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Green synthesis of copper oxide nanoparticle using *Azolla pinnata* extract

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Abstract- This study explores the green synthesis of copper oxide nanoparticles (CuO-NPs) using the aqueous extract of *Azolla pinnata*, an eco-friendly and sustainable approach to nanoparticle production. The process leverages the natural abundance and low cost of *A. pinnata*, aligning with green chemistry principles to minimize environmental impact. The synthesized CuO-NPs were characterized using various techniques, including X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS). The results revealed that the CuO-NPs possess a face-centered cubic structure with crystalline sizes ranging from 20-50 nm. The nanoparticles exhibited a predominantly spherical shape with uniform size distribution, and a capping organic layer was observed, enhancing stability. The colloidal stability of the nanoparticles, attributed to a negative zeta potential, prevents aggregation and ensures long-term stability. This research demonstrates the effectiveness of *A. pinnata* extract in synthesizing stable and well-characterized CuO-NPs, providing a promising alternative for sustainable nanotechnology applications in catalysis, biomedicine, and environmental remediation.

Key words: Characterization, Silver nanoparticles, Characterization, *Ocimum sanctum* leaf, Green synthesis

INTRODUCTION

Nanoscience is a rapidly advancing field dedicated to creating, enhancing, and applying structures at the nanoscale. The remarkable progress in nanotechnology has paved the way for significant advancements in materials science and engineering. This includes breakthroughs in Surface-Enhanced Raman Scattering (SERS), quantum dots, nanobiotechnology, and applications in chemistry and microbiology.[1-4] Metal nanoparticles, in particular, have found extensive use in diverse areas such as optics, mechanics, electronics, space industries, chemical industry, biomedical sciences, drug-gene delivery, energy science, catalysis, optoelectronic devices, nonlinear optical devices, and photoelectrochemical applications.

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Nanotechnology is considered the pinnacle of manipulating matter at the molecular, atomic, and supramolecular scale. As defined by the National Nanotechnology Initiative (NNI), nanotechnology involves manipulating matter that has at least one dimension sized within the range of 1 to 100 nanometers, enabling novel applications. To put this into perspective, a nanometer is one billionth of a meter, so 1 nm is equal to 10^{-9} m. Materials with dimensions within the range of 1 to 100 nm are commonly referred to as nanoparticles. In the field of nanotechnology, there are two major approaches used for the synthesis and fabrication of nanostructures. The "bottom-up" approach involves synthesizing nano-structures by stacking atoms onto each other, leading to the formation of crystal planes that are further stacked onto each other, ultimately resulting in the synthesis of the desired nanostructure.[5]

In nanotechnology, there are two fundamental approaches for synthesizing nano materials: top-down and bottom-up methods. The top-down method involves converting bulk molecules into nano-sized fragments of the same molecules. This can be thought of as sculpting the material from a larger, bulk form. The bottom-up method, on the other hand, involves the self-assembly of molecules and atoms to create the desired nanostructures. In the top-down method, physical processes are used to break down bulk materials into smaller nanoparticles. This can be achieved through techniques such as grinding, milling, or etching. The resulting fragments are typically of the same material as the bulk, but at a much smaller size. In contrast, the bottom-up method involves the formation of nanoparticles through self-assembly. Molecules and atoms come together to form clusters, which then further assemble to create nanoparticles. This self-assembly process is driven by physical forces at the nano scale and ultimately leads to the formation of larger, stable structures. Examples of bottom-up approaches include the epitaxial growth of quantum dots, where precise control of atomic layers leads to the formation of nanoscale structures. Another example is the formation of nanoparticles through colloidal dispersion, where particles are suspended in a liquid and come together to form larger structures. Both top-down and bottom-up methods have their advantages and limitations, and researchers often employ a combination of both approaches to achieve desired nano materials with specific properties.

