ORIGINAL ARTICLE

Green Synthesis of Copper Oxide Nanoparticles Decorated with *Borassus flabellifer* Leaf Extract for Anti-bacterial and Antioxidant Applications

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Abstract

Introduction: Nanotechnology has provided a wide range of applications, including copper-containing nanoparticles (CuO NPs) for batteries, gas sensors, superconductors, solar energy conversion, food, pharmaceutical, agriculture, and organic-inorganic nanostructure composites. **Materials and Methods:** *Borassus flabellifer* fresh leaves were collected, ground into powder, boiled, centrifuged, and characterised using IR spectra, XRD pattern, TEM analysis and UV-Visible spectrophotometer. The antibacterial and antioxidant properties of CuO NPs were tested using the disc diffusion method, and the DPPH technique was used to calculate the scavenging potential. **Results and Discussion:** UV-Visible Spectroscopy was used to identify CuO NPs formation and to establish the presence of surface plasmon resonance (SPR). FTIR studies showed the presence of Hydroxyl/amines, C=O Carbonyl/amide groups, alkanes, phenols, carboxylic acid groups, and alcohols. X Zeta potential and TEM analysis were used to verify the size and shape of the produced CuO NPs. **Conclusion:** Using BF leaf extract, this article describes a green method for producing CuO NPs. CuO NPs were spherical in shape and demonstrated excellent antibacterial activity against both gram-positive and gram-negative bacteria, as well as significant antioxidant activity. These CuO NPs have the potential to be useful tools in biomedical research.

Key words: Anti-bacterial and antioxidant, *Borassus flabellifer*, copper oxide nanoparticles

INTRODUCTION

In the few decades, nanotechnology has provided a wide range of applications. Metal and metal oxide nanoparticles have applications in biomedical, chemical, physics, and electronics due to the large surface area to volume ratio of nanoparticles allows them to perform unique activities in optical, magnetic roles, catalysts, and sensing as well as biology.[1] Compared to their bulk material, the copper-containing nanoparticles exhibit special characteristics.[2] With a band gap of 1.7 eV, Copper Oxide nanoparticles (CuONPs) are p-type semiconductors.[3] CuONPs are therefore used as batteries, gas sensors catalysts, superconductors, solar energy conversion, food, pharmaceutical, agriculture, and organic-inorganic nanostructure composites.[4,5] The CuONPs also show promise as antibacterial, antioxidant, and anticancer medications.

The common techniques for the synthesis of CuONPs involve chemical or physical procedures such as sol-gel, hydrothermal, and solid-state reactions.[6] Although these techniques may be adjusted to generate well-defined nanoparticles with desired morphologies and sizes, the environment is put in danger by the hazardous solvents and byproducts.[7] The need for specialized equipment, such as ball grinding, ball milling, polyol method-irradiation, hydrothermal route, electrodeposition, and the high energy needs of some of these processes, such as the vapor transport method, which is effective at temperatures as high as 1,400°C, result in additional high costs of investment.[8,9] However, green synthesis, which involves using biomaterials as a reducing agent to create nanostructures, has become a popular method since it is simple, affordable, and safe for the environment.

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The green synthesis method requires less energy and less specialized equipment, which results in large cost savings.[10] In addition, they are quicker to react and more effective. To scale up such plant extract-based techniques for industrial manufacturing, further work must be done on them. Aloe vera leaves, tamarind leaves, Calotropis procera leaves cinnamon leaves, lemon grass leaves, Cassia auriculata leaves, and neem leaves are only a few examples of plants that have been used in the green synthesis of CuONPs.[11,12]

In this paper, we describe the green and cost-effective synthesis of CuONPs utilizing *B. flabellifer* (BF) leaf extract. The BF belongs to the family Arecaceae, and the nutritional and commercial relevance of this family has increased significantly.[13] Fruits and root tubers are very nutrient-dense in many different ways, including sugars, calcium, iron, and polyphenols. Many components, especially the leaves and bark, have significant commercial value.[14] To make thatch, hats, baskets, writing surfaces, and for weaving, people employ leaves.[15] The BF leaf extract has revealed that it contains a mixture of several groups such as cellulose, polyphenol pectin, pentosan sulfate, hemicellulose, and lignin.[16] BF plants have reportedly included biological processes and pharmacological purposes, including antioxidant, bactericidal, diuretic, and anthelmintic properties wound healing and immunomodulatory.

