Green Synthesis of Copper Oxide Nanoparticles Using *Rauvolfia serpentina* Root Extract and its Characterization

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Abstract: ***The development of sustainable and environmentally benign methods for nanoparticle synthesis is of growing importance in nanotechnology. In this study, copper oxide (CuO) nanoparticles were synthesized using Rauvolfia serpentina root extract, a dark brown coloured solution acts as a natural reducing and stabilizing agent. The color change of the reaction mixture (dark brown to dark maroon red) and the biosynthesized plant extract show a characteristic absorption peak at 334 nm in UV–Vis spectroscopy, confirming nanoparticle formation. The sharp peaks in the FTIR analysis at 1083 cm⁻¹ and 1045 cm⁻¹ indicated the involvement of phytochemicals such as phenolics, alkaloids, and flavonoids in the reduction and capping processes. X-ray diffraction (XRD) analysis revealed that the nanoparticles exhibited a monoclinic CuO crystal structure (JCPDS 48-1548) with crystallite sizes in the 20–30 nm range, as estimated by the Scherrer equation. Scanning electron microscopy (SEM) revealed predominantly spherical nanoparticles with average particle sizes ranging from 40 to 80 nm, consistent with XRD findings. These findings highlight the potential applications of biogenic CuO nanoparticles in catalysis, antimicrobial activity, sensors, environmental remediation, and energy storage, while aligning with the principles of green chemistry.***

Keywords: *Rauvolfia serpentina*, Copper oxide nanoparticles

1. Introduction

In the last few decades, the field of nanotechnology has become one of the most active areas of customizable materials science [1], with wide practicability in various clinical applications, due mainly to the specific size-dependent properties exhibited by the resulting nanomaterials as a direct consequence of a controlled synthesis procedure [2]. Metal and metal oxide nanoparticles have made significant contributions to biomedical sensing, imaging, diagnosis, and treatment in recent years. Silver, copper, and gold are the three most prevalent metals in use today. Among these metal nanoparticles, Copper is a low-cost, high-yielding material that can be used in biomedical and environmental remediation applications [3]. Copper and copper oxides-based nanomaterials have garnered considerable attention in recent years due to their intriguing properties, which make them applicable in a wide variety of fields, including sensors [4,5], catalysts [6,7], electronics [8], optics [9], solar cells [10], environmental remediation [11,12], antimicrobials [13,14] etc. Using plant extracts to synthesize CuO NPs has been proven to be cost-effective, fast, simple, and environmentally friendly [15].

In the present work, the synthesis, characterization, and multidisciplinary properties of CuO nanoparticles prepared via a greener route at low temperature using ethanolic root extract of *Rauvolfia serpentina* as a fuel.

Rauvolfia serpentina was one of the medicinal species of flowering plants, which belongs to the family Apocynaceae. It was also called ‘Indian snakeroot’ or ‘sarpagandha’ [16]. In Ayurveda, the powdered form of the root of *Rauvolfia serpentina* has been used for the treatment of mental illness, snake bites, and feverish illnesses [17]. *R. serpentina* is used as a therapeutic agent to treat insomnia, high blood pressure, anxiety, and epilepsy. Reserpine is an effective indole alkaloid first isolated from the Sarpagandha plant, which is widely used as an antihypertensive [18]. In *Rauvolfia serpentina,* the following alkaloids are present: ajmalicine, ajmaline, ajmalimine, indobine, deserpidine, indobinine, reserpiline, reserpine, rescinnamine, serpentinine, rescinnamidine, yohimbine, and serpentine [19].

2. Materials and Methods

Sample Collection: (Materials)

The roots of *Rauvolfia serpentina* used as the green source in this synthesis process (Fig. 1) were procured from Indian Jadibooti (100% Pure Ayurvedic Herbs), Delhi. It was taxonomically identified, and a voucher specimen was deposited in the Herbarium of Cluster University, Silver Jubilee Government College (A), Kurnool. The collected dry roots were cut into fine pieces and stored in an airtight container at room temperature until further use. All the chemicals were purchased from Sigma-Aldrich Chemicals, India.