Plants have gained attention for nanoparticle synthesis due to their eco-friendliness, ease of availability, low cost, and simple downstream processes. Various plants, such as *Albizia lebbeck, Euphorbia esula, Acalypha indica, Aloe barbadensis* etc., have been investigated for their ability to synthesize nanoparticles, including CuO-NPs and Ag nanoparticles. The choice of plant, phytochemical composition, solvent medium, extraction procedure, reaction temperature, and pH can influence the synthesis process. Plant extracts act as reducing, controlling, and stabilizing agents, incorporating biological components like proteins, amino acids, vitamins, polyphenols, enzymes, and polysaccharides in the synthesis process.[6-9]

In this paper, we have conscientiously documented the bio/green synthesis of copper nanoparticles using extracts from *Azolla pinnata*, commonly known as mosquito fern, is a floating aquatic fern found predominantly in warm and tropical water bodies.

MATERIALS & METHODS

Plant Material

Azolla pinnata, also known as velvet fern, is a type of aquatic fern belonging to the Salviniaceae family. It is indigenous to various regions across Asia, Africa, and Australia, thriving in freshwater habitats. Characterized by its rapid growth and ability to cover water surfaces, *Azolla pinnata* is recognized for its dense, green foliage that gives it a velvety appearance. Despite being considered a weed in some contexts, it plays a valuable role in ecosystems and agriculture. This fern is notable for its high protein content, making it a useful feed for livestock in certain areas. Furthermore, *Azolla pinnata* capacity to fix atmospheric nitrogen enables it to act as a natural biofertilizer, enriching soil nutrients and supporting sustainable agriculture practices. Its unique ability to synthesize copper nanoparticles through its aqueous extract has garnered interest in the field of nanotechnology, paving the way for the development of environmentally friendly and stabilized copper nanoparticles for various applications.

Preparation of plant extract

30 g of fresh *Azolla pinnata* fronds were gathered, thoroughly cleaned initially with tap water and subsequently with deionized water to eliminate any foreign particles. The leaves were then finely chopped and placed into a 250 ml Erlenmeyer flask filled with 120 ml of deionized water, allowing them to infuse overnight. Following a 24-hour period, the resulting orange-tinted extract underwent filtration using Whatman filter paper No.1, followed by a secondary filtration under vacuum using a 0.20 µm pore diameter filter paper. The final filtrate was then collected, securely sealed, and stored at 4°C in a refrigerator, ready for future utilization in the crafting of copper nanoparticles.

Synthesis of copper nanoparticles

In the process of green synthesis, 100 ml of the *Azolla pinnata* filtrate was heated until it reduced to half its original volume, leaving 50 ml of concentrated extract. This concentrated, hot extract from *Azolla pinnata* was then introduced to 450 ml of a 1 mM solution of copper sulfate. Subsequently, 1N sodium hydroxide (NaOH) was added meticulously, drop by drop, to the mixture while it was being continuously stirred, with the aim of adjusting the mixture's pH to 11. As this adjustment took place, the color of the solution began to change noticeably and rapidly, indicating the initiation of nanoparticle formation. This step is crucial in the synthesis process, as the pH

adjustment directly influences the size and stability of the resulting copper nanoparticles

Characterization of the CuO nanoparticles

The analysis of the copper nanoparticles was conducted using a diverse array of techniques to comprehensively assess their characteristics. X-Ray Diffraction (XRD) techniques were pivotal in delineating the crystalline structure, offering vital insights into the atomic configuration of the nanoparticles. Imaging methods, such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), provided intricate details regarding the nanoparticles' shape, size, and distribution. To ascertain the chemical composition, Energy-Dispersive X-ray spectroscopy (EDX) was employed, delivering precise elemental analysis. The surface chemistry of the nanoparticles was explored using Fourier-transform infrared spectroscopy (FTIR), which identified the functional groups adorning the nanoparticle surfaces. Additionally, the hydrodynamic size distribution in liquid environments was measured through Dynamic Light Scattering (DLS), offering a closer look at the nanoparticles in suspension. Collectively, this elaborate array of characterization methods furnished a detailed examination of the copper oxide nanoparticles' structure, chemical makeup, and dynamic behavior in solutions, facilitating a richer understanding of their potential applications and mechanisms of action.[10]