The present study focuses on the green synthesis and characterization of CuONPs made from BF leaf extract utilizing a reducing and stabilizing agent. Ultraviolet (UV), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and transmission electron microscopy (TEM) analyses were used to characterize the produced CuONPs. In addition, the synthesized CuONPs were tested for antioxidant and anti-bacterial activity.

EXPERIMENTAL

Materials and methods

Fresh leaves of BF were collected from our campus premises, Nalgonda University, Nalgonda, Telangana, India, and authenticated. Copper nitrate was purchased from Sigma-Aldrich; all reagents were purchased from the S-D fine chemicals and used without further purification.

Preparation of aqueous extract of BF

BF leaves were collected from our campus promises at Nalgonda University, Telangana, India. Green Fresh Leaves after being collected, the BF leaves were cleaned under running water, dried in the shade, and then stored. Using a mixer grinder, the dried leaves were ground into powder. In a 250 mL beaker, 1 g of dry leaves was boiled at 80 C for 30 min with 100 mL of double distilled (DD) water. Centrifugation at 10,000 rpm for 10 min removed the leaf debris from the solution, and the fresh supernatant recovered was utilized for nanoparticle creation.

Synthesis of CuONPs

The CuONPs were synthesized by a microwave-assisted method. 5 mL of copper nitrate in an aqueous solution at 8 mM purity was included in 10 mL BF leaf extract at room temperature and continuously stirred using a magnetic stirrer. The reaction mixture was subjected to microwave irradiation for 6 min at 600 W power. The color of the reaction mixture gradually shifted from bluish-to-black indicating the formation of CuONPs. The resulting precipitate was obtained by centrifugation and washed with ethanol and distilled water, respectively, and finally freeze-dried.[10]

Characterization

Different methods were used to characterize the synthesized CuONPs. A UV-Visible spectrophotometer was used to record the Ultra-Violate Visible absorption spectra (UV-3600, Shimadzu, Japan). The IR spectra were analyzed by IRAffinity-1 (Shimadzu), and the XRD pattern was on a Rigaku-Miniflex powder X-ray diffractometer with Cu-Ka radiation. A JEOL 2000 FX-II TEM was used to capture TEM pictures.

Anti-bacterial activity

The disc diffusion method was used to test the produced CuONPs' antibacterial capabilities. As model test strains Staphylococcus sp. and *Escherichia coli*, two Gram-positive and Gram-negative bacteria, respectively, were chosen. Prepared Luria-Bertani agar medium was added to Petri dishes that had undergone sterilization. Staphylococcus sp. and *E. coli* were dispersed on the Petri plates individually in a laminar air flow hood after the medium had a chance to harden. 15, 25, 35, and 50 µL (100 µg/mL) of the CuONPs applied to each well on the two plates using a micropipette. The discs were incubated at 37 C for 24 h while being air-dried in a laminar hood. The bacteria's zone of inhibition was then determined.[9]

Antioxidant capacity

Using the 2,2-diphenylpicrylhydrazyl (DPPH) technique, the ability of synthesized CuONPs to scavenge free radicals was determined. The color of the DPPH free radical is red, but when it becomes a hydrazine molecule, it changes to yellow. Under dark conditions, different CuONPs (20 µL, 40 µL, 60 µL, 80 µL, 100, and 120 µg/mL) were combined with a 0.1 mM DPPH solution in DMSO. It was possible to directly quantify the scavenging potential of the CuONPs after 15 min of incubation at room temperature by observing the decrease in color intensity at 517 nm. As the reference, ascorbic acid (12 mg/mL DMSO) was employed.[17]

DPPH scavenging effect (%) = $(Abs_{control}-Abs_{sample})/Abs_{control} \times 100$

RESULTS AND DISCUSSION

UV-Visible spectroscopy

CuONPs formation is primarily indicated by the combination of leaf extract and copper sulfate changing from blue to dark green following microwave irradiation. To identify this creation and to establish the presence of particular surface plasmon resonance (SPR), UV-visible spectroscopy analysis was used. Due to metal oxide's SPR, a broad peak in the UV-visible spectrum at 430 nm was observed.[18] This wavelength matched previously published values rather well. According to another study, the production of SPR at 415 nm can be used to monitor the synthesis of CuONPs [Figure 1]. The spherical form of CuONPs may be responsible for these fluctuations in the SPR, and the size distribution has an impact on both the redshift and the SPR.[2] Assuming that the synthesized CuONPs were spherical, a single SPR peak was seen in the current investigation.

