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Research Article

Heterophyllum

Eco-Friendly Synthesis of Copper Nanoparticles Using Aconitum Heterophyllum Extract and Its Antibacterial Evaluation

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Copper nanoparticles (CuNPs) were successfully synthesized via an eco-friendly green method using Aconitum heterophyllum extract, which acts as a reducing as well as stabilizing agent. The biosynthesis involved the reaction of copper sulfate with the plant extract in deionized water, forming well-dispersed, uniform, and stable CuNPs. This rapid and cost-effective method is environmentally benign and enhances the functional properties of the nanoparticles. The synthesized CuNPs were characterized using UV-Vis spectroscopy (revealing a distinct absorption peak), high-resolution transmission electron microscopy (HR-TEM) for morphological analysis, and energy-dispersive X-ray spectroscopy (EDX) for elemental composition. Antimicrobial tests confirmed the potent antibacterial activity of the CuNPs, demonstrating significant inhibition zones against both Gram-positive and Gram-negative bacterial strains. This study highlights A. heterophyllum as a sustainable and effective bioresource for CuNP synthesis with promising applications in nanotechnology, biomedical treatment, and antimicrobial formulations.

Keywords: Heterophyllum, Formulations, Copper Nanoparticles

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1. Introduction

The growing incidence of infectious diseases, coupled with the alarming rise in antibiotic-resistant microbial strains, has intensified the search for effective alternative antimicrobial agents. Among these, metal nanoparticles have emerged as promising candidates due to their broad-spectrum antimicrobial activity and unique physicochemical properties [1]. Monometallic nanoparticles have been extensively utilized in various research fields owing to their antimicrobial, antioxidant, photocatalytic, and anti-biofilm properties. Copper nanoparticles (Cu NPs), in particular, have demonstrated potent antibacterial effects against a range of pathogenic microorganisms, including Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and various Streptococcus species [2,3]. Owing to these properties, Cu NPs have been widely integrated into commercial products such as plastics and textiles for antimicrobial applications. Additionally, their effectiveness has been exploited in biomedical fields, notably in the development of wound dressings [4]. Nanotechnology has emerged as a fascinating area of research due to its potential to generate nanoparticles (NPs) with high uniformity and unique properties, making them highly valuable in fields such as optical sensing, drug delivery, catalysis, adsorption, water purification, and nanomedicine [5]. These nanoparticles can be prepared through different methods, including chemical, physical, and biological approaches.

But chemical and physical synthesis routes are often complex, costly, and less environmentally friendly. Techniques like chemical reduction, electrochemical methods, and pyrolysis require sophisticated procedures and involve hazardous and expensive reducing agents such as borohydride and hydrazine derivatives, leading to toxic byproducts and environmental risks [6]. The biosynthesis of copper nanoparticles (CuNPs) has been widely recognized by researchers as a superior alternative to physical and chemical synthesis methods, due to its environmentally friendly nature and cost-effectiveness [7].

The most common method for synthesizing metal nanoparticles is by reducing metal ions in a solution.While chemical and physical methods can yield pure and well-defined nanoparticles, they are often costly and pose potential environmental risks [8–9].

Green chemistry offers significant promise in guiding the sustainable advancement of nanotechnology by promoting the design of eco-friendly nanomaterials and the development of greener manufacturing techniques. Green synthesis emphasizes minimizing hazardous waste, utilizing sustainable processes, and incorporating environmentally benign chemicals, solvents, and renewable resources [10–11] In this study, we further explore the antibacterial potential of Cu NPs against E. coli, Staphylococcus aureus, thereby contributing to the growing evidence supporting their use as versatile and effective antimicrobial agents.

2. Materials and Methods

Materials: Copper sulfate pentahydrate purchased from Sigma-Aldrich. Aconitum heterophyllum were obtain from the local market. De-ionized and distilled water was obtained from our laboratory at Ramsheth Thakur College.

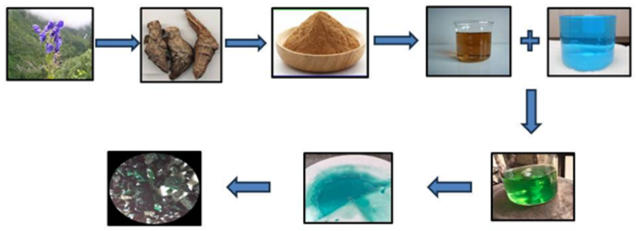
Preparation of Plant Extract

The harvested bark samples of the Aconitum heterophyllum plant were cleaned using deionized water and then dried. 4g of bark specimens were ground by using a conventional grinder, and stored at 4˚C. The powder of Aconitum heterophyllum was mixed with 200ml of deionized water.The extract was passed through Whatman filter paper for filtration. Following this, an equal volume of ethanol was added to precipitate the mucilage present in the extract. The mixture was then centrifuged at 3000 rpm for 10 minutes to remove the mucilage. The resulting supernatant was collected and stored at 4°C.