RESULTS & DISCUSSION

XRD crystallography was utilized to determine the crystalline structure of the synthesized copper oxide nanoparticles (CuONPs), using *Azolla* fronds extract as a mediating agent (Table 1). The X-ray diffractogram, as shown in the corresponding figure, revealed distinct Bragg's reflection planes across a 2θ range of 20 to 90°. Notable diffraction peaks at 2θ = 38.9°, 43.54°, 56.98°, 74.3°, and 84° matched the lattice planes (Miller indices) of 110, 111, 211, 200, and 311 for CuONPs, respectively (Figure 1). These peaks confirm the face-centered cubic (fcc) structure of the nanoparticles, aligned with the Joint Committee on Powder Diffraction Standards (JCPDS) database reference.[11]

The presence of broad peaks in the XRD pattern indicated the nanoparticles' small crystalline size. The Bragg's diffraction peaks conformed to the JCPDS card No. 04-0836 for CuO, suggesting the particles' multi-phase characteristic. Sharp peaks in the pattern may reflect the

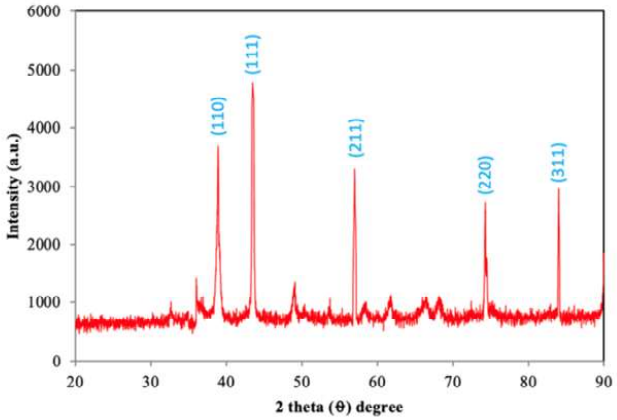

Figure 1. XRD patterns of *Azolla pinnata* leaf extract mediated synthesized CuONPs.

influence of the capping agent used to stabilize the nanoparticles, whereas unassigned peaks (*) might derive from the crystallization of biomolecules on the nanoparticle surface. Similar instances of Bragg's reflection for CuONPs have been reported in literature.[12-14] The lattice constant was derived from the diffraction pattern (α = 0.78 Å), with the d-spacing values and crystalline sizes for each peak detailed in the provided table. Utilizing the Scherrer equation:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where, D represents the average crystalline size (Å), k is a constant set to 1, λ is the X-ray radiation wavelength (λ = 1.54 Å), β is the angular line full width at half maximum (FWHM), and θ is the Bragg angle. The average size of CuONPs was determined to fall within the range of 20-50 nm, as presented in the table.

Table 1- Parameter calculation for average size calculation for nanoparticle

2θ	Integral Result of B FWHM	d-spacing	(*hkl*)	crestalline size (nm)
38.9	0.158	2.31	110	47.70
43.54	0.160	2.08	111	46.39
56.98	0.182	1.61	211	38.41
74.3	0.228	1.28	200	27.87
84	0.254	1.15	311	23.29

Dynamic Light Scattering (DLS) analysis was utilized to assess the hydrodynamic diameter, polydispersity index (PDI), and surface zeta potential of copper nanoparticles synthesized within a colloidal aqueous medium, employing *Azolla pinnata* in the process (Figure 2 a-c). The size distribution, as indicated in the relevant figure, demonstrated that these eco-friendly produced copper

nanoparticles varied in size from 15 to 95 nm, with a PDI of 0.238. A PDI close to '0' indicates a uniform particle size distribution, while '1' suggests a wide variance in sizes, pointing to a moderately diverse size distribution among the nanoparticles. The DLS method calculates the hydrodynamic diameter, reflecting the size of a theoretical sphere that moves through a fluid at the same speed as the nanoparticle. This measure includes not just the nanoparticle's core but also any molecules bound to its surface, like stabilizers, and the hydration layer, thus typically yielding a larger size than microscopic techniques would show. The average hydrodynamic diameter was determined to be 70 nm.