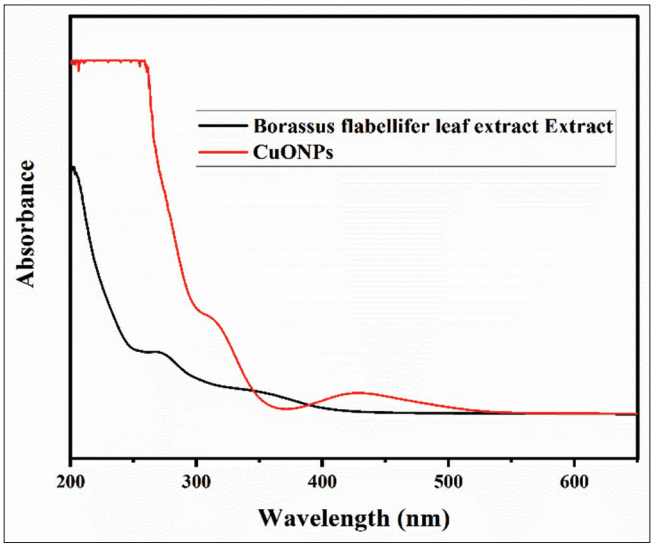

Figure 1: Ultraviolet-visible spectra of *Borassus flabellifer* leaf extract and copper oxide nanoparticles

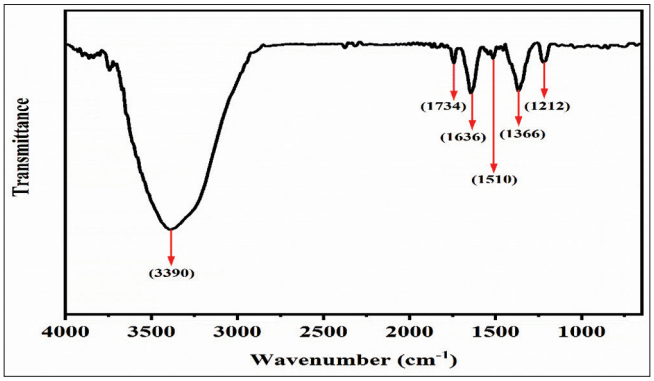

Figure 2: Fourier transform infrared spectra of synthesized copper oxide nanoparticles

FTIR studies

Figure 2 shows the CuONPs FTIR spectrum. The peak at 3390 cm^{-1} this peak represents the stretch of Hydroxyl/amines, and the peaks at 1734 cm^{-1} the absorption peaks represent the C=O Carbonyl/amide groups. The peaks shown at 1636, 1510, and 1366 cm^{-1} these various bands demonstrate the existence of alkanes, phenols, carboxylic acid groups, and alcohols, which are necessary for the capping materials used in plant extract to reduce Cu^{+2} to CuONPs.[9] The Hydroxyl and carbonyl might be responsible for the reduction stabilization of CuONPs.

XRD analysis

The XRD studies of prepared CuONPs from BF leaf extract are shown in Figure 3, it reveals that succession of diffraction peaks at 2θ of 31.81, 36.55, 42.40, 45.40, 56.35, 62.17, 66.68, and 75.32, which were assigned to planes (110), (111), (200), (202), (020), (113), (022), and (004) planes, respectively.[19] The extremely crystalline character of the nanoparticles is shown by their crisp and narrow diffraction peaks, which are in good agreement with those of powder CuONPs acquired from the international center of diffraction data card (JCPDS).

The Scherrer formula, $D = 0.9\lambda/\beta\cos\theta$, was used to determine the average crystallite size of CuONPs. Here, β is the full width at half maximum of the X-ray spectrum, and wavelength (λ) is the wavelength. θ is the peak at the angle of diffraction. Its size of 12 nm, which indicates that it is nanocrystalline, was discovered.

Zeta potential

The stability and surface charge of the prepared CuONPs are described by the zeta potential values. The BF leaf extract-capped CuONPs were discovered to have a zeta potential of – 16.8 mV [Figure 4], which is a definite indication that the nanoparticles are negatively charged and extremely stable [8].

