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In Vitro Ecofriendly synthesis of Copper Nanowires from the leaf extract of *Leucaena leucocephala* and its Antibacterial activity

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ABSTRACT: The eco-friendly method for the synsthesis of CuO nanoparticle by using leaf extract of *Leucanena leucocephala*. The formation and characterizations of the synthesized nanomaterial was monitored by UV- Visible spectrophotometer, SEM and EDAX. Investigation on the antibacterial effect of the synthesised CuO nano particles were performed against, pathogenic bacteria *Esherichia Coli*, *Staphlococcus aureus* reveals high efficacy as a strong antibacterial agent.

I. INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles usually referred to as particles with a size approximately extending from 1 nm up to 100 nm in length in at least one dimension[1] , exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The application of nanoscale materials and structures is an emerging area of nanoscience and nanotechnology. In recent years, much attention has been paid to metal nanoparticles, which exhibit novel chemical and physical properties owing to their extremely small dimensions and high specific surface area.

Copper (Cu) is a transition metal with a distinct red-orange colour and metallic luster having atomic number 29 and atomic mass 63.546. It is relatively more abundant metallic element of the Earth's crust (the 8th) having special properties of high electrical conductivity, high thermal conductivity, high corrosion resistance, good ductility and malleability, and its reasonable tensile strength makes it an essential element to the functioning of society and has played several important roles in society for thousands of years. Copper is a good conductor, can joined to itself very easily and has better corrosion resistance and is a more abundant hence cheaper material to use. This properties, has made copper the number one material used in modern household water piping and associated plumbing and the metal of choice for most vehicle radiators and air conditioners.

Leucanena leucocephala has a local name wild tamarind. It is Fabaceae – mimsoideae family. Its leaves have a high nutritive value. It is used as forage food fodder. Biosynthesis of metal nanoparticles by plant extracts is currently under exploitation. The uses of *Azadirachta indica* (Neem)[2], *Emblica officinalis* (amla, Indian Gooseberry)[3], mangostem leaf[4],*Chenopodium album*[5,] have already been reported. Studies have indicated that biomolecules like proteins, phenols, and flavonoids not only play a role in reducing the ions to the nanosize, but also play an important role in the capping of the nanoparticles [6].

In our paper in vitro ecofriendly synthesis copper nanowire from the leaf extract of *Leucanena leucocephala* and their potential application in antibacterial effect against clinically isolated pathogens.

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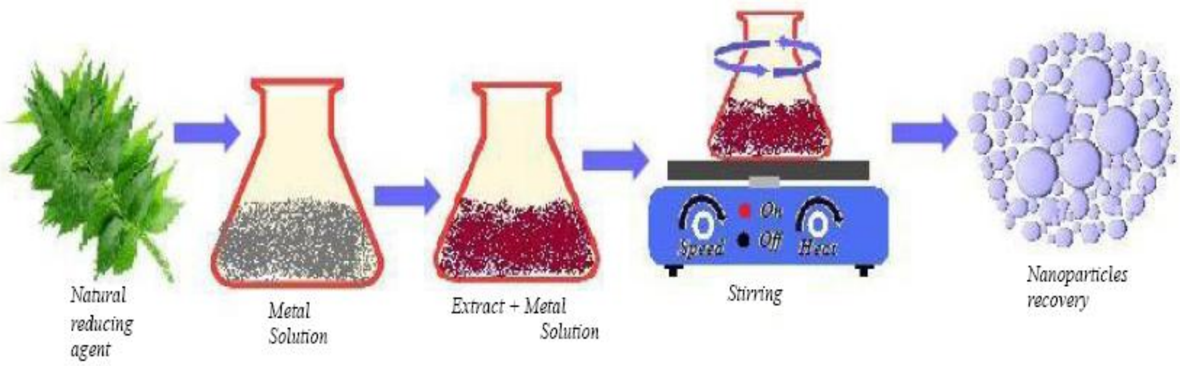
II. MATERIALS AND METHOD

A. Preparation of plant leaf extracts

25 gm fresh leaf of *Leucaena leucocephala* plant were separately washed thoroughly with de-ionized water to remove dirt particles if any adsorbed on the surface of the leaves and the washed samples were air-dried. The dried leaves were crushed with mortar and pestle. The mashed sample of fresh leaves was then mixed with 100 ml of sterile double distilled water (DDW) in a 250 ml Erlenmeyer flask and kept at 65^{0}C for 30 min and then filtered off using Whatmann No.1 filter paper. The resulting sample leaf extract was stored at 4^{0}C[7].

Fig 2.1 Process involved in nanoparticles synthesis by plant extract



B. Biosynthesis of CuO nanoparticles

In a typical synthesis of copper nanoparticles, 10 ml of fresh leaf extract was added to 100 ml of 0.01 M $CuSO_4.5H_2O$ aqueous solution and the mixture was kept at 56^{0}C with constant stirring on a magnetic stirrer for 6h. The suspension produced was centrifuged at 3000 rpm for 10 min and the supernatant liquid was decanted off and the residue was repeatedly washed with 10 ml of de-ionized water. Centrifugation-decantation-washing processes were repeatedly done six times to remove impurities if any on the surface of the copper nanoparticles. The obtained precipitate was dried in an oven at 50^{0}C for 24h. The as-synthesized copper nanoparticles were then kept for further characterization by FTIR, XRD and antibacterial studies.

III. METHODS OF CHARACTERIZATION

A. UV-Visible Spectra Analysis

The CuO nanoparticles were characterized by UV-Visible spectroscopy, one of the most widely used techniques for structural characterization of CuO nanoparticles. The visual observation showed colour change in reaction mixture (metal ion solution + leaf extract). UV-Visible absorption spectrophotometer with a resolution of 1nm between the range 200 and 800 nm was used. The CuO nanoparticle examined by measuring the UV-Visible spectrum of the reaction mixture on diluting a miniature amount of the sample into glass distilled water. 1 ml of the sample was pipetted into a test tube and diluted with 2ml of deionized water and subsequently analysed at room temperature. UV-Visible spectral analysis has been done by using a SANYO SP65 GALANAKAMP, UK spectrophotometer.

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B. SEM and EDAX analysis

Scanning electron microscopy (SEM) analysis was carried out using JEOL JSM 6390 MODEL, JABAN model machine. The SEM was used to observe the size, shape and morphology of the CuO nanoparticles. Thin film of the sample was prepared on carbon coated tape by adhering small amount of dried fine powder of the sample on the grid, the extra sample was removed with the help of blotting paper and the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The SEM analysis was used to determine the structure of the reaction products during biosynthesis of CuO nanoparticles. EDAX (Energy Dispersive Analysis of X Rays) analysis was conducted with the same instrument to confirm the elemental composition of the synthesized CuO nanoparticles.

C. Antibacterial Activity Studies

The antibacterial assays were done for Gram-negative *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus* by paper disc diffusion method. Nutrient agar media were used to cultivate bacteria.

D. Preparation of inoculums

The test bacterial strains were transferred from the stock cultures as streaked on Nutrient Agar (NA) plates and incubated for 24h. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved nutrient agar that was cooled to about 45^0C in a water bath mixed by gently swirling the flasks. The medium was then poured to sterile Petri plates, allowed to solidify and used for the biotest [8].

A fresh culture of inoculums of each culture was streaked on nutrient agar media in a petri dish. 50µl aliquots containing 5 mg/ml as-synthesized CuO nanoparticles were impregnated using micropipette on paper discs of 6 mm in diameter.

E. Preparation of test solutions

The sample was prepared for bacterial test and labeled for *Leucaena leucocephala* mediated CuO nanoparticles. Then the sample of the as-synthesized CuO nanoparticles solution was prepared at concentration of 5 mg/ml by dissolving them in DMSO (dimethylsulfoxide). Zones of inhibition were measured after 24h of incubation. The magnitude of antibacterial effect against, gram negative *Escherichia coli* and gram positive bacteria *Staphylococcus aureus* was determined based on the inhibition zone measured in the disk diffusion test.

IV. RESULTS AND DISCUSSION

A. UV-Visible Absorption Spectroscopic Study

UV-Visible spectra of aqueous $CuSO_4.5H_2O$ mediated with Leucaena *leucocephala* leaf extract are shown in Figure 4.1. CuO nanoparticles using leaf extract were indicated by the change of color from greenish to brown. The prepared CuO nanoparticles using the leaf extract was observed 557 nm (Surface Plasmon absorption peak)[9].

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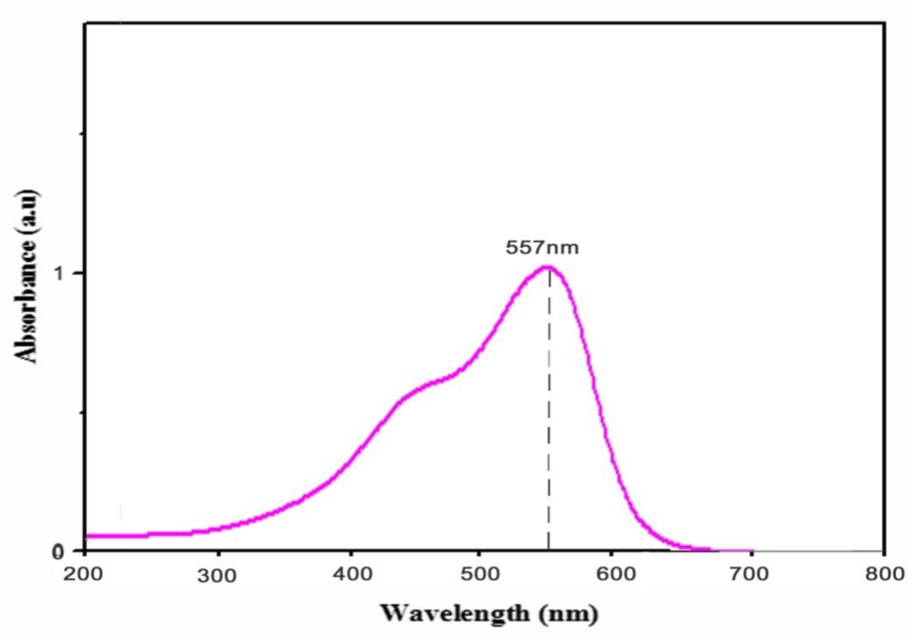
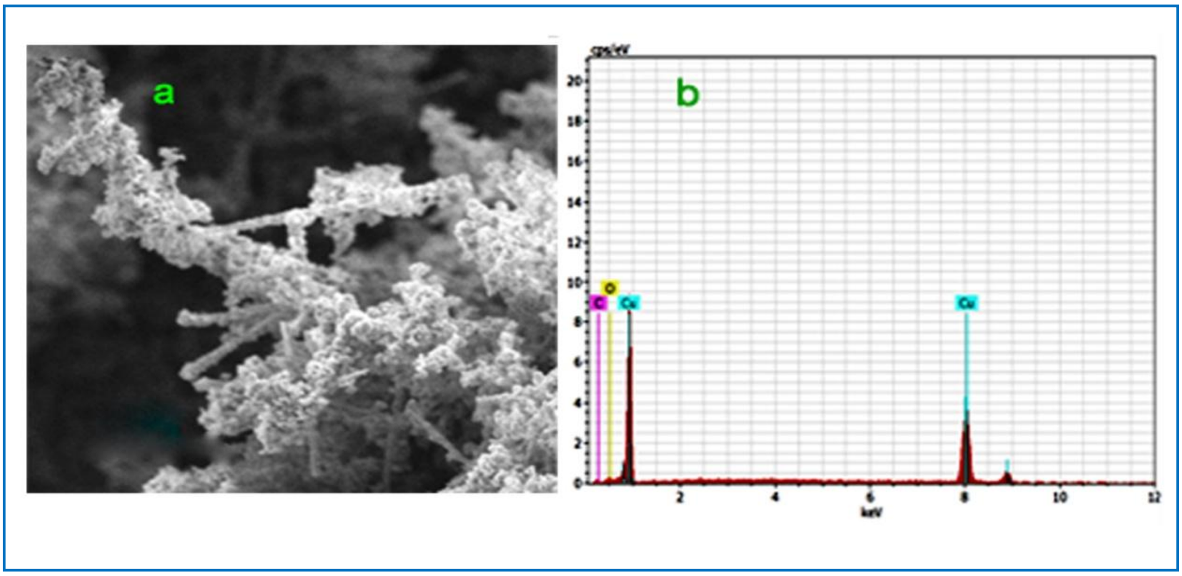
Figure 4.1 UV-visible spectra of aqueous copper sulfate pentahydrate solution mixed with *Leucaena leucocephala*.



B. Morphological analysis

To study the structural morphologies of CuO nanoparticles, the scanning electron microscopy (SEM) technique was applied and the obtained morphological images of corresponding nanoparticles were depicted in Figure 4.2a. The prepared CuO nanoparticles were composed of wires (nanowires) and each nanowire exhibited average length of 85nm. For the confirmation of elements present in the prepared CuO nanoparticles (nanowires), EDAX (Energypersive Analysis of X Rays) analysis was carried out and depicted in Figure 4.2b.The presence of Cu and O element in the obtained results ensures the prepared nanoparticles (nanowires) omposed by CuO.

Figure 4.2 (a) SEM and (b) EDAX analysis of CuO nanoparticles.



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C. Antibacterial Activities of CuO Nanoparticles

The antibacterial activities of *Leucaena leucocephala* leaf extract mediated CuO nanoparticles were performed against two pathogenic bacteria, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* using the paper disk diffusion method. The values of zone of inhibition (mm) sample of the as-synthesized CuO nanoparticles are presented in Table 4.1.

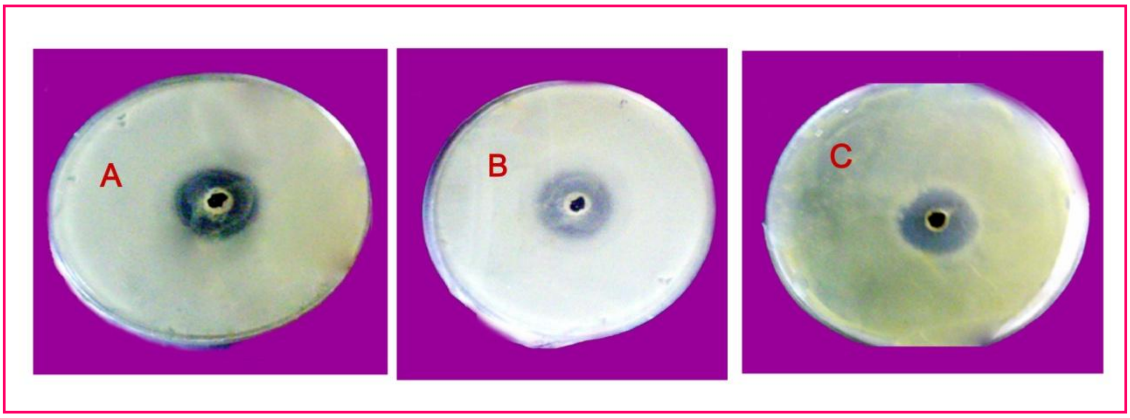
Table 4.1 Zone of inhibition (mm) of *Leucaena leucocephala* mediated CuO nanoparticles

Sample	Test Organism	Zone of inhibition (mm)
Concentration of CuO nanoparticles (50µl)	Standard (50 µg) (Ampicillin)	10.27
	Escherichia coli	12.33
	Staphylococcus aureus	15.25

Although the as-synthesized CuO nanoparticles of the present study are less active against *Escherichia coli* and *Staphylococcus aureus* as compared to the reference standard drug, but still they exhibited good antibacterial activity (Table 4.1). Results shown that the test solutions are significant indicating that CuO nanoparticles exhibit good biocidal activity. This corroborates with the previous observations of other researchers[7].

The study shows that CuO nanoparticles synthesized *via* green route are promising antibacterial agent against the pathogens which are highly toxic to multidrug resistant bacteria hence have a great potential in biomedical applications. There is a variation in the measured zones of inhibition as a function of applying concentrations of CuO nanoparticles suspension and nature of the bacteria employed. These results clearly demonstrate that the as-synthesized CuO nanoparticles are promising antibacterial agents against the pathogenic bacteria.

Fig 4.3(A) Standard (Ampicillin), (B) *Escherichia coli* (C) *Staphylococcus aureus*



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V. CONCLUSION

In conclusion, this greener approach toward the synthesis of CuO nanoparticles, using plant leaf material, has many advantages such as ease with which the process can be scaled up, economic viability, environmentally benign and renewable, there is no need to use high pressure, energy, temperature and toxic chemicals. Applications of eco-friendly CuO nanoparticles in bactericidal, wound healing and other medical and electronic applications are potentially exciting for their large-scale synthesis. Toxicity of CuO nanoparticles on human pathogen bacteria opens a door for new range of antibacterial agents.

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