nanoparticles size was less. In ZnO nanoparticles absorption peak was found at 374nm leads the individual SPR bands for ZnO nanoparticles with less size.

FT-IR spectroscopy

Different functional groups were present, according to FT-IR analyses. It was evident that ZnO vibrations were present in the band at 422.08 cm^{-1} . The vibrational band for CuO at 428.83 cm^{-1} .

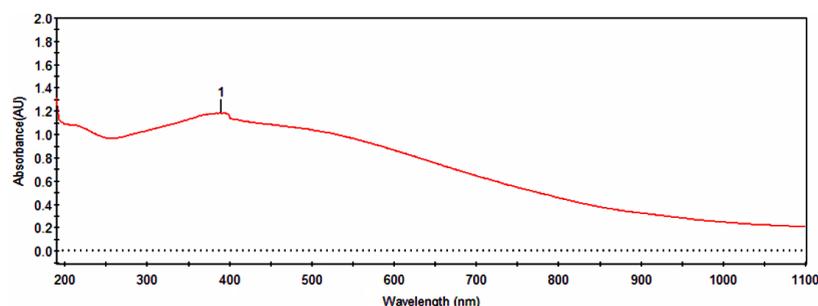


Fig. 1. UV-Visible spectrum of CuO nanoparticle

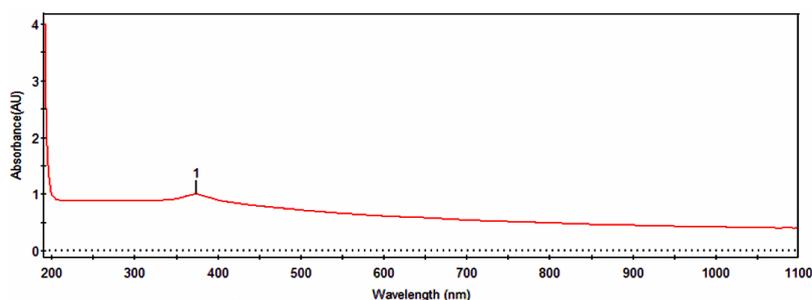


Fig. 2. UV-Visible spectrum of ZnO nanoparticle

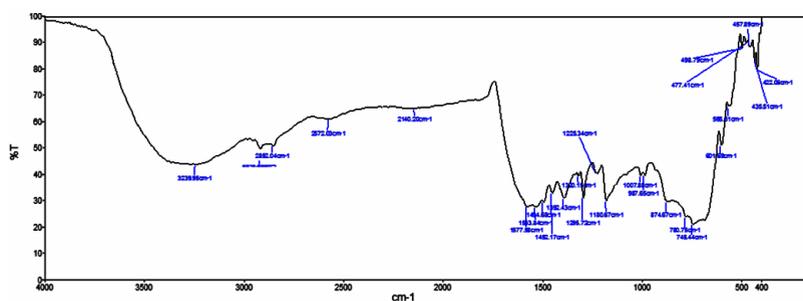


Fig. 3. FT-IR spectrum of CuO nanoparticle

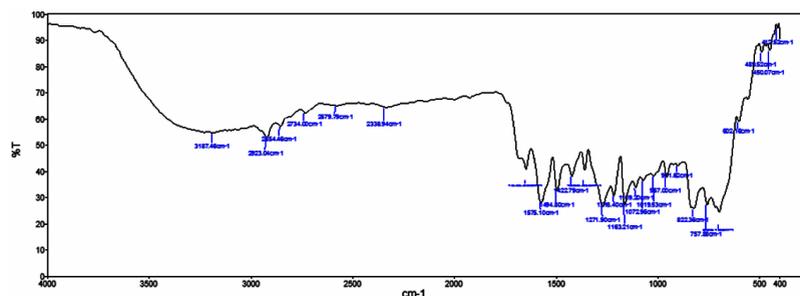


Fig. 4. FT-IR spectrum of ZnO nanoparticle

SEM and EDAX characterization of metal

The ZnO and CuO nanoparticles are synthesized by using *Catharanthus roseus* 5 μ m and 3 μ m in size and both were spherical shape.

