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Green synthesis of copper oxide nanoparticles using *Cinnamomum malabatrum* leaf extract and its antibacterial activity

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Development of green nanotechnology has attracted significant attention of researchers towards the eco-friendly green synthesis of nanoparticles. The present study reports a green synthesis of copper oxide nanoparticles (CuO NPs) using the aqueous leaf extract of *Cinnamomum malabatrum* in appropriate conditions. It is observed that the leaf extract can act as reducing and stabilizing agents to synthesize CuO NPs. The synthesized NPs are characterized using XRD. The XRD pattern clearly suggests the crystalline nature and monoclinic structure of the synthesized composition. The average crystalline size is calculated using Debye-Scherrer relation and it was found to be 17nm. The FT-IR analysis is carried out in order to investigate the biomolecules present in the synthesized CuO NPs. The presence of CuO NPS is confirmed with EDX. The TEM and SEM images reveal the spherical shape of the NPs and the size of the NPs calculated is consistent with XRD results. The antibacterial activity of these NPs is tested against four human pathogenic bacteria both Gram-positive (*S.aureus*) and Gram-negative *(E.coli, P.aeruginosa*, and *P.mirabilis*). The synthesized CuO NPs show significant antibacterial activity against all the four bacteria.

Keywords: Antibacterial activity, *Cinnamomum malabatrum,* CuO NPs, Green synthesis

In recent years, nanotechnology has attracted significant attention of researchers from various fields including biotechnology, physics, chemistry, material sciences, engineering and medicine as it is a promising area of the scientific and technological development for future. Nanotechnology is a deliberate manipulation of atoms and molecules to form structures on the scale of nanometres [one billionth of a metre]. It is a vast and rapidly growing field. In general, the size of a NP spans the range between 1 and 100 nm. These NPs typically exhibit unusual and fascinating properties or behaviours due to their extremely small size [1].

Out of different NPs, transition metal oxide NPs have broad applications in many areas. The copper oxide NPs have been of great interest due to their wide range of applications in high-T_c superconductors[2], sensors[3], catalysts[4], giant magnet resistance material[5], solar energy devices[6] and in medicine[7]. They are used as antioxidants, drug delivery agents and imaging agents in the field of biomedicine[8]. Further CuO NPs are strong antimicrobial agents and they are introduced in textiles and coatings for the development of different types of wound dressings and textiles. The antimicrobial property of CuO NPs is considered to be one of the important properties, which might have a lot of potential in animal and human medicine[9]. CuO NPs are stable, robust and their shelf life is longer when compared with organic antimicrobial agents[10].

Green synthesis indicates the utilization of biological routes, for example, those including microorganisms[11], plants[12] and so on for the synthesis of nanoparticles. Green synthesis is regarded as a better alternative to physical and chemical methods as it is easy, efficient, eco-friendly, cost-effective and free of chemical contamination for medical and biological application where purity is a factor of serious concern. Green synthesis of NPs using leaf extract have been a developing area of research and is favourable over other environmentally benevolent biological processes as it eliminates the elaborate processes of maintaining cell culture and it can also meet large scale production[13-15]. *Cinnamomum malabatrum* is a tree distributed in south India and endemic to the region. It belongs to the family Lauraceae. The essential oil collected from the leaves has been shown to be antioxidant[16], antibacterial[17], antifungal[18] and antidiarrheal activity[19]. The phytochemical examination of the leaves reports the presence of phenolics and flavonoid[20].

The present study reports the synthesis and characterization of CuO NPs using *Cinnamomum malabatrum* leaf aqueous extract. The obtained NPs were analyzed by X-Ray Diffraction (XRD), Fourier Transform Infrared (FT-IR) Spectroscopy, Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) and Transmission Electron Microscopy (TEM). Further, the antibacterial activity of the synthesized CuO NPs was examined against four human pathogenic bacteria both Gram-positive (*S.aureus*) and Gram- negative (*E.coli, P.aeruginosa* and *P.mirabilis*) bacteria using Agar well diffusion method.

Experimental Section

Experimental site

Cinnamomum malabatrum leaves were collected from Kozhikode district, Kerala during the month of November 2018. Healthy leaves were selected and separated from the rest. XRD, FTIR, SEM with EDX and TEM measurements of the synthesized product were done at Sophisticated Test & Instrumentation Centre (STIC), Cochin University Campus, Kerala.

