

Antibacterial Activity of Copper Nanoparticles Synthesized by *Bambusa arundinacea* Leaves Extract

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Abstract: A novel bio-fabrication of copper nanoparticles (CuNPs) using *Bambusa arundinacea* leaves aqueous extract solution, and their prospective biological activities are reported. Scanning and Transmission Electron Microscopy study of synthesized powder confirms the formation of Cu NPs with a size range 15-30 nm. The Dynamic Light Scattering (DLS) study further confirms the average particle size as 24.4 nm, and the observed zeta potential value of -16.1 mV affirms the high stability of Cu NPs. The synthesized Cu NPs exhibited potent antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*. This method provides a safe, clean, eco-friendly, and economical way to produce Cu NPs.

Keywords: green synthesis; nanoparticles; electron microscopy; antibacterial activity.

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1. Introduction

In recent years, the green synthesis of nanoparticles has been attracted much attention due to its reliable, sustainable, eco-friendly, cost-effective, and non-toxic protocol [1,2]. The main drawbacks of thermal, physical, and chemical methods of nanoparticle preparation are the use of sophisticated instruments, high-temperature synthesis, toxic solvents, and quite expensive. The main advantages of the green chemistry method over chemical and physical approaches are simple to control the reaction, cost-effective, low-temperature synthesis, non-toxic chemicals, easy to scale-up, good production rate, and eco-friendly. The green synthesis was used to prepare different metal oxide and metal nanoparticles in the literature [3]. Among all metal nanoparticles, copper (Cu) nanoparticles are superior due to their unique chemical and physical properties, such as high thermal conductivity, electrical conductivity, and biological activity. Copper is highly toxic to organisms and non-toxic to animal cells, and it is considered a useful bactericidal metal. Copper NPs have been used in different applications such as health, food, medical, consumer, and some industrial sectors. In this manuscript, green synthesis of Cu NPs with a novel plant extract is reported for the first time to the best of our knowledge.

The green synthesis of Cu NPs has been a promising research area in biomedicine, biosensors, and pharmacy in recent times. Green synthesis of Cu NPs focuses on the employment of fungi, bacteria, and plant extracts, as reducing and capping agents [4-6]. Among all, nanoparticle preparation with plant extracts has been attracted massive attention due to the accessibility of a biological entity. The different plant extracts such as *Azadirachta indica*,

Cissusarnotiana, *Green and black tea*, *Eichhornia crassipes*, *Falcaria vulgaris*, *Millettiapinnata*, *Perseaa mericana*, *Quisqualis indica*, *Ziziphusspina-christi* were reported for the Cu NPs synthesis in recent years [7]. To the best of the authors' knowledge, Cu NPs synthesis with *Bambusa* plant leaves (BPL) extract is not reported. The BPL extract holds natural bio-compounds like flavonoids, tannins, aldehydes, ketones, alkaloids, saponins, polysaccharides, and additional nutritional compounds [8, 9]. In this green synthesis, the plant extract act as a strong natural reducing agent and stabilizer for Cu NPs. The phytoconstituents like flavonoids, phenolic acids, tannins, saponins, glycoside, alkaloid, and polysaccharides are responsible for reducing Cu metal salts into Cu NPs. This process's main advantages are (1) easy mixing of extract solution with an aqueous solution of metal salt precursor; (2) prepared Cu NPs are less toxic and biocompatible. The surface of the prepared Cu NPs has some bonded biomolecules; thus, they show good antibacterial activity against pathogenic bacteria.

In the present study, we have used BPL extract to synthesize Cu NPs and evaluated their antibacterial activity against four pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*.

2. Materials and Methods

2.1. Materials.

The *Bambusa* leaves were collected from in and around the campus of the National Institute of Technology Warangal, India. The Cupric acetate ($\text{Cu}(\text{CH}_3\text{COO})_2$) procured from Sigma Aldrich was used without further purification. The bacterial cultures of *Escherichia coli* (MTCC-433), *Staphylococcus aureus* (MTCC-1430), *Bacillus subtilis* (MTCC-441), and *Proteus vulgaris* (MTCC-426) were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India.

2.2. *Bambusa arundinacea* leaves extract preparation.

The *Bambusa* leaves were washed several times with double distilled water and dried at room temperature. The leaves were cut into small pieces and put into the glass beaker containing 100 ml distilled water. The leaves extract was generated by boiling the leaves in the water for 30 minutes at 80°C. The extract was filtered with the help of Whatman filter paper. The collected, filtered extract solution was stored for further usage for Cu NPs synthesis.