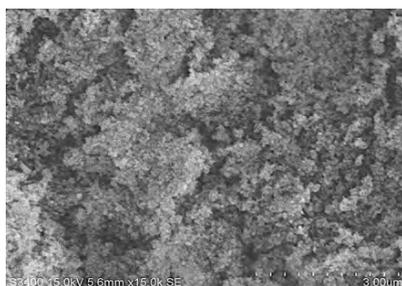


Fig. 5. Scanning Electron Microscope picture of ZnO Nanoparticles

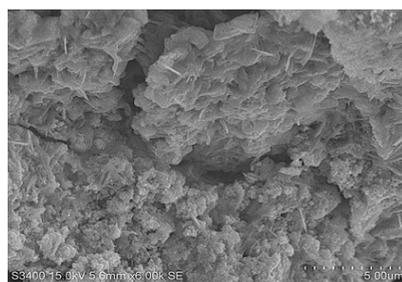


Fig. 6. Scanning Electron Microscope picture of

CuO nanoparticles

EDAX analysis verified the elemental composition of the produced CuO and ZnO Nanoparticles. CuO Nanoparticles were produced as the appearance of the copper and oxygen peaks in the EDAX spectrum, while ZnO Nanoparticles were produced as the zinc and oxygen peak.

XRD analysis

Figure 9 displays the XRD image of CuO Nanoparticles produced from *Catharanthus roseus* flower extract. The CuO Nanoparticles' monoclinic structure was revealed by the diffraction peaks $2\theta=33.53^\circ$, 35.82° , 38.78° , 48.99° , 55.75° , 58.64° ,

62.26° and 67.34° , which were correspondingly indexed to the (110), (111), (111), (112), (020), (113), (311), and (220) planes. The diffraction peaks that were obtained corresponded to the JCPDS (048-1548) of typical CuO NanoParticles.

Figure 10 illustrates the XRD pattern of *Catharanthus roseus* flower extract made from ZnO Nanoparticles. The diffraction $2\theta=32.630$, 34.720 , 36.480 , 45.360 , 53.750 , 56.840 , 63.260 , and 66.460 was indexed to the monoclinic planes of ZnO Nanoparticles in the (100), (002), (101), (104), (102), (110), (103), (200), (112), (201), (004), and (202), respectively. The diffraction peaks that were obtained matched JCPDS21.