Preparation of Cinnamomum malabatrum leaves extract

The selected leaves were washed in running tap water several times and then washed with distilled water 2-3 times in order to remove dust particles. About 10 g of chopped leaves were weighed and taken in a beaker of 200 mL. Distilled water (100mL) was added to it. It was then boiled at 85°C for 15-20 min. By this time the aqueous part turns pale yellow. After cooling to room temperature the extract was filtered through Whatman no.1 filter paper. The filtrate was collected as an extract.

Synthesis of CuO NPs

About 1.8756g of Copper(II) nitrate trihydrate salt was weighed accurately and made up to 100 mL in a standard flask in order to prepare 0.1M copper nitrate solution. To synthesize CuO NPs 100 mL plant extract was added to 100 mL 0.1M copper nitrate solution and stirred continuously for 1 h at 85°C by using magnetic stirrer. By this time the colour of the solution was green and the brownish black coloured precipitate was settling down. The precipitate was collected and washed repeatedly with distilled water to remove the impurities. The obtained precipitate was dried and grounded using pestle and mortar. The obtained black coloured CuO NPs were stored in a properly labeled airtight container for further characterization.

Characterization

The XRD pattern of the synthesized CuO NPs was recorded on a Bruker AXS D8 Advance X-ray diffractometer with a 2θ range from 10° to 80° with high-intensity Cu Kα radiation (λ=1.5406Å). The FTIR spectra analysis was on a Thermo Nicolet Avatar 370 spectrometer with 4 cm^{-1} resolution in the range of 4000-400 cm^{-1}. Scanning Electron Microscope was recorded by JEOL model JSM-6390 LV and Energy Dispersive X-ray analysis was done by OXFORD XMXN. The structural characterization of the CuO NPs was analyzed by HR-TEM operating at an acceleration voltage of 200kV (Jeol/JEM 2100). Additionally, the antibacterial activity of the synthesized CuO NPs against various bacterial pathogens both Gram-positive (*S.aureus)* and Gram-negative (*E.coli, P.aeruginosa* and *P.mirabilis*) were investigated.

Determination of antibacterial activity

The antibacterial activity of synthesized CuO NPs was tested against four human pathogenic microbes *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis*. The antimicrobial test was done using Agar well diffusion method. For this, all bacteria were cultured on nutrient broth and maintained in fresh sets for 24 hours. Nutrient agar plates were swabbed with sterile swabs dipped in culture. The particles were used to make 1mL of 1% w/v aqueous solution. 2mm wells were cut into the agar and 10 l of the solution was introduced into the test plate set. The control set and the test set plates were placed under incubation for 24 hours at 38°C. The antimicrobial activities were evaluated y measuring the zone of inhibition in millimetres (mm).

Results and Discussion

X-ray diffraction analysis

XRD pattern of the synthesized CuO NPs using the aqueous leaf extract of *Cinnamomum malabatrum* is shown in Fig. 1. It has shown a series of diffraction peaks at 2θ of 32.64°, 36.56°, 38.64°, 42.30°, 43.34°, 48.83°, 50.40° and 61.37° which were correlated with (110), (002), (111), (-202), (020), (202), (-113) and (113) planes respectively. The sharp peaks shown in the XRD pattern confirmed crystalline nature and the peak positions showed the monoclinic structure of CuO which was assured by the International Centre for Diffraction Data (ICDD) card no 89-5895[21]. The average crystalline size of

synthesized CuO NPs was calculated using Debye-Scherrer equation $D= K\lambda/\beta\cos\theta$, where K is known as Scherrer constant (shape factor), ranges from 0.9 to 1.0, λ is X-ray wavelength (1.54Å) and β is the full width half maximum (FWHM) of the peaks at the diffracting angle θ. The average grain size was estimated to be about 17nm.

FT-IR analysis

The FT-IR measurement was recorded in the range of 400-4000 cm^{-1} in order to estimate the biomolecules present in the synthesized product.