Synthesis of Copper Nanoparticles

To synthesize copper nanoparticles (CuNPs), 100 mL of Aconitum heterophyllum aqueous extract was mixed with 1 g of copper sulfate pentahydrate. The mixture was stirred magnetically at room temperature (27°C) for 2 hours. Within 10 minutes, the initial blue color of copper sulfate pentahydrate changed to pale green, indicating the reduction of Cu(II) ions to elemental copper (Cu) and the formation of CuNPs, the pH was maintained at 7.0 by using NaOH. The colour of the solution changed from pale green to pale blue during stirring at 70 °C for 1 hour.

Afterward, the solution was centrifuged for 1 hour and then filtered. The resulting solid precipitate was washed three times with deionised water, followed by a final wash with 100% ethanol to facilitate the separation of CuNPs. The precipitate was then dried at 80 °C for 1 hour and stored at 4 °C for further applications.



Characterization of Biosynthesized Copper Nanoparticles

UV–visible spectroscopy: The formation of copper nanoparticles (CuNPs) was preliminarily confirmed by a visible color change in the reaction mixture, shifting from pale green to pale blue, indicating nanoparticle synthesis. The transformation was further confirmed through UV–visible spectroscopy. The absorption spectrum of the biosynthesized CuNPs was measured within the wavelength range of 300–700 nm using a Shimadzu spectrophotometer. A distinct surface plasmon resonance (SPR) peak appeared at 421 nm, indicating the successful formation of copper nanoparticles. **Scanning electron microscopy (SEM) :-** The surface morphology of the synthesized copper nanoparticles (CuNPs) was examined using scanning electron microscopy (SEM).The SEM images revealed that the CuNPs predominantly exhibited a spherical shape with an average diameter of approximately 200 nm(shown in fig.2).

Transmission electron microscopy (TEM): Further insight into the internal structure and precise size of the nanoparticles was obtained through transmission electron microscopy (TEM), presented TEM analysis confirmed the spherical morphology and indicated an average particle size of about 50 nm. The particle size distribution, ranged from 50 nm to 200 nm, shown in fig.3 with a relatively narrow distribution centered around 50 nm, suggesting uniformity in particle formation.

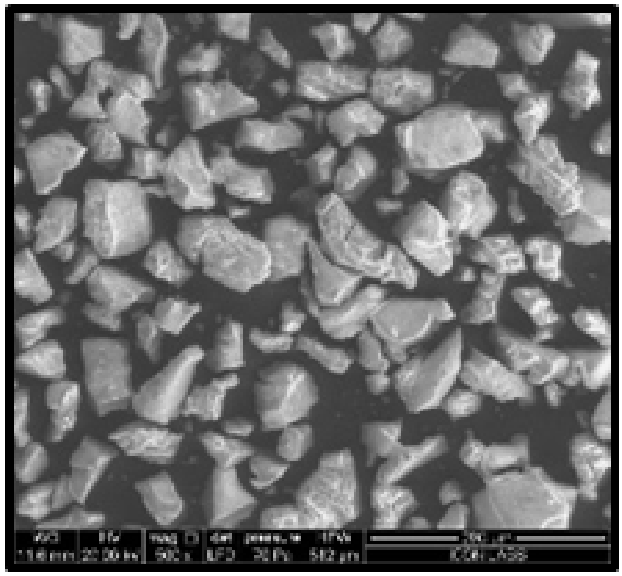


Figure 1

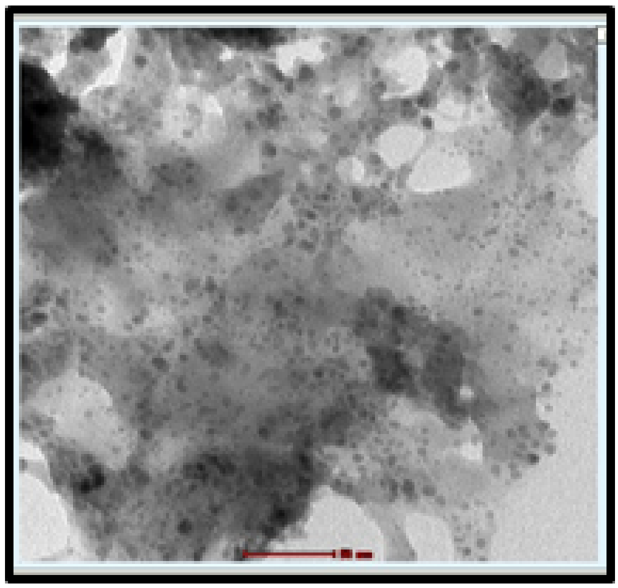


Figure 2

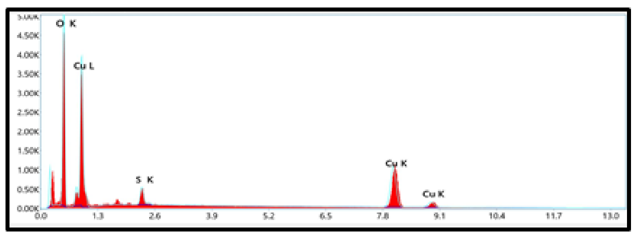
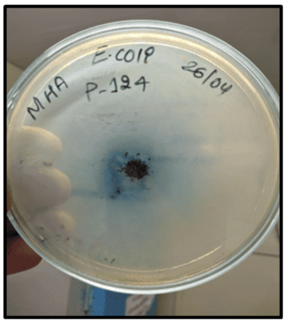