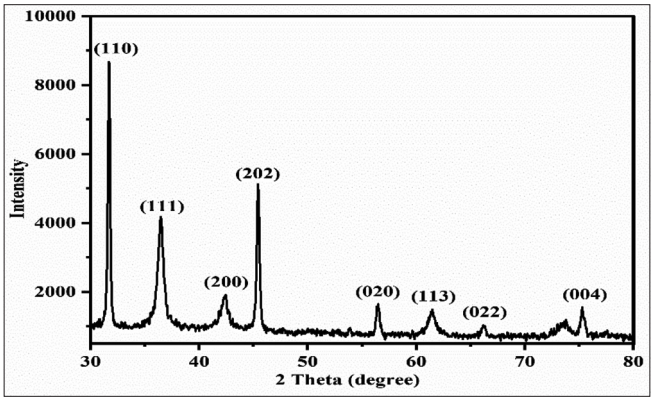

Figure 3: X-ray diffraction spectra of synthesized copper oxide nanoparticles

TEM analysis

TEM analysis verified the size and shape of the produced CuONPs. According to Figure 5, the produced CuONPs were mostly spherical. The average particle size of CuONPs falls in the range of (5–25) [Figure 6]. The average size distribution, according to a histogram derived by taking into account 141 nanoparticles, is 14 ± 2 nm.

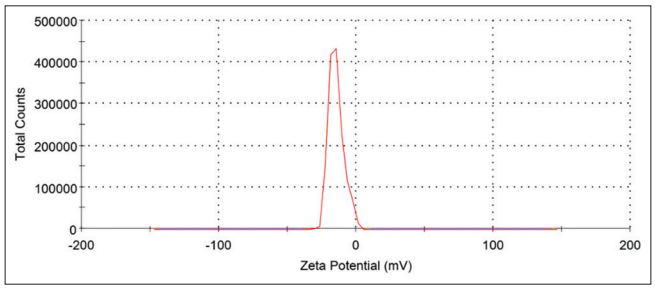

Figure 4: Zeta potential measurement of synthesized copper oxide nanoparticles

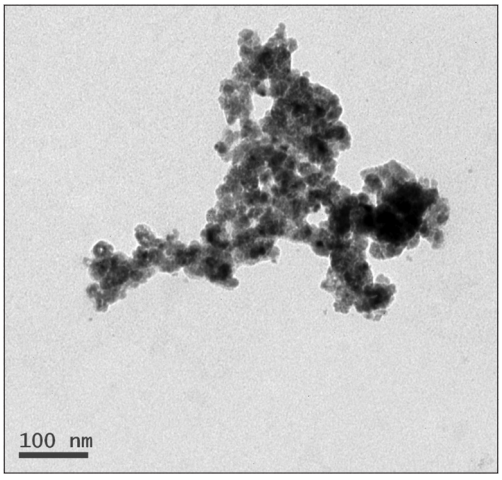

Figure 5: Transmission electron microscopy image of synthesized copper oxide nanoparticles