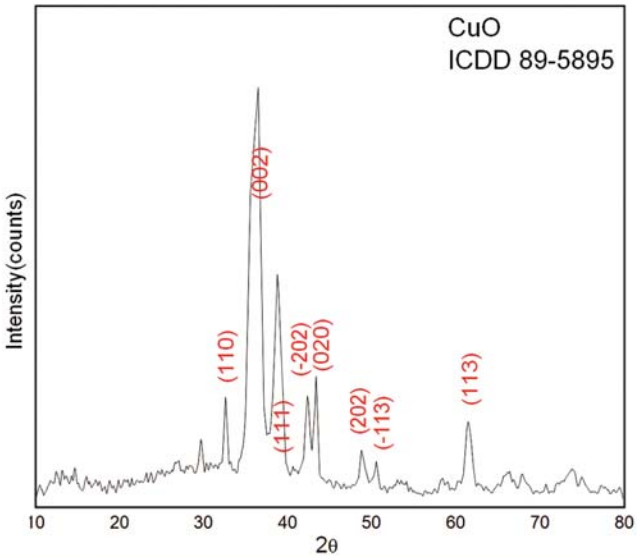

Fig. 1 — XRD pattern of the synthesized CuO NPs

Figure 2 illustrates the FT-IR spectra of synthesized CuO NPs using *Cinnamomum malabatrum* leaf aqueous extract. The peaks at the 3420.61 cm^{-1}, 2923.86 cm^{-1} and 2356.18 cm^{-1} can be attributed to a hydroxyl group (-OH) stretching, C-H stretching (sp^3) and N-H stretching vibrations respectively. The peaks at 2198.64, 1619.83 and 1596.20cm^{-1} are corresponding to C≡C stretching, C=O stretching and aromatic C=C stretching vibrations respectively. Additionally, the presence of peaks at 1426.14, 1113.91 and 853.19 cm^{-1} are associated with C=N stretching, C-O stretching and C-Cl absorption respectively. Further the peaks at 617.40 and 516.00cm^{-1} are due to the Cu-O stretching in the monoclinic structure of CuO NPs. These peaks confirm the presence of flavonoid and other phenolics in the plant leaves extract which could be responsible for the reduction of copper ions[13].

Scanning electron microscopy (SEM) analysis

The surface morphology of the synthesized CuO NPs was characterized by SEM technique at different magnification levels. The SEM images are shown in Fig. 3. The synthesized composition is heterogeneous in nature. The SEM images show the presence of some large granular particles which could be due to aggregation or overlapping of smaller particles with sizes around 100nm[4]. This could be due to nanoparticle oxidation[22].

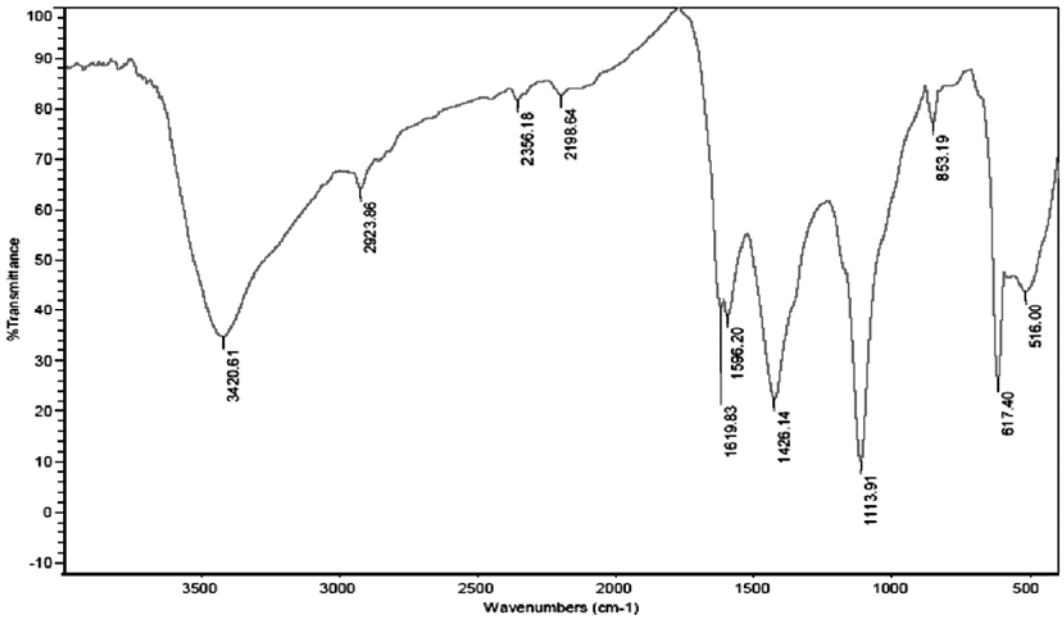

Fig. 2 — FT-IR spectra of the synthesized CuO NPs

Energy dispersive X-Ray (EDX) analysis

The chemical characterization of the synthesized CuO NPs was done by EDX analysis. The EDX spectrum of the synthesized CuO NPs is shown in Fig. 4 The EDX spectrum revealed the presence of carbon(C), oxygen(O), magnesium(Mg),aluminium(Al), silicon(Si), sulphur(S), chlorine(Cl), potassium(K), iron(Fe) and copper(Cu). The major constituents are Cu (51.86%) and O(21.71%). This confirms the presence of CuO NPs. The constituent elements are represented in Table 1. It is clear from Fig. 5, that the EDX spectrum confirmed the successful formation of CuO NPs with the aqueous leaf extract of *Cinnamomum malabatrum*. The presence of the other elements could be due to impurities from the leaf extract[23].

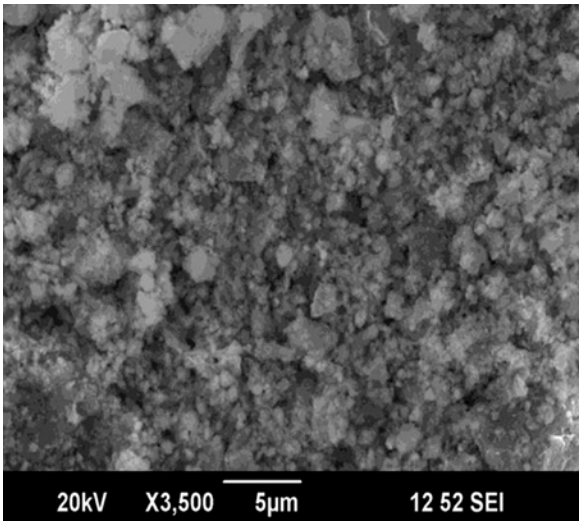

Fig. 3 — SEM images of the synthesized CuO NPs

HR-TEM Analysis

HR-TEM measurement has been carried out in order to investigate the structural characterization of the synthesized CuO NPs derived from *Cinnamomum malabatrum* aqueous leaf extract. Figure 5a shows the HR-TEM image of the synthesized CuO NPs and Figure 5b shows the selected area electron diffraction (SAED) pattern. The SAED pattern clearly suggests the polycrystalline nature of the synthesized CuO NPs. The SAED pattern has shown small spots making up rings which can be attributed to the diffraction of transmitted electrons through the monoclinic CuO NPs in different orientations[23]. The interplanar spacing (d-spacing) was calculated to be 0.271nm indicating the preferred (110) orientation. The diffraction rings correspond with planes (110),

Table 1 — Elemental composition of the synthesized CuO NPs

Element	Line Type	Wt%	Atomic%
C	K series	20.84	42.63
O	K series	21.71	33.33
Mg	K series	0.64	0.65
Al	K series	0.47	0.43
Si	K series	0.42	0.37
S	K series	0.43	0.33
Cl	K series	0.5	0.34
K	K series	2.61	1.64
Fe	K series	0.52	0.23
Cu	K series	51.86	20.05
Total		100	100

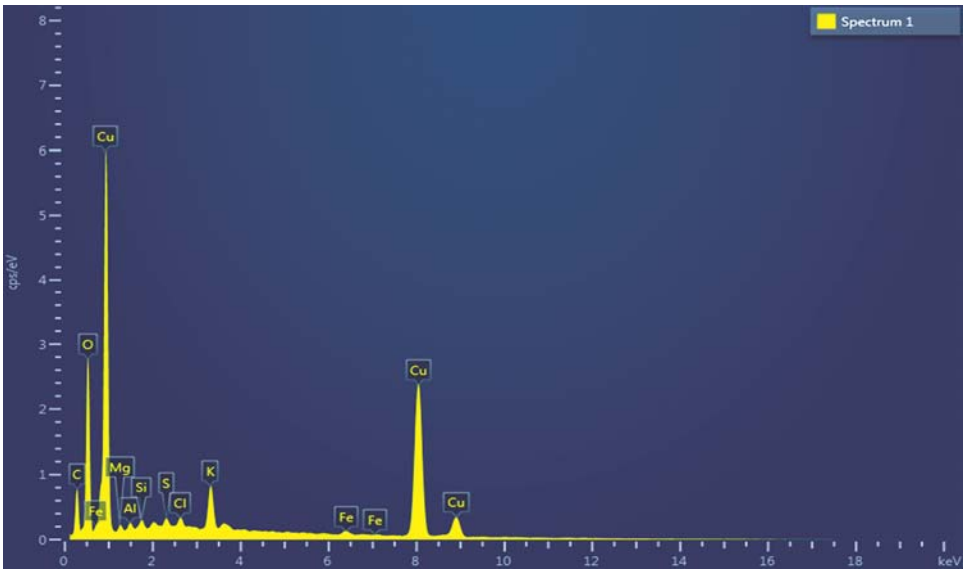

Fig. 4 — EDX spectra of the synthesized CuO NPs

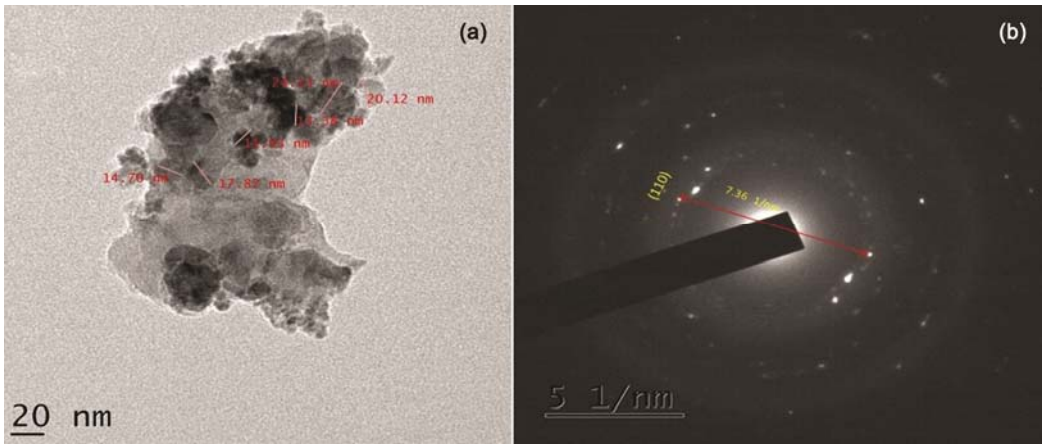

Fig. 5 — HR-TEM image of CuO NPs, 6b. SAED pattern of CuO NPs

Table 2 — Antibacterial activity of CuO NPs on pathogenic bacterial strains

Sample	Control zone Diameter (mm)	Test zone Diameter (mm)
Escherichia coli	6	32
Staphylococcus aureus	0	32
Pseudomonas aeruginosa	6	36
Proteus mirabilis	0	(Test 1) 12 (Text 2)12

(-112), (020) and (-113) are correlated for CuO NPs. The HR-TEM image showed spherically shaped CuO NPs with a size range of 11nm to 24nm.

Antibacterial activity

The antibacterial activity of the synthesized CuO NPs was tested against four human pathogenic bacteria both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis*) by Agar well diffusion method. In Agar well diffusion method the CuO NPs showed significant antibacterial activity against all four bacterial strains. These NPs show higher antibacterial activity against

Pseudomonas aeruginosa followed by *Escherichia coli, Staphylococcus aureus* and *Proteus mirabilis*. The zone of inhibition is given in Table 2. Usually, the size of the bacterial cells is in the range of micrometres. Those have cellular membranes containing pores, which is in the nanometre range. The antibacterial activity of the synthesized CuO NPs could be due to the fact that these NPs may have a size less than the pore size of the bacteria and consequently it is easy for the NPs to cross the cell membrane without any obstruction[24].

Conclusion

The present study reports for the first time green synthesis of CuO NPs by using aqueous leaf extract of *Cinnamomum malabatrum*. Green synthesis is the most promising strategy of nanoparticle synthesis in an economical and eco-friendly manner. The XRD analysis confirms the crystalline nature and monoclinic structure of the synthesized CuO NPs, and the average crystalline size was found out to be 17nm. FT-IR results show that the reduction and stabilization of NPs could be due to biomolecules present in the leaf extract. The morphology and size of the synthesized CuO NPs were investigated by SEM and TEM analysis. The chemical composition of the synthesized sample was investigated by EDX analysis. The EDX spectrum confirms the presence of CuO NPs. Further, investigations on the antibacterial activity of the synthesized CuO NPs against four human pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* reveals the high antibacterial efficiency of the synthesized CuO NPs.

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