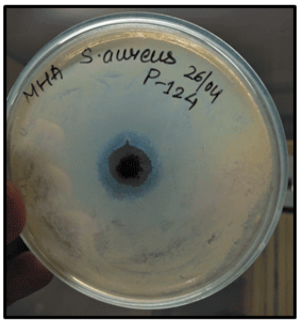
Figure 3

Energy-dispersive X-ray spectroscopy (EDX) was performed to determine the elemental composition of the synthesized copper nanoparticles (CuNPs). The EDX spectrum, shown in Fig. 4, confirmed the presence of copper as the major elemental component, with a prominent signal corresponding to Cu at approximately 1 keV.

The quantitative analysis indicated that copper constituted approximately 56% by weight of the total composition, verifying the successful synthesis of CuNPs. Minor signals from other elements may be attributed to residual biomolecules from the plant extract or the supporting substrate used during analysis. The strong copper peak and minimal impurities confirm the high purity and elemental integrity of the biosynthesized nanoparticles.

Antimicrobial Activity: The antimicrobial efficacy of the synthesized sample was evaluated using the agar well diffusion method against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) bacterial strains at a concentration of 10^6 CFU/mL. The results demonstrated measurable zones of inhibition for both tested organisms, indicating potential antibacterial activity.





Zone of Inhibition 106 CFU/ml		
Copper nanoparticles	E. coli (2.9× 105 CFU/ml)	S. aureus(1.5× 105 CFU/ml)
	11.03	11.82

3. Results and Discussion

Synthesis and Characterization of Copper Nanoparticles (CuNPs)

The successful biosynthesis of copper nanoparticles (CuNPs) was initially indicated by a visible color change in the reaction mixture from pale green to pale blue, suggesting nanoparticle formation. This observation was further supported by UV–visible spectroscopy, which revealed a characteristic surface plasmon resonance (SPR) peak at 421 nm (Fig. 1). This SPR peak is consistent with the formation of CuNPs, confirming the synthesis process.

Surface Morphology and Particle Size Analysis

The morphology of the synthesized CuNPs was examined using scanning electron microscopy (SEM).

The SEM images (Fig. 2) showed that the nanoparticles predominantly exhibited a spherical shape with an average diameter of approximately 200 nm. However, to gain more precise information on particle size and internal structure, transmission electron microscopy (TEM) analysis was conducted.TEM analysis (Fig. 3) further confirmed the spherical nature of the CuNPs and revealed a significantly smaller average particle size of approximately 50 nm, with a particle size distribution ranging from 50 nm to 200 nm. The relatively narrow distribution centered around 50 nm suggests a uniform size and effective synthesis methodology.

Elemental Composition

To determine the elemental makeup of the nanoparticles, energy-dispersive X-ray spectroscopy (EDX) was performed. The EDX spectrum (Fig. 4) exhibited a prominent peak at approximately 1 keV, which corresponds to copper, confirming its presence as the major component. Quantitative analysis showed that copper accounted for about 56% by weight of the total composition. Minor peaks corresponding to other elements were observed and are likely due to residual phytochemicals from the plant extract or the sample substrate. The dominance of the copper peak and minimal impurities indicate high purity of the synthesized nanoparticles.

Antimicrobial Activity

The antimicrobial potential of the CuNPs was assessed using the agar well diffusion method against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) at a bacterial concentration of 10^6 CFU/mL. The results demonstrated clear zones of inhibition, indicating effective antibacterial activity. Specifically, the zone of inhibition measured 11.03 mm for E. coli and 11.82 mm for S. aureus, demonstrating the CuNPs' potential in antimicrobial applications. These findings affirm that the biosynthesized CuNPs possess significant antimicrobial properties, possibly due to their small size and high surface area, which enhances their interaction with bacterial cell membranes.

4. Conclusion

The present study successfully demonstrated the biosynthesis of copper nanoparticles (CuNPs) using a green synthesis approach.

The formation of CuNPs was confirmed by a visible color change and further validated by UV–visible spectroscopy, showing a distinct SPR peak at 421 nm. Morphological analyses using SEM and TEM revealed that the nanoparticles were predominantly spherical, with an average size ranging from 50 nm to 200 nm, and a relatively uniform distribution centered around 50 nm. Elemental analysis through EDX confirmed the presence of copper as the primary component, with high purity and minimal impurities. Moreover, the synthesized CuNPs exhibited notable antimicrobial activity against both Escherichia coli and Staphylococcus aureus, indicating their potential applicability as effective antibacterial agents. These results highlight the efficacy of biosynthesized CuNPs in nanomedicine and other antimicrobial applications, promoting a sustainable and eco-friendly route for nanoparticle synthesis.

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