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Synthesis of copper oxide nanoparticles using leaf extract of *Simaroubaglauca* and its antibacterial study

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The present study is aimed at synthesizing extract CuO NPs using *Simaroubaglauca* plant by green synthesis method. The synthesized CuO NPs have been characterized by XRD, FTIR, SEM, EDAX and HR TEM techniques. XRD analysis showed that the particles are crystalline and monoclinic in nature. The average size of the particle is 31.1 nm. SEM images indicate that the particles area almost spherical in nature with some agglomeration. EDAX analysis and HRTEM confirmed the formation of CuO NPs. FTIR results indicated the presence of phytochemicals present in the plant extract, which is responsible for the formation of CuO NPs. Further, the antibacterial study of CuO NPs against four human pathogenic bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* is carried out. The study showed that CuO NPs have significant antibacterial property against these human pathogenic bacteria.

Keywords: Antibacterial study, Copper oxide nanoparticles Green synthesis, *Simaroubaglauca* leaf extract

The green synthesis of nanomaterials of different sizes and shapes are attractive because of its advantage over other methods such as cost-effectiveness, non-toxicity and lower reaction temparatures[1-2]. *Simaroubaglauca*, commonly known as "The Paradise Tree" or "King Oil Seed Tree" or "LaxmitaruTree", belongs to the family of *Simaroubaceae*. *Simaroubaglauca*, has got critical attention due to its medical and pharmacological interactions like antidysenteric, haemostatic, antipyretic, antihelmentic, anticancerous and antiparasitic. The fruit, pulp, leaf and seed of *S.glauca* possess also exhibit analgesic, antiviral, stomachic, tonic, vermifuge and emmenagoguephysiognomy[3]. The leaf extract of it consists of calcium oxalate, Alkaloids, Carbohydrates, Proteins, Terpenoids, Steroids, Saponins, Phenolic compounds and tannins[4]. Among this, the phenolic compounds can be used for the production of metal ion and formation of CuO nanoparticles under appropriate conditions[5].CuO nano particles has got wide range of applications in technology and development also, especially in high-temperature superconductors, gas sensors, wood preservatives and solar cells[6]. Further, it can be used as an antimicrobial, antibiotic and antifungal agent when incorporated with plastics and textiles[7]. Here, the present study reports the synthesize CuO nano particles (CuO NPs) from the aqueous leaves extract of *Simaroubaglauca* plant. The investigation is mainly focused on the biomolecules responsible for the development of CuO NPs and its antibacterial properties. The as obtained CuO NPs were analyzed by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction analysis (XRD), High-resolution transmission electron microscopy(HR-TEM), X-ray photoelectron emission spectra and Scanning electron microscopy(SEM) and energy-dispersive x-ray spectroscopy (EDS). Besides, the antibacterial features of CuO NPs were also discussed using disc diffusion method.

Experimental Section

Preparation of *S.glauca* Leaf Extract

To prepare the leaf extract of *S.glauca* plant, leaves (10g) were thoroughly washed using distilled water, dried and finely chopped. The finely chopped material was allowed to boil for 10-15 min at 80°C with 100 mL of de-ionized water in a 250 mL Erlenmeyer flask and then cooled down to room temperature. The resulting solution is passed through a filter paper to remove any solid particles and then again filtered through Whatman filter paper (Fig. 1). 100 mL of *S.glauca* plant extract was added dropwise into 100 mL of 0.1 mm aqueous solution of copper