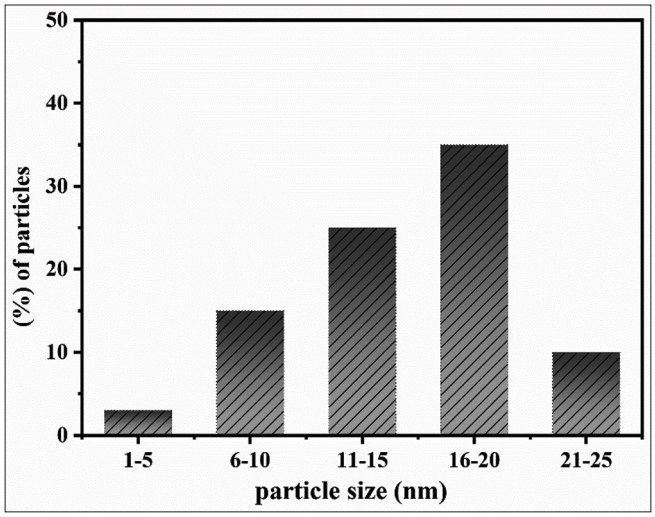

Figure 6: Size distribution synthesized copper oxide nanoparticles

Anti-bacterial activity

Gram-negative *E. coli* and Gram-positive *Staphylococcus aureus* both exhibit strong antibacterial action against the BF leaf extract-capped CuONPs. It showed that bacterial growth in both cases was reduced as the concentration of CuONPs was raised. CuONPs' higher overall surface area per unit volume boosts their antibacterial effectiveness. Figure 8 depicts the zone of inhibition of CuONPs against *E. coli* and *S. aureus* bacteria. The findings revealed that CuONPs made from BF leaf extract was more efficient against Gram-negative bacteria than Gram-positive bacteria.[20] There are several publications available on the antibacterial activity' underlying mechanisms.[21] According to the theorized process, copper ions will be liberated from the nanoparticles and might connect to the negatively charged bacterial cell wall, causing it to burst, which would cause protein denaturation and cell death [Figure 7]. However, in CuONPs Samples, Azam *et al.*, Line *et al.*, and Nawaz *et al.* postulated a similar sort of process.[15]

Antioxidant studies

Using ascorbic acid as a reference, the DPPH radical scavenging experiment evaluates the considerable antioxidant

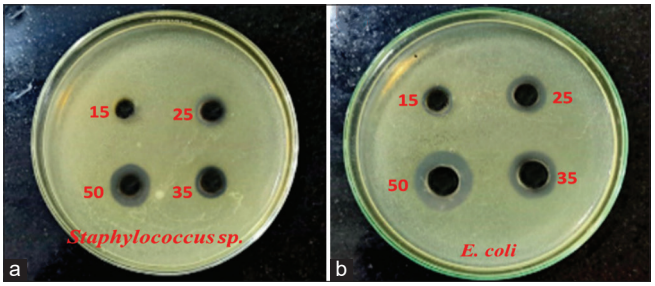

Figure 7: Antibacterial activity of *Borassus flabellifer* leaf extract capped copper oxide nanoparticles using (a) *Staphylococcus aureus* and (b) *Escherichia coli*

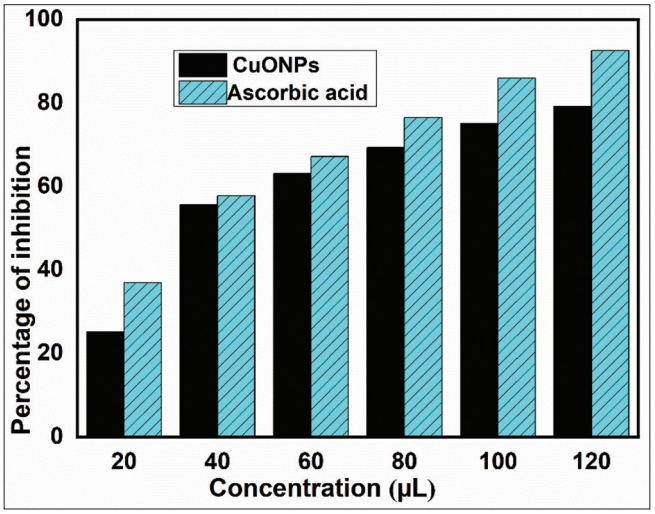

Figure 8: 2,2-diphenylpicrylhydrazyl scavenging activity of synthesized copper oxide nanoparticles was compared with ascorbic acid standard

potential of Synthesized CuONPs. It is a convenient and cost-effective strategy. The DPPH is a red-colored and stable free radical that absorbs at 517 nm. Antioxidant chemicals that give hydrogen and get reduced cause it to react. As a result of this process, the color becomes yellow and the absorbance at 517 nm decreases.[21] Because the surface area of the nanoparticles rises with increasing substrate concentration, the percentage of inhibition increases. At concentrations 20, 40, 60, 80, and 100 1st 120 µL proves a scavenging rate of 25.25%, 55.6%, 63.1%, 69.3%, 75.1%, and 79.2%, respectively. The free radical scavenging activity synthesized CuONPs is very close to the standard ascorbic acid. In agreement with our work, earlier research found that metal oxide nanoparticles showed potent antioxidant activities and effectively slayed several free radicals, including DPPH.[22]

CONCLUSIONS

This article describes a green method for producing CuONPs utilizing the BF leaf extract. The creation of CuONPs was indicated by the 415 nm SPR band in UV-Vis spectra. The green synthesized CuONPs were spherical, ranging from 4 to 25 nm, according to the findings of the TEM. The prepared CuONPs showed excellent anti-bacterial activity against Gram-positive (*S. aureus*), and Gram-negative (*E. coli*). CuONPs also showed significant antioxidant activity. In conclusion, these uses of green synthesized CuONPs might be powerful tools in the development of new biomedical research.

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