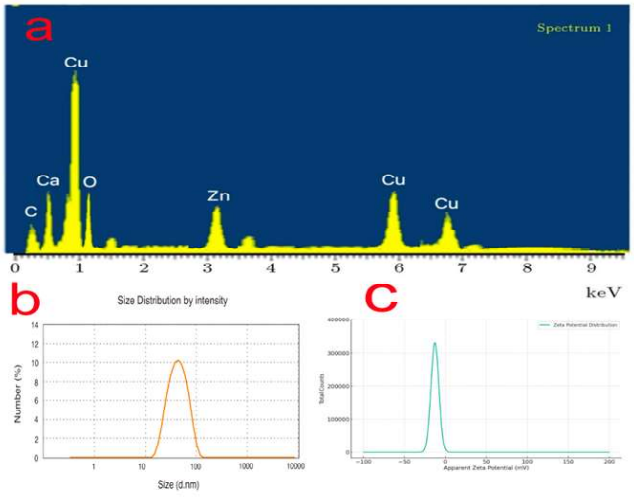

Figure 2. Energy dispersive spectroscopy (a), Size distribution (b) and Zeta potential of CuONPs.

Regarding the nanoparticles' surface charge, a negative zeta potential of -12.5 mV, with a deviation of ±4.65 mV, was recorded. The substantial absolute value of the zeta potential signals a strong electric charge on the nanoparticle surfaces, creating repulsive forces that help prevent particle clumping. This property is essential for the colloidal solution's stability, which was evaluated by observing the nanoparticle mixture at ambient temperature over a series of days. The pronounced repulsion due to the zeta potential plays a vital role in maintaining the nanoparticles dispersed and stable in the solution.

The characterization of copper oxide nanoparticles synthesized using *Azolla pinnata* was thoroughly investigated using Transmission Electron Microscopy (TEM), as highlighted in the provided figures. The TEM analysis demonstrated that the nanoparticles primarily display a spherical shape, with a tendency to form small clusters (Figure 3). These nanoparticles were found to be remarkably consistent in shape, possessing an average diameter of 36.73 nm. The presence of a few larger particles was observed, though they were relatively rare in the sample. TEM imagery also unveiled a slender organic layer surrounding the clusters, serving as a stabilizing capping agent. This feature emphasizes the nanoparticles' superior dispersion across the aqueous solution, facilitated by the biological reduction process.[15] Furthermore, the figures include the Selected Area Electron Diffraction (SAED) pattern for the copper oxide nanoparticles, showcasing diffraction spots that form concentric rings, indicative of CuO (110, 111, 211, 200, 311) lattice reflections. This evidence supports the highly crystalline structure of the synthesized nanoparticles.[16,17] A histogram depicted in the figures reveals that a majority of the nanoparticles are sized between 20-50 nm, aligning with the observed uniformity and crystalline clarity of the copper oxide nanoparticles produced through this green synthesis method.

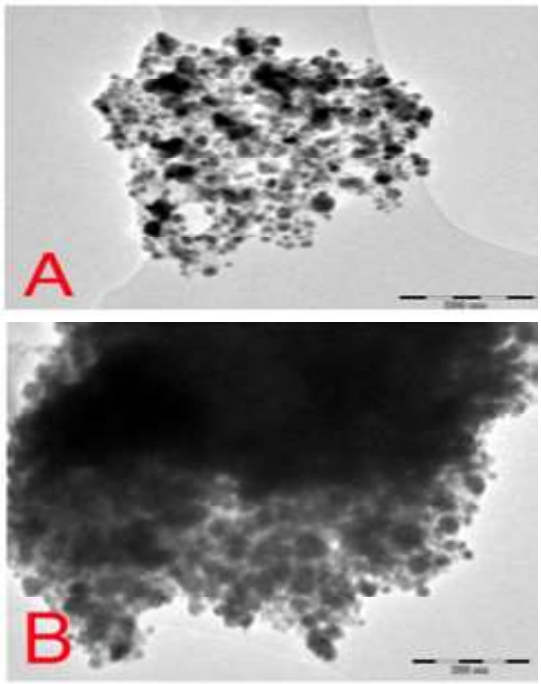

Figure 3. TEM micrograph of CuONPs synthesized from leaf extract

The surface morphology of nanoparticles synthesized with the aid of *Azolla pinnata* leaf extract was examined using field emission scanning electron microscopy (FE-SEM), as shown in the provided figure. The SEM images clearly showcase particles with a uniform spherical shape, highlighting the efficacy of the synthesis process (Figure