2.3. Biosynthesis of Cu NPs.

Initially, *Bambusa arundinacea* leaves extract was prepared. Subsequently, cupric acetate 0.01M solution was prepared in a beaker at 60°C. In the next step, 25ml of aqueous extract of *Bambusa* leaves was added to the cupric acetate solution. The resulting solution was stirred at 65°C for four hours to get the Cu NPs. The color of the solution has been started to change from blue to bluish-green after 30 min of reaction, indicating the formation of Cu NPs [10-12]. The obtained powder was washed with an ethanol solution to remove unwanted compounds. The resulting powder was characterized by different techniques.

2.4. Characterization techniques of Cu NPs.

The morphology and size of the prepared Cu NPs were analyzed with the scanning electron microscope (SEM, Zeiss) and transmission electron microscope (TEM, JEOL-2100).

The crystalline nature of the Cu NPs powder was confirmed by X-ray diffraction (XRD, Bruker D8 advance). UV-spectrophotometer was used for confirmation of the Cu NPs formation. Particle size distribution, the zeta potential, and polydispersity index (PDI) of Cu NPs were measured by particle size analyzer (Horibha SZ-100). FTIR spectrometer was used to determine the functional chemical groups (Perkin Elmer-400).

2.5. Antibacterial activity.

The antibacterial activity of green synthesized Cu NPs was analyzed using the Disc diffusion method. The bacterial inoculums were firstly grown in LB broth for 24 h at 28°C and 200 rpm. It was then re-suspended in LB medium until the optical density (OD) is adjusted to 0.1 at 600 nm, which corresponds to 10^8 Colony Forming Units (CFU)/mL. Spread plate technique was used in which 100 μ L of each bacterial suspension was spread over nutrient agar medium. A set of four concentrations (50, 100, 150, and 200 μ L) of Cu NPs colloidal solution in the disc were prepared and used in the study. The four different concentrations of the Cu NPs in respective discs were placed over the lawn of bacterial culture and standard. All the agar plates were incubated in the bacteriological incubator for 24 h at 37°C. The zone of inhibition (ZOI) around the discs was determined with a measuring scale (mm).

3. Results and Discussion

3.1. Characterization of Cu NPs.

The FESEM image of the obtained powder shown in figure 1(a) confirms the formation of Cu NPs and most of the particles agglomerated in nature. Further, TEM was employed to get information about the shape and size of the NPs. It is evident from figure 1(b), the synthesized Cu NPs are spherical with smooth surfaces. The histogram shown in the inset of figure 1(b) represents the particle size distribution and indicating that the average size of Cu NPs is 23 nm. The size of the Cu NPs dependent on the plant extract concentrations, the phytochemicals involved in the formation of Cu NPs.

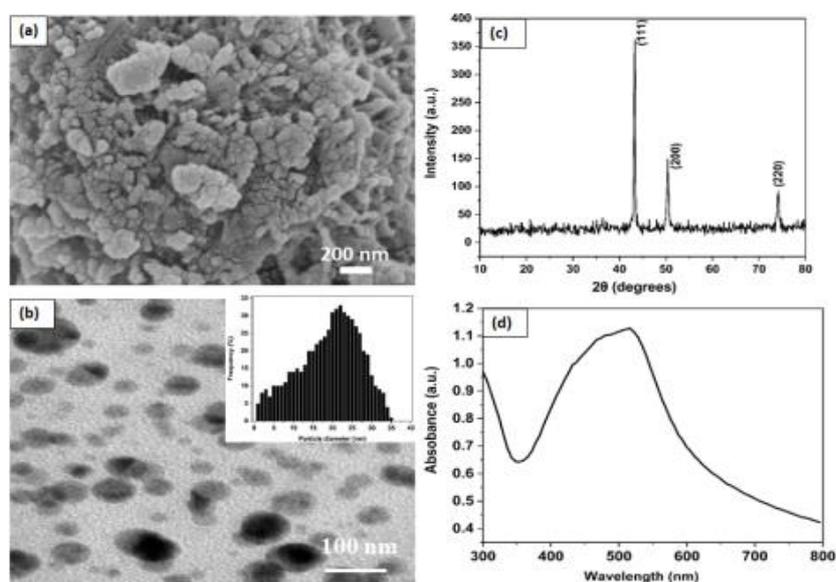


Figure 1. (a) FESEM image of green synthesized Cu NPs, (b) TEM image of the Cu NPs, Inset of (b) histogram of particle size distribution, (c) XRD pattern of the Cu NPs powder, (d) UV-Vis absorption spectra of the Cu NPs.

are observed at 3427, 2918, 2091, 1602, 1394, 1258, 1059, 795 and 599 cm^{-1} . The peaks at 2918, 1602, and 1059 cm^{-1} indicate the C-H asymmetric stretching, C=O aromatic vibrations, and C=C stretching, respectively [15]. The peak at 2091 cm^{-1} confirms the carboxylate ions [16]. The peak positions at 1394 and 1258 cm^{-1} correspond to the organic and aromatic molecule derivatives like phenolic, alkaloids, flavonoids, tannins, aldehydes, and ketones present in the plant extract the peaks at 795 and 599 cm^{-1} identified as the aromatic ring of amino acids. The peak observed at 3427 cm^{-1} may be assigned to OH or NH stretching of phenolic compound. The involvement of phytochemicals in the formation of Cu NPs is confirmed with the FTIR analysis. Hence we can conclude these phytochemicals play a significant role in synthesizing Cu NPs [17].