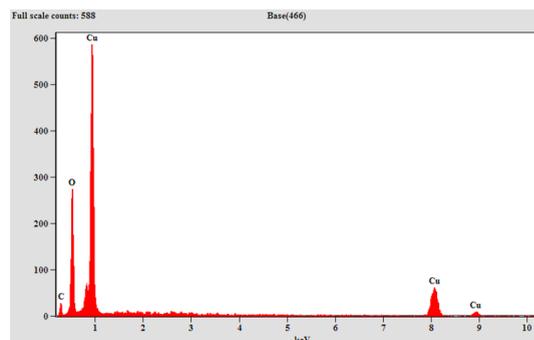


Fig. 7. EDAX - CuO Nanoparticles

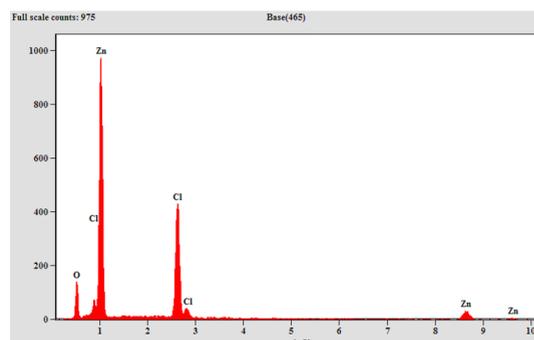


Fig. 8. EDAX - ZnO Nanoparticles

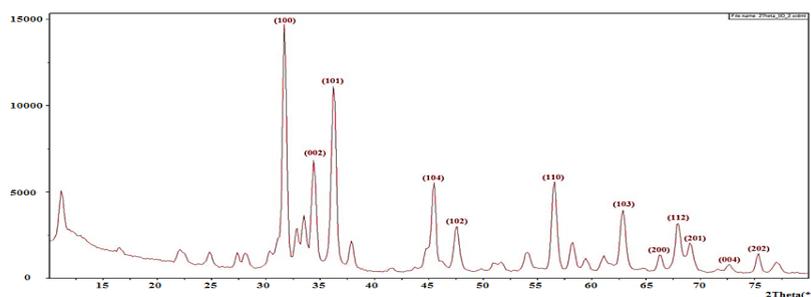


Fig. 9. XRD spectrum of CuO Nanoparticles

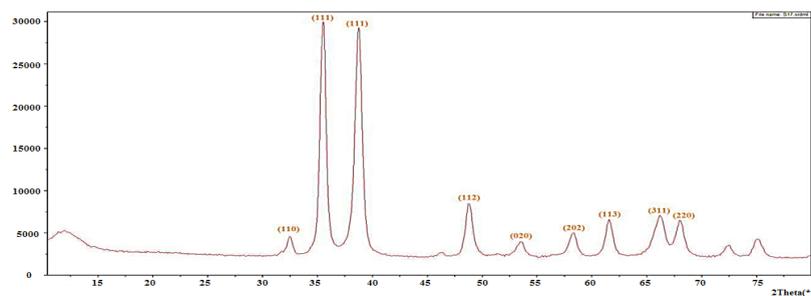


Fig. 10. XRD spectrum of ZnO Nanoparticles

Antioxidant activity

In vitro antioxidant property of synthesized CuO and ZnO Nanoparticles from *Catharanthus roseus* flower extract by DPPH method is shown in Table 4. Ascorbic acid was utilized as a standard. The antioxidant activity at various concentrations say, 20, 40, 60 and 80 $\mu\text{g/mL}$ are shown. The

percentage inhibition(%) for standard ascorbic acid at 80 $\mu\text{g/mL}$ is 98.23 whereas for CuO nanoparticles is 77.27 and for ZnO nanoparticles is 78.1. The results clearly indicates that copper oxide and zinc oxide nanoparticles synthesized using *Catharanthus roseus* have high capacity in controlling the free radicals.

Table 4: Antioxidant activity of metal oxide nanoparticles by DPPH method

Concentration, $\mu\text{g/mL}$	CuO Nanoparticles	ZnO Nanoparticles	Ascorbic acid
20	22.72 \pm 0.59	21.87 \pm 0.53	41.0 \pm 0.90
40	40.90 \pm 0.86	43.75 \pm 0.06	68.10 \pm 0.60
60	63.63 \pm 0.45	56.25 \pm 0.93	84.64 \pm 0.80
80	77.27 \pm 0.40	78.12 \pm 0.45	98.23 \pm 0.30

Values are expressed as Mean \pm SE (n=3)

Antibacterial activity

Copper and zinc oxide nanoparticles' antibacterial potential was evaluated. Disk diffusion method was utilized to test the antibacterial assessment of copper and zinc oxide nanoparticles towards *Staphylococcus aureus* B23 and *Pseudomonas aeruginosa* 424. Both *Gram-positive* and *Gram-negative* bacterial organisms had their zones of inhibition established, and they were compared to those of

conventional chloramphenicol. The synthesized CuO nanoparticle showed significant activity (zone of inhibition 12.83mm at 100 $\mu\text{g/mL}$) compared with standard (zone of inhibition 15.00mm) for gram positive pathogen. The synthesized ZnO nanoparticle showed significant activity (zone of inhibition 11.33mm at 100 $\mu\text{g/mL}$) compared with standard (zone of inhibition 15.83mm) for *Gram-positive* pathogen. CuO nanoparticle showed significant activity (zone

of inhibition 10.27mm at 100µg/mL) compared with standard (zone of inhibition 15.93mm) for *Gram-positive* pathogen. ZnO nanoparticle showed significant activity (zone of inhibition 10.43mm at 100 µg/mL) compared with standard (zone of inhibition 14.67mm) for Gram-negative

pathogen. Both of these two have remarkable antimicrobial activity than ethanolic flower extract of *Catharanthus roseus* in both bacterial microbes. The antibacterial action of synthesized metal oxide nanoparticles was shown in the Table 5,6,7 and 8.

Table 5: Antibacterial potential of copper oxide nanoparticles towards *Staphylococcus aureus*

GPB	Nanoparticles	Zone of inhibition	Ethanol Extract/Control	Zone of inhibition
<i>Staphylococcus aureus</i> B23	Positive Control	15.00±0.00	Positive Control	16.00±0.29
	Negative Control	0.12±0.06	Negative Control	1.11±0.02
	CuONp /25	6.53±0.32	EECO /25	4.60±0.31
	CuONp /50	8.23±0.15	EECO /50	6.93±0.23
	CuONp /75	8.50±0.29	EECO/75	7.17±0.44
	CuONp /100	12.83±0.44	EECO/100	9.13±0.24

Values are expressed as Mean ± SE (n=3)

Table 6: Antibacterial potential of zinc oxide nanoparticles towards *Staphylococcus aureus*

GPB	Nanoparticles	Zone of inhibition	Ethanol Extract/Control	Zone of inhibition
<i>Staphylococcus aureus</i> B23	Positive Control	15.83±0.17	Positive Control	16.43±0.30
	Negative Control	0.11±0.02	Negative Control	1.38±0.01
	ZnONp /25	2.83±0.17	EECO /25	1.67±0.35
	ZnONp /50	6.17±0.17	EECO /50	2.07±0.12
	ZnONp/75	9.17±0.60	EECO/75	6.17±0.66
	ZnONp/100	11.33±0.33	EECO/100	9.07±0.58

Table 7: Antibacterial potential of copper oxide nanoparticles towards *Pseudomonas aeruginosa*

GNB	Treatment/Control	Zone of inhibition	Treatment/Control	Zone of inhibition
<i>Pseudomonas aeruginosa</i> , 424	Positive Control	15.93±0.23	Positive Control	15.93±0.23
	Negative Control	0.21±0.03	Negative Control	1.48±0.03
	CuONp /25	3.23±0.15	EECO /25	2.23±0.15
	CuONp /50	6.40±0.26	EECO /50	4.40±0.26
	CuONp /75	8.67±0.18	EECO/75	6.67±0.18
	CuONp /100	10.27±0.18	EECO/100	8.27±0.18

Values are expressed as Mean ± SE (n=3)

Table 8: Antibacterial potential of zinc oxide nanoparticles towards *Pseudomonas aeruginosa*

GNB	Treatment/Control	Zone of inhibition	Treatment/Control	Zone of inhibition
<i>Pseudomonas aeruginosa</i> , 424	Positive Control	14.67±0.17	Positive Control	16.13±0.09
	Negative Control	0.11±0.04	Negative Control	1.52±0.01
	ZnONp/25	0.57±0.28	EECO /25	1.67±0.12
	ZnONp/50	6.07±0.07	EECO /50	3.60±0.12
	ZnONp/75	8.73±0.27	EECO/75	7.27±0.18
	ZnONp/100	10.43±0.30	EECO/100	9.40±0.06

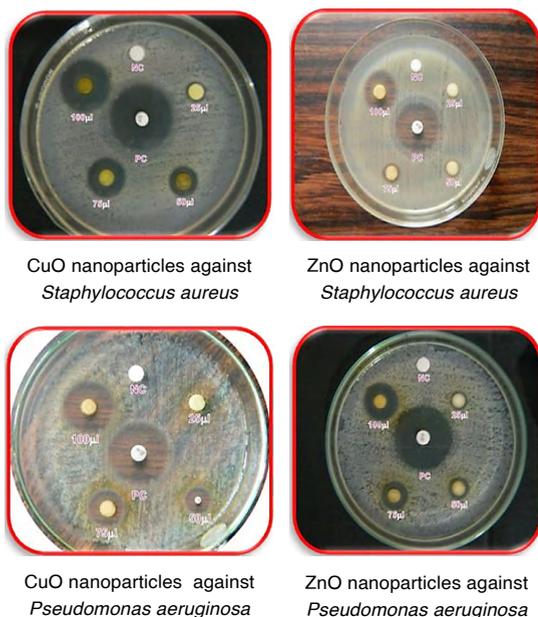


Fig. 11. Antibacterial activity of Copper oxide and Zinc oxide nanoparticles

CONCLUSION

The copper and zinc oxide nanoparticles were produced through fresh flower extract of *Catharanthus roseus*. The synthesized nanoparticles were confirmed by using various spectrometric techniques. The antioxidant study by DPPH method showed good result compared with standard ascorbic acid. The *In vitro* antibacterial activity depicts the effective antibiotic action of both of these metal oxide nanoparticles. It concludes that CuO and ZnO nanoparticles helps for medication development.

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Conflict of Interest

All the authors declare that there is no conflict of interest.

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