4). The analysis of particle size distribution indicated an average diameter of around 36.73 nm for the copper oxide nanoparticles, but also revealed the occurrence of larger particles in the sample, suggesting potential aggregation during the synthesis of copper oxide nanoparticles (CuONPs).[18]

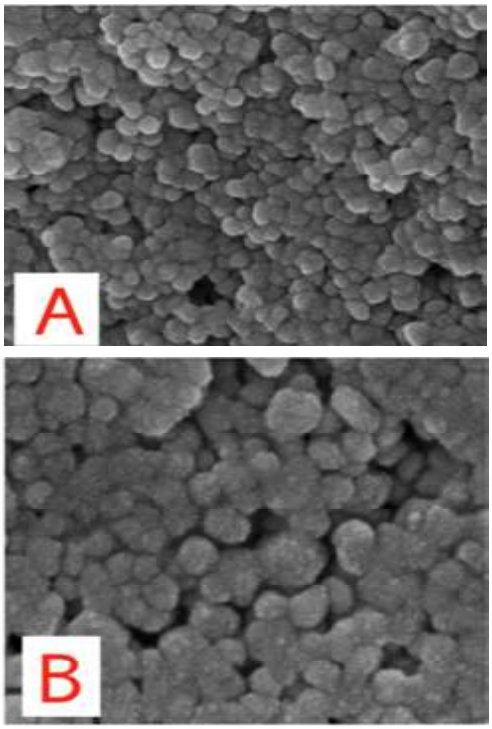

Figure 4. SEM micrograph of CuONPs synthesized from leaf extract

Detailed observations of the spherical copper oxide nanoparticles' surface features were obtained from FE-SEM micrographs at 1 mm (for broader perspective) and 100 nm (for detailed scrutiny), revealing the particles' distinctly rough surface texture. Additionally, the chemical makeup of the synthesized nanoparticles was analyzed using energy-dispersive X-ray spectroscopy (EDS). The EDS spectrum identified the presence of copper (Cu) alongside elements from organic compounds surrounding the nanoparticle aggregates, including oxygen (O), carbon (C), chlorine (Cl), potassium (K), and calcium (Ca). Quantitative analysis showed copper as the major component, constituting 73.43%, followed by oxygen with 5.35 %, and minor contributions from carbon, chlorine, potassium, and calcium. The detection of copper confirms the synthesis of CuONPs, while carbon, oxygen, and chlorine are likely derivatives of the *Azolla pinnata* extract, as illustrated in the associated figure (c, d). Significantly, the EDS analysis verifies the metallic nature of the CuONPs and their purity from other impurities.

CONCLUSION

The green synthesis of copper oxide nanoparticles (CuO-NPs) using the aqueous extract of *Azolla pinnata* demonstrates a promising, eco-friendly approach to nanoparticle production. The use of *Azolla pinnata* not only leverages its natural abundance and low cost but also aligns with sustainable and green chemistry principles, minimizing environmental impact. The study successfully synthesized CuO-NPs with desirable characteristics, confirming the potential of plant-mediated synthesis. Characterization techniques, including X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS), collectively revealed that the synthesized CuO-NPs possess a face-centered cubic structure with a crystalline size ranging from 20-50 nm. The nanoparticles exhibited a predominantly spherical shape with uniform size distribution, as confirmed by TEM and SEM analyses. The presence of a capping organic layer, which enhances nanoparticle stability, was also noted. The synthesized nanoparticles demonstrated notable stability in colloidal solutions, attributed to their negative zeta potential, which prevents aggregation by creating repulsive forces among particles. This stability is crucial for their potential applications in various fields, including catalysis, biomedicine, and environmental remediation. Overall, this research highlights the effectiveness of *Azolla pinnata* extract in synthesizing stable, well-characterized CuO-NPs. The successful implementation of this green synthesis method opens avenues for further exploration and application of plant-based nanoparticle synthesis, promoting environmentally friendly practices in nanotechnology and photocatalytic treatment of waste water.

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