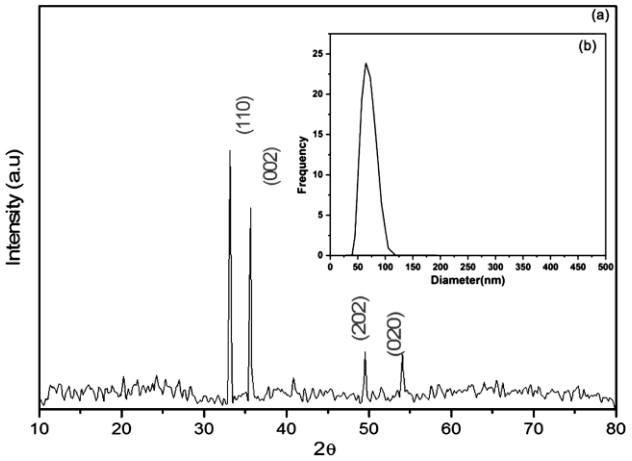

Fig. 1(a) — XRD pattern of CuO NPs indicating the growth of particles in monoclinic cristal structure 2(b) Particle size distribution estmated using Zeta potential.

nitrate with constant stirring at 85°C for 1hr to 2 h. The color of the mixture gradually changed from bluish to brownish black, indicating the formation of CuO NPs. The formed brownish black precipitate is allowed to settle down, filtered and is subjected to microwave heating at 900 W for 10 min. It is grounded to fine powders for further analysis. XRD analysis of CuO NPs was investigated with Bruker AXS D8 Advance diffractometer using Cu-kα radiation (λ=1.5406 Å) and 2θ ranges from 10° to 80°. The FTIR spectra analysis was done by using FTIR spectrometer (Thermo Nicolet, Avatar 370) in the range of 4000-400 cm^{-1} with resolution 4 cm^{-1}. The morphology, size and chemical composition were studied using SEM-EDAX (JEOL Model JSM 6390LV with OXFORD XMX N) equipment.HR-TEM images were obtained using a Joel/JEM 2100 instrument. The X-ray photo- electron spectra (XPS) is recorded by equipment Thermo Scientific Multilab2000callibrated with a standard Mg Kα (1253.688eV) radiation. The CuONPs synthesized using *S.glauca* leaf extract were tested for antibacterial activity by disc diffusion method against four human pathogenic bacteria such *as Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* (Gram negative) and *Staphylococcus aureus* (Gram positive). All the bacteria were refined in nutrient stock and kept up in new arrangements of 24 h. Nutrient agar plates were swabbed with sterile swabs dunked in cultures. Sterile Whatman filter paper No. 1 plates were acquainted with 1% w/v fluid arrangement of the particles and incubated under steady overnight to impregnate the discs with the particles and dried under laminar airflow. The dry

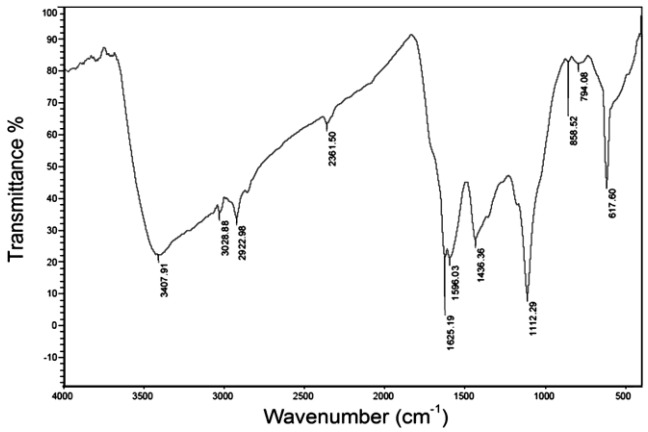

Fig. 2 — FTIR spectrum of biosynthesized CuO NPs.

discs with the impregnated particles were put on the swabbed plates before incubation tenderly squeezed against the agar surface. The control set and the test set plates were put under incubation for 24 h at 38°C. The plates were observed for the zone of inhibition and the diameter was noted.