3.2 Antibacterial activity of Cu NPs.

Figures 4(a)–(d) show the antibacterial activity of different concentrations of Cu NPs against four pathogenic bacteria of *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, respectively. The central white spot in figures 4 (a)-(d) is the standard sample used as reference measurement. The measured zones of inhibition diameter (in mm) around the discs were summarized for different concentrations of Cu NPs shown in figure 4(e). The diameter of the zone of inhibition gives information about the magnitude of the susceptibility of microorganisms. It is observed that Cu NPs demonstrated a high degree of antibacterial activity for *Escherichia coli* and *Bacillus subtilis*. Whereas, in the case of *Proteus vulgaris* and *Staphylococcus aureus*, moderate activity is observed for Cu NPs.

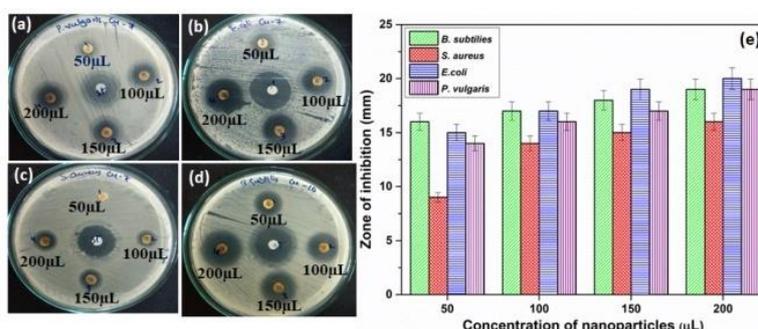


Figure 4. Antibacterial activity of Cu nanoparticles at different concentrations against (a) *Proteus vulgaris*, (b) *Escherichia coli*, (c) *Staphylococcus aureus*, (d) *Bacillus subtilis*, (e) graphical representation of zone of inhibition values at different concentration of Cu NPs.

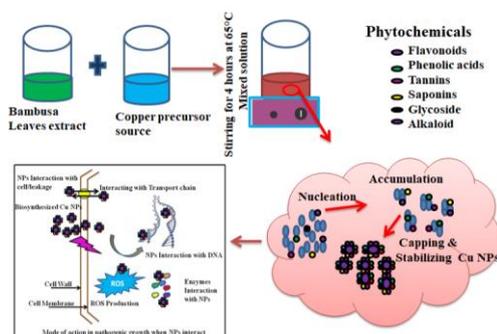


Figure 5. Schematic diagram of Cu nanoparticles synthesis and antibacterial activity.

There are different accepted mechanisms for the antibacterial activity of metal nanoparticles [18, 19]. The most-reported mechanism is based on the reactive oxygen species generation (ROS). Cu NPs also dramatically enhance the cellular ROS level that influences

lipid peroxidation, protein oxidation, and DNA destruction and finally kills the microorganism cells [20-22]. The ROS contain radical compounds such as hydroxyl (-OH), superoxide radical (O_2^-), singlet oxygen (1O_2), and hydrogen peroxide (H_2O_2), which destroy the bacteria. To conclude, the green synthesized Cu NPs demonstrated significant antibacterial activity against various organisms. The schematic diagram of the biosynthesis of Cu NPs using *Bambusa arundinacea* aqueous extract and antibacterial activity is shown in figure 5.

4. Conclusions

In summary, *Bambusa arundinacea* aqueous extract was used successfully to synthesize the Cu NPs for the first time. The synthesized nanoparticles were characterized by using SEM, TEM, XRD, UV-Visible, and DLS techniques. The synthesized nanoparticles were spherical in shape and crystalline in nature, with an average particle size ~24.4 nm. The green synthesized Cu NPs exhibited excellent antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*. The antibacterial activity was also tested for different concentrations of the Cu NPs and found increased activity with the increase of concentration. Further, the application of green synthesized Cu NPs can be extended to antioxidant, anticancer, and wound healing activities.

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Conflicts of Interest

The authors declare no conflict of interest.

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