Figure 1: ***Rauvolfia serpentina roots*** **Soxhlet root extract** **Biosynthesized CuoNPs**

Preparation of the *Rauvolfia serpentina* root extract:

Dried roots were cut into small pieces and then powdered with the help of an electric grinder. For preparation of the ethanolic root extract of *Rauvolfia serpentina*, 20 g of powdered root was taken in a Soxhlet apparatus (Fig. 1) and extracted with ethanol (95%) for about 6 hr at 35°C. The alcoholic root extract of *Rauvolfia serpentina* was stored in a refrigerator at 4°C for further use.

Biosynthesis of the *Rauvolfia serpentina root* extract with ethanolic solution of CUO NPs:

To biosynthesize CUO nanoparticles, the isolated root extract acted as a green source. For the fabrication of CUO nanoparticles, we first prepared 100 mL of a 1 mM ethanolic solution of CuO nanopowder. Then, 25 mL of isolated root extract was placed into a conical flask. The flask was then placed on a magnetic stirrer. 70 mL (1 mM) of CuO nanopowder solution was added dropwise to the root extract. The biosynthesis of CuO in the reaction was supervised by observing the colour change from light brown to dark brown.

3. Results and Discussion

UV–Visible Spectroscopic Analysis:

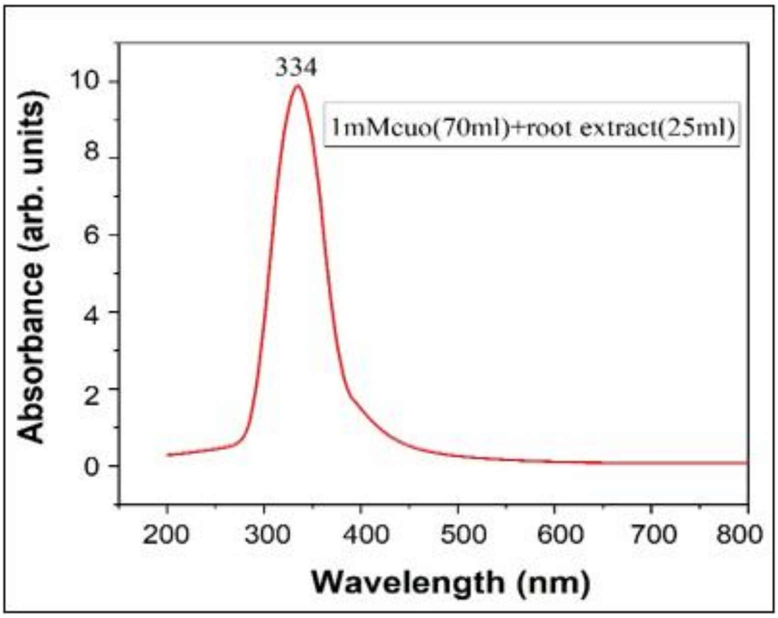

Figure 2: UV–Vis spectrum of *root* extract of *Rauvolfia serpentina* biosynthesized with CuO Nps

The formation of CuO NPs was followed through measurement of its SPR peak using a UV–Vis spectrophotometer. As shown in Fig. 2, the maximum absorption peak occurred at 334 nm, attributed to the SPR absorption band of CuO NPs. The SPR absorption band of CuO NPs at 334 nm confirmed the formation of CuO NPs. The position of the peak indicates the formation of nanoscale particles, with stability imparted by phytochemicals in the extract. As the wavelength increased, the absorbance intensity decreased, suggesting that NPs formation did not occur at a large wavelength. CuO NPs formed in agreement with studies that proposed that CuO NPs formed between 200 and 350 nm [20].

Fourier Transform Infrared Spectroscopic Analysis (FTIR):