Results and Discussion

Structural studies and vibrational spectra

The X-ray diffraction pattern of CuO NPs is displayed in Fig. 2(a), the Bragg's reflections at the positions 33.19°, 35.67°, 49.50° and 54.08° are indexed as the diffraction pattern from the planes of (110), (002), (202) and (020) respectively(ICDD: 89-5895) and are indexed in the figure. The results affirm the formation of CuO NPs in monoclinic crystal structure[8]. Using the Debye Scherrer equation, the mean crystalline size (D) of the CuO NPs was calculated.

$$D=0.9\lambda/(\beta\cos\theta) \quad \ldots (1)$$

where λ is the wavelength of X-ray radiation, β is the full width half maximum(FWHM) of the peaks at diffraction angle θ. The estimated average crystalline size of CuO NPs is31.1nm and this indicates its nano-crystalline nature[10]. Fig. 1(b), the inset, show the particle size distribution anlyzed using Zeta potential analyzer and the average particle size was found to be 71.27 nm.

The FTIR spectra was recorded in the range of 400-4000 cm^{-1}. Fig. 3 represents FTIR peaks of CuO NPs at 3407 cm^{-1}, 3028 cm^{-1}, 2922 cm^{-1}, 2361 cm^{-1}, 1625 cm^{-1}, 1596 cm^{-1}, 1436 cm^{-1}, 1596 cm^{-1}, 1436 cm^{-1}, 1112 cm^{-1}, 858 cm^{-1}, 794 cm^{-1} and 617cm^{-1}. At 3407 cm^{-1}, the broad absorption band is mainly arouse fromhydrohyle groups present on the surface of CuO nanostructures[11]. This suggest that water-soluble

antioxidant phenolic compounds, especially flavonoids of *S.glauca* leaf extract were responsible for the production of CuO NPs[12]. The vibrations located at 3028 cm^{-1} and 2922 cm^{-1} are C-H stretching vibrations, whereas the presence C≡N attributed to the absoption at 2361cm^{-1}.The peaks at 1625 cm^{-1} represent carbonyl group (C=O) and the presence of reducing sugar like fructose & sucrose is represented by peaks 1596 cm^{-1} and 1436 cm^{-1}. These peaks also suggested the presence of flavonoids and other phenolics in the plant leaves extract which could be responsible for the metal ions reduction and formation of the corresponding metal NPs[13]. The absortption at 1112 cm^{-1} is due to C-F stretching vibrations. The existence of bands at 858 cm^{-1} and 794 cm^{-1} is due to C-Cl absorption.The band at 617 cm^{-1} is recognized to Cu-O absorption and confirms the formation of CuO NPs.

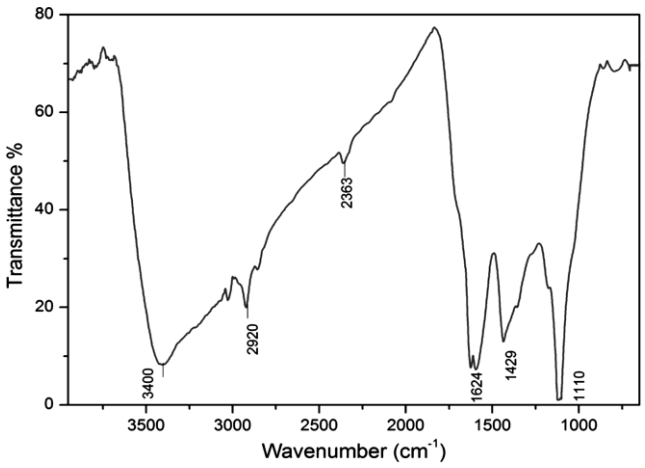

Fig. 3 — FTIR spectrum of *S.glauca* leaf extract.

Surface morphology and chemical compostion

SEM analysis, diaplyaed in Fig. 4(a) showed the morphology and structural properties of CuO NPs, the desnse growth of spherical shaped CuO NPs is identified from the images. Energy dispersion X-ray spectroscopy(EDAX) is shown in Fig 4b demonstrate the chemical composition in the samples and the constitents was composed of 51.48% for Cu and 20.11% for Oxygen. During sample preparation,

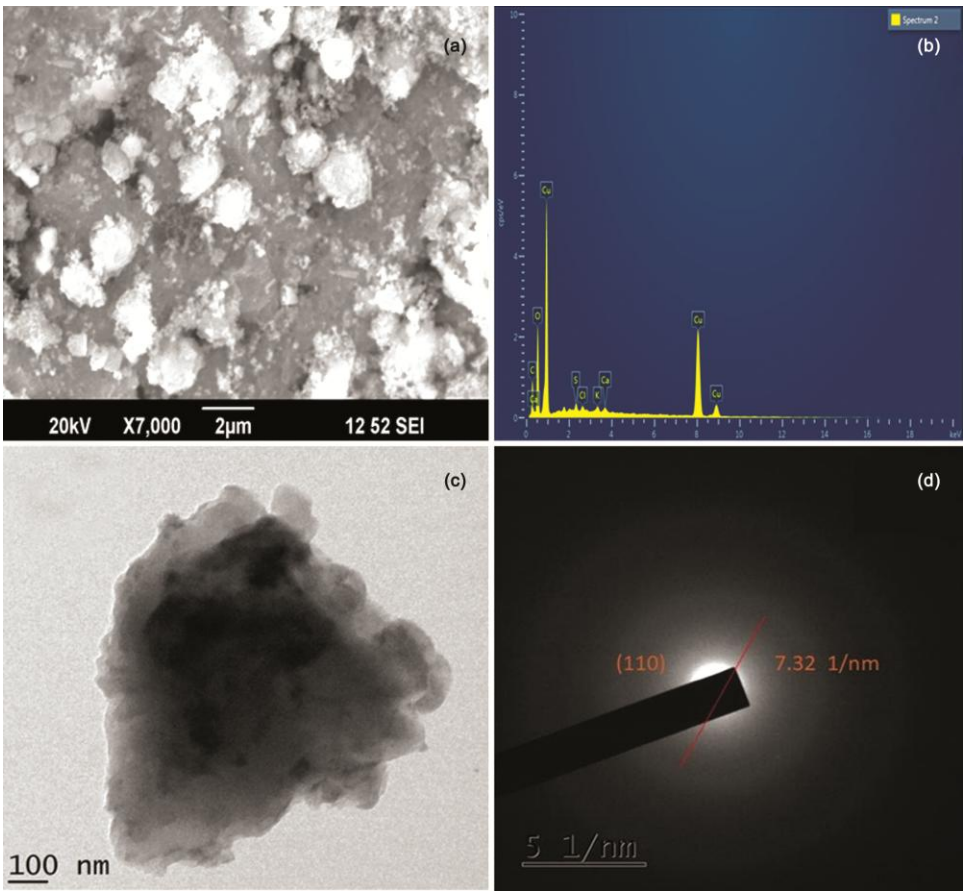

Fig. 4 (a) — SEM images of CuO NPs, (b) EDAX analysis of CuO NPs, (c) HRTEM image of CuO NPs and (d) SAED pattern of CuO NPs.

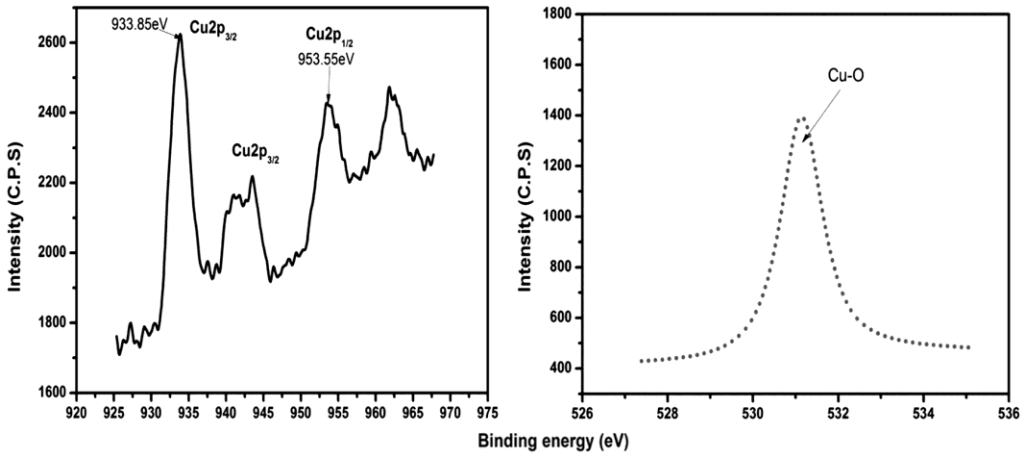

Fig. 5 — XPS of CuO NPs showing the presence of Cu and O1s, the symmetry in peak shape confirm the Cu ions are of single oxidation state

excess oxygen is present due to the physical absorption of oxygen from the environment[13]. Cl is present in the species as a result of the plant-mediated process.The protein capping over the CuO NPs may be responsible for the presence of sulfides[15].

HR-TEM image (Fig 4c) indicates agglomerated CuO NPs which are non uniform spherical in shape. SAED pattern of CuO NPs obtained using *S.glauca* leaf reveals that different diffraction rings of monoclinic CuO NPs are present in the sample[16]. The diffraction rings are prominently from planes (110), (200), (221) and (221), that corresponds to CuO. The measured d-spacing of 0.273 nm was found to be in good agreement with a calculated d spacing of (110) plane, i.e 0.27 nm[17]. The XPS spectra recorded and shown in Fig. 5, the presence of Cu and Oxygen are idenfied prominently. In figure, the binding energies 933.5eV and 953.5 eV are the core level emission of electron from Cu2p3/2 and Cu2p1/2 states of CuO. The Cu2p3/2 at 933.5 has a satellite peak with doublet at 942 and 944 eV. The symmetry in peak shape suggest the Cu ions are in its most stable 2+ oxidation state. The binding energy curve at 539 eV corresponds to O1s, this is also found to be in sigle electronic state with copper[18].

Antibacterial activity

The antibacterial activity against four human pathogenic bacteria such *as Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* are studied using disc diffusion method. *Staphylococcus aureus* is Gram +ve bacteria and *as Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* are Gram-ve bacteria. The results are shown in Table 1. *Staphylococcus aureus* (12 mm)

Table 1 — Zone of Inhibition

Sample	Control zone Diameter (mm)	Test zone Diameter (mm)	MIC (µg/mL)
Escherichia coli	0	11	30
Staphylococcus aureus	0	12	60
Pseudomonas aeruginosa	0	4	120
Proteus mirabilis	0	10	90

ishaving the highest zone of inhibition(ZOI) followed by *Escherichia coli* (11 mm), *Proteus mirabilis* (10 mm) andleast was noticed for *Pseudomonas aeruginosa* (4 mm).The discs filled with corresponding bacteria alone,which is taken as control, did not show any zone of inhibition. From the obtained results, Gram-ve bacteria seemed to have more resistance towards bacteria than Gram +ve bacteria.This is because, Gram-ve bacteria have an outer membrane which is made largely of lipopolysaccharides,make it difficult for the NPs to penetrate inside,destroy the cell wall,which then leads to cell death[18-20]. Gram +ve bacteria doesn't have an extra outer membrane.

Conclusion

In this work, CuO nano particles were synthesised from the leaf extract of *Simaroubaglauca*. The structural analysis of the compound confirm the monoclinic crystal structure of CuO powders and vibrational analysis shows the presence Cu and O prominently and energy dispersive spectra also suggest the same result, however the presence of trace elements and impurities also found in the species, however the method is cost effective Surface studies reveals that the compounds are composed of spherical nano particles. Antibacterial study proved that CuO

nano particles can be a promising candidate for medical and pharmaceutical applications.

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