

Antioxidant and Antimicrobial Potential of Green Synthesized Copper Oxide Nanoparticles Using *Phyllanthusniruri*

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ABSTRACT

Phyllanthusniruri leaves extract was effectively used for the synthesis of copper oxide nanoparticles as a natural reducing agent. The XRD and SEM -EDAX analysis confirmed the formation of copper oxide nanoparticles. Based on SEM images, copper oxide nanoparticles were spherical in shape with agglomerated particles. The antibacterial activity of copper oxide nanoparticles were analyzed with *E.coli* and *S. aureus*, high antibacterial activity was observed against both the bacteria. The copper oxide nanoparticles have good potential of antioxidant activity.

Keywords

Copper oxide nanoparticles, *Phyllanthusniruri*, *E.coli* and *S. aureus* and antioxidant activity

INTRODUCTION

Metal oxide nanoparticles:

Metal oxide nanoparticles have particular interest due to their unique electronic, optical and magnetic properties. The transition metal oxides comes under the class of semiconductors possess enormous applications in electronics, solar energy transformation, magnetic storage media, catalysis and gas sensors. The nanotechnology is one of the enthusiastic field in modern materials science [1]. Nanotechnology is based on synthesise of nanomaterials (1-100 nm) and its applications in various fields especially for industrial revolution [2].

Copper oxide nanoparticles (semiconductors) are potential in electronic devices, chemical sensors, solar cells and antimicrobials [3] and vital application in the field of photocatalytic activity and photocells due to its stability, low cost and etc.,[4],[5],[6].

Design and fabrication of nanoparticles have gained interest in medicinal field owing to their distinct size-dependent, catalytic, optical, mechanical and electrical properties with its innovative high-tech applications when compared to their bulk counterparts [5]. Though enormous researches have been carried out in metals such as silver and gold, their limitations lie on its elevated cost [6]. Hence, researchers focused to replace the metals which are of high cost with other metals for the nanomaterial synthesis. Now a days CuO nanoparticles have significant attention for its effective applications in biological field [7], [8][9][10][11].

MATERIALS AND METHODS:

2.1.Plant Scientific classification

Family: Phyllanthaceae

Kingdom: Plantae

Species: *P. niruri*

Order: Malpighiales

The medicinal valuable plants of *Phyllanthusniruri* leaves were collected from Thoothukudi, Tamilnadu, India. The fresh leaves were completely washed with distilled water to eliminate the dust particles and shade dried for one week. After complete drying of the leaves, it was powdered by the use of a kitchen blender and packed in an airtight container for storage at room temperature.

2.2 .Preparation of leaves extract:

For each plant, 5 g of powdered leaf sample was added to the 50 mL of double distilled water in 250 mL beaker and kept in the water bath for 15 min at 60 °C. After cooling, the solution was mixed well with magnetic stirrer for 20-30 min to improve the extraction efficiency. The extract was filtered using Whatman No.1 filter paper to separate the leaf debris and the obtained clear leaves extract filtrate was stored at 4°C to use it further for nanoparticle synthesis studies.

2.3. Preparation of Copper sulphate solution:

1 mM copper sulphate solutions were prepared by exactly weighing the required amount for 100 mL. The prepared salt solutions were then used for green synthesis of CuO nanoparticles.

2.4. Synthesis of CuO nanoparticles:

The pH of 10mL of *Phyllanthusniruril* eaves extract was adjusted to 9 using 0.1 N NaOH. Exactly 10 mL of 0.1 M cupric sulphate solution was mixed with pH adjusted leaves extract in a beaker and boiled. Immediately a black colloid was obtained. The black colloid thus obtained was centrifuged at 13,000rpm, washed several times with distilled water and dried black solid CuO nanoparticles (CuO NPs) were used in all for further studies.

CHARACTERIZATION OF CuO NANOPARTICLES

3.1. UV-Vis Spectroscopy Analysis

The formation of nanoparticles by the reduction of ions in the salt solution was analyzed for 4 days at regular intervals of 24 hrs. UV-vis spectroscopy analysis of CuO nanoparticles was carried out using UV spectrophotometer.

3.2. Energy Dispersive X-Ray (EDX)Analysis

The presence of elemental copper was confirmed with EDX analysis. This is done by using the EDX detector coupled with TECNAI Transmission electron microscope.

3.3.X-RAY Diffraction (XRD)Analysis

The crystalline nature of CuO was characterized by X-ray diffractometer (X'Pert power, Germany) instrument using Cu K α radiation in the 2 θ range of 10-80°.

3.4. FTIR Analysis

The fine powder of CuO nanoparticles was analyzed by FTIR spectroscopy to determine the functional groups present in the synthesized nanoparticles. FTIR analysis was done using Perkin-Elmer Spectrum Two model instrument. The sample was mixed with KBr salt and measurements were recorded at a scan range of 400- 4000 cm⁻¹ with the resolution of 2 cm⁻¹.

3.5. Scanning Electron Microscopy (SEM)

Further Morphology study was done by using SEM (FEI Quanta FEG 200). The dried biomass of CuO was subjected on a carbon coated copper grid by just coated a very

small amount of the sample on the grid and then performed the SEM.

3.6. Antibacterial Assay By Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the test sample against microorganisms. The determination of the anti-bacterial activity of sample can be determined by using Muller Hinton Agar medium (HIMEDIA- M173). The sterilized 15- 20 mL of Mueller-Hinton agar was poured on glass Petri plates of same size and allowed to solidify. After the solidification, the wells (4 wells/ plate) were made with a sterile cork borer of diameter 8 mm (20 mm apart from one another) were punched aseptically in each plate. The standardized inoculum of the test organism was uniformly spread on the surface of these solidified media using sterile cotton swab. The test volumes (20 μ L & 40 μ L) of the sample at desired concentrations were added to the first 2 wells, 1well with 80mcg of Gentamycin as positive control and other one with DMSO as negative control. Then, the agar plates were incubated in incubator under 37°C for 24 hr. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm.

3.7. Antifungal Assay By Agar Well Diffusion Method

Agar well diffusion assay was done to detect the presence of anti-fungal activities of the test samples. Once the Rose Bengal agar medium had solidified, four wells, each 9 mm in diameter, were cut out of the agar; a sterile swab was used to evenly distribute fungal culture over the agar surface. The plates were allowed to dry for 15 minutes and the test sample (40 and 80 μ L) was added into the wells T1 & T2. In the positive well (+) the control drug, clotrimazole was added (20 μ l from 10mg/ml stock) and in the negative well (-) the solvent used for the sample dilution was added. The plates were incubated at room temperature for 24 hours after which they were examined for zone of inhibition.

3.8. DPPH Radical Scavenging Assay

Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the concentration (1mg/1000 μ l). The solution of DPPH in methanol 60 μ M was prepared fresh daily before UV measurements. This solution (4ml) was mixed with various concentrations (6.25, 12.5, 25, 50 & 100 μ L) of samples. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula.

% Inhibition = (Absorbance of Control at 0 minute - Absorbance of Test) / Absorbance of Control at 15 minutes' x 100

Where C= absorption of control sample (t= 0 min), C= absorption of control (t=15 min), T=absorption of test solution.

RESULTS AND DISCUSSION

4.1. UV-Vis Spectroscopy Analysis:

The UV spectral analyses for the synthesized CuO NPs were done. The color change from yellow to brown color primarily indicates the conversion of Cu into CuO NPs. The UV spectrum of the synthesized CuO NPs evidently indicates the progression and stability as shown in the Fig. 1. The

surface plasmon resonances of the nanosized copper oxide particles were confirmed by the appearance of maximum absorbance at the 202nm.

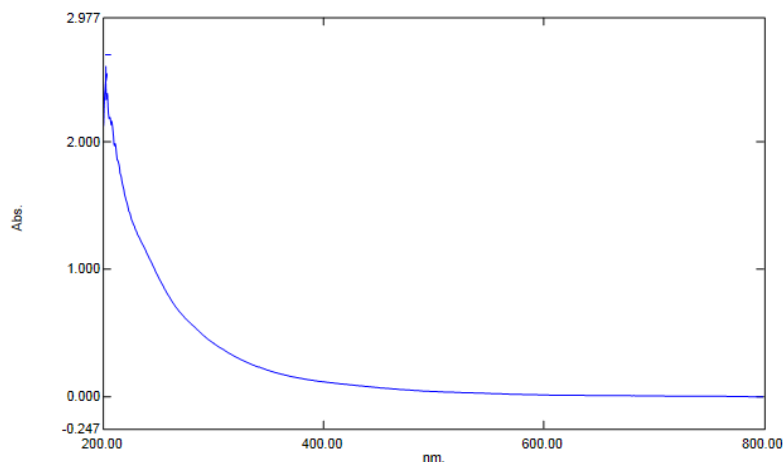


Fig - 1

4.2. FTIR Analysis:

The FT-IR transmittance analysis was carried out to determine the functional groups present in the synthesized copper oxide nanoparticles from *Phyllanthusniruri*. The FT-IR analysis shows different characteristics peaks at 618.4cm^{-1} , 1108.03cm^{-1} , 1188.07cm^{-1} , 1384.79cm^{-1} , 1440.73cm^{-1} , 1606.59cm^{-1} between the range $400 - 4000\text{cm}^{-1}$ (Fig. 2). The slight broad band at 3443.66cm^{-1} corresponds to the N-H stretch due to amine group and the peak at 1606.59cm^{-1} shows the presence C=C bending due to the presence of aromatic secondary metabolites. The band at 1384.79cm^{-1} corresponds to the presence of C-N stretch due to amine group and the peak at 1108.07cm^{-1} shows the presence of =C-H bending due to the alkene group. The prominent peak at 618.4cm^{-1} confirms the presence the Cu-O vibration in the synthesized CuO NPs.

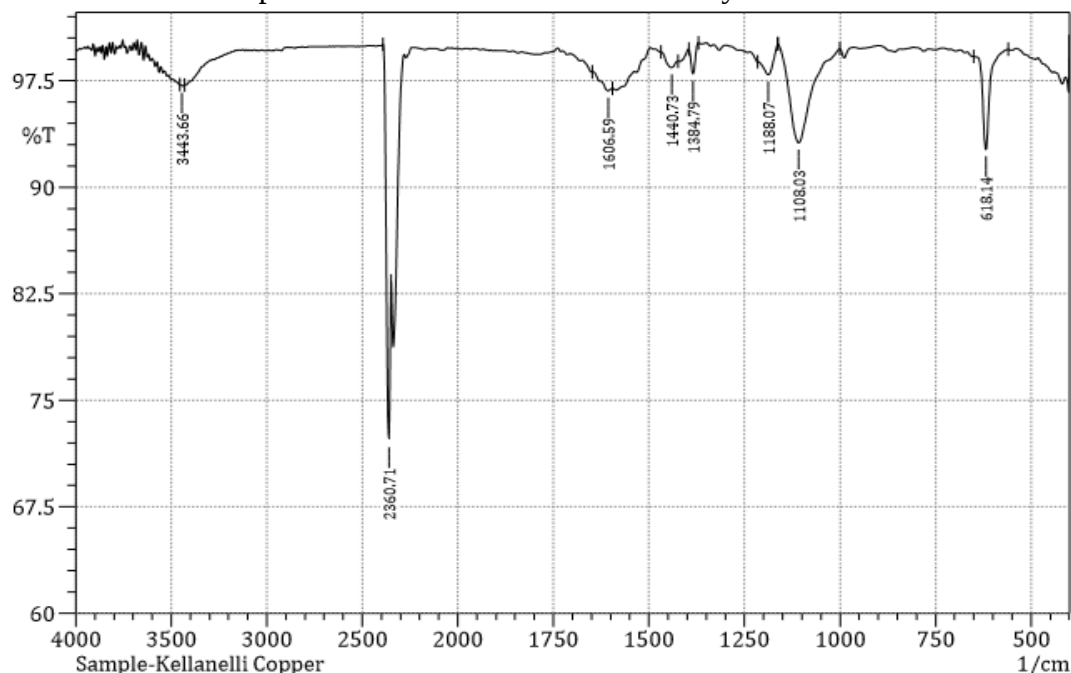


Fig.2

4.3.EDX Analysis:

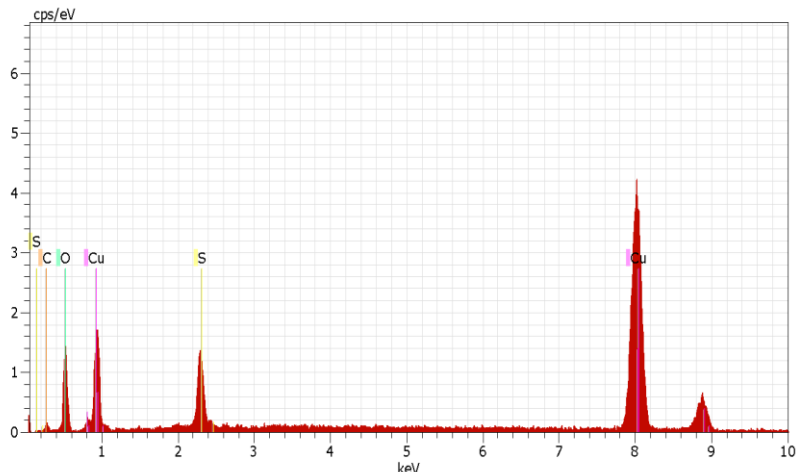


Fig – 3

The strong Cu peak in the EDX spectrum confirms the presence Cu. In the present study the EDX peak of Cu with O as the mixed components present in the reaction medium.

4.4.XRD and SEM analysis of CuO nanoparticles:

The X-Ray Diffractometer (XRD) analyses were done for the synthesized *Phyllanthusniruri* CuONPs confirm the crystalline nature of synthesized nanoparticles(Fig .4). The XRD analysis of the CuO NPs was interrelated with the Joint Committee on Powder Diffraction Standards (JCPDS), which confirmed the crystalline nature of CuO NPs (JCPDS 96- 901-5924).

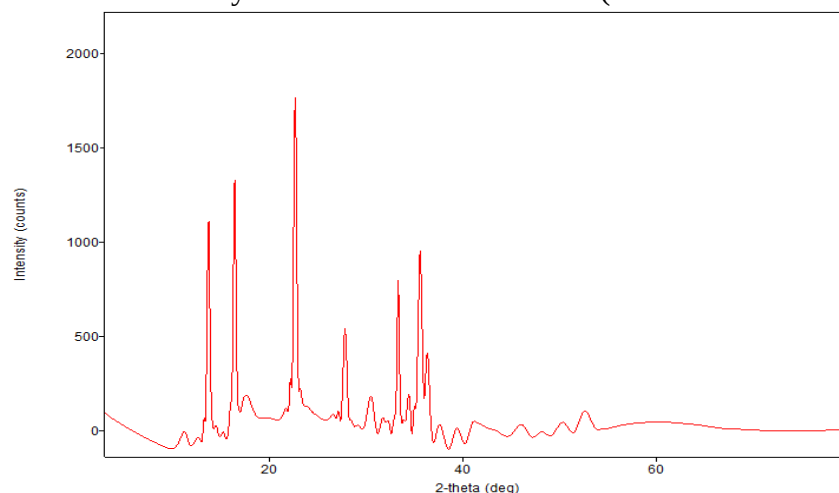


Fig. 4

The morphological studies for the synthesized *Phyllanthusniruri* mediated CuO NPs were ascertained by SEM analysis. The Scanning Electron Microscope (SEM) analysis for the synthesized nanoparticles indicated the formation of CuONPs agglomerated cluster forms as shown in the (Fig.5).

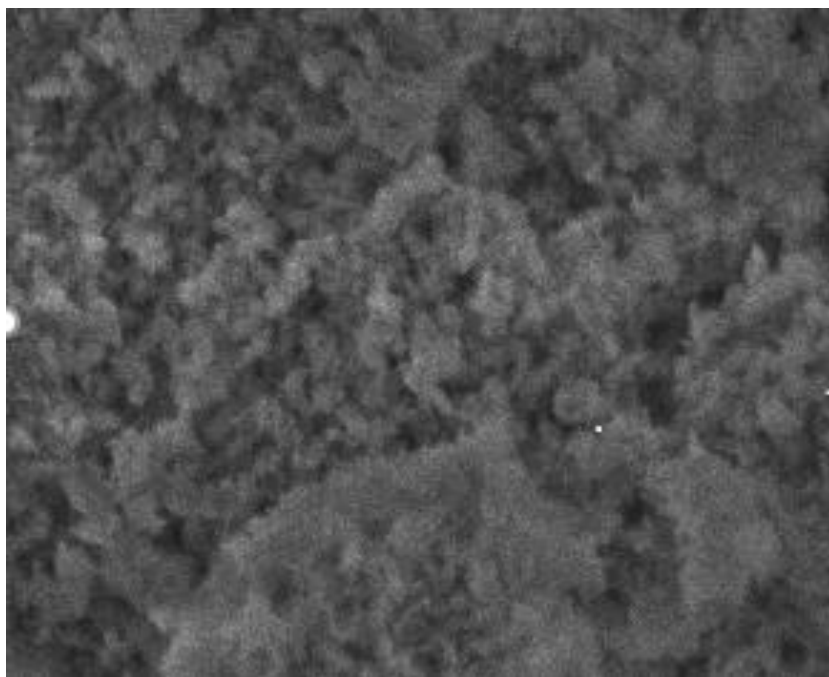


Fig- 5

4.5. Antibacterial activity

The CuO nanoparticles synthesized using *Phyllanthus niruri* was tested for antibacterial activity against a gram positive bacteria *Staphylococcus aureus* and a gram negative bacteria *E.coli*. CuO nanoparticles have known to exhibit antibacterial activity against *E.coli* with inhibition zone of 13 mm and 23 mm for 20µl and 40µl respectively (Table1 & Fig6). However, it failed to show activity against gram positive *Staphylococcus aureus* at low concentration and less activity of 10mm inhibition zone at 40µl concentration. The possible mechanism of antibacterial activity of CuO nanoparticles due to bacterial cellwall synthesis inhibition. Since the cell wall of gram positive bacteria is more thicker than gram negative bacteria, the CuO nanoparticles inhibit gram negative bacteria more than gram positive bacteria. Secondly, being a positively charged particle, it penetrates the negatively charged bacterial cell membrane readily and kills the bacteria by inhibiting protein synthesis

Table 1. Antibacterial activity

S.No	Sample	Organism	Zone of inhibition(mm)			
			Standard Gentamycin (80mcg)	Negative control	Test 1 (T1) (20 µL)	Test 2 (T2) (40 µL)
1.	<i>P.niruri</i> CuO nanoparticles	<i>E.coli</i>	30	-	13	23
		<i>S. aureus</i>	26	-	-	10

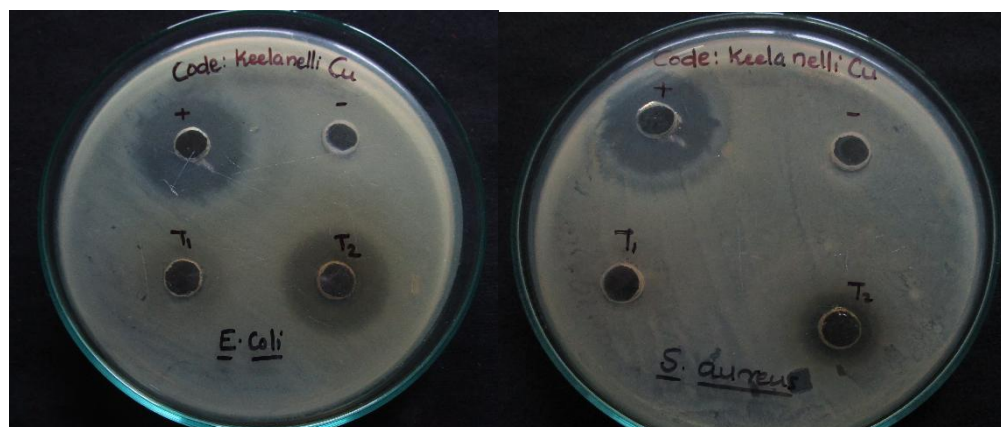


Fig.6 - . Antibacterial activity

4.6. Antifungal activity

CuO nanoparticles shown antifungal activity against *Candida albicans* and *Aspergillusniger* with inhibition zone of 15mm and 19mm respectively at 40μl concentration (Table2. and Fig7).The possible mechanism of antifungal activity is due to cell wall disruption or protein synthesis. The result shown that CuO nanoparticles to be a promising molecule to combat *Candida albicans*, an opportunistic pathogen and respiratory pathogen *Aspergillusniger*

Table2. Antifungal activity

S.No	Sample	Organism	Zone of inhibition(mm)			
			Standard Clotrimazole (200mcg)	Negative control	Test 1 (T1) (20 μL)	Test 2 (T2) (40 μL)
1.	<i>P.niruri</i> CuO nanoparticles	<i>C. albicans</i>	18	-	14	15
		<i>A.niger</i>	20	-	16	19

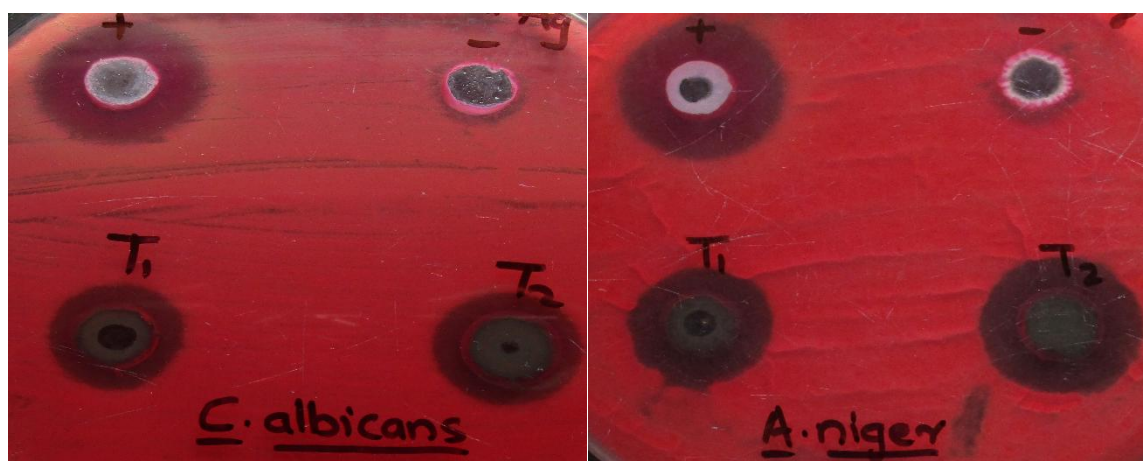


Fig7. Antifungal activity

4.7. Antioxidant activity

Antioxidants are the natural or synthetic molecule that neutralizes the free radicals or free electrons. The presence of free radicals or electrons in the living system cause damage to DNA,

RNA and protein results in severe damage of the biomolecules. Hence, antioxidants are the potent molecules that conserve the protein and DNA from deleterious effect. The antioxidant potential of CuO nanoparticles was shown in Table3. At 6.25µl, the sample shown 29.34 % inhibitory activity and maximum inhibitory activity of 90 % at 100 µl concentration.

Table3.

Sample	Concentration (µL)	OD at 515nm	% of Inhibition
Control at zero minute		1.548	
Control at 15 minute		1.537	
<i>P.niruri</i> CuO nanoparticles	6.25	1.097	29.34
	12.5	0.980	36.96
	25	0.785	49.64
	50	0.438	72.22
	100	0.164	90

CONCLUSION

This study offers an economic and ecofriendly method to synthesize CuONps using *P.niruri* as a good reducing and stabilizing agent. The absorption peaks at 202 nm in the UV-visible spectroscopy confirmed the formation of CuO nanoparticles. The active compounds responsible for the formation of nanoparticles were explained by FT-IR spectroscopy. The antibacterial activity of copper oxide nanoparticles were analyzed with *E.coli* and *S. aureus*, high antibacterial activity was observed against both the bacteria. It also proved to have excellent antifungal activity. The copper oxide nanoparticles have good potential of antioxidant activity and hence used for biomedical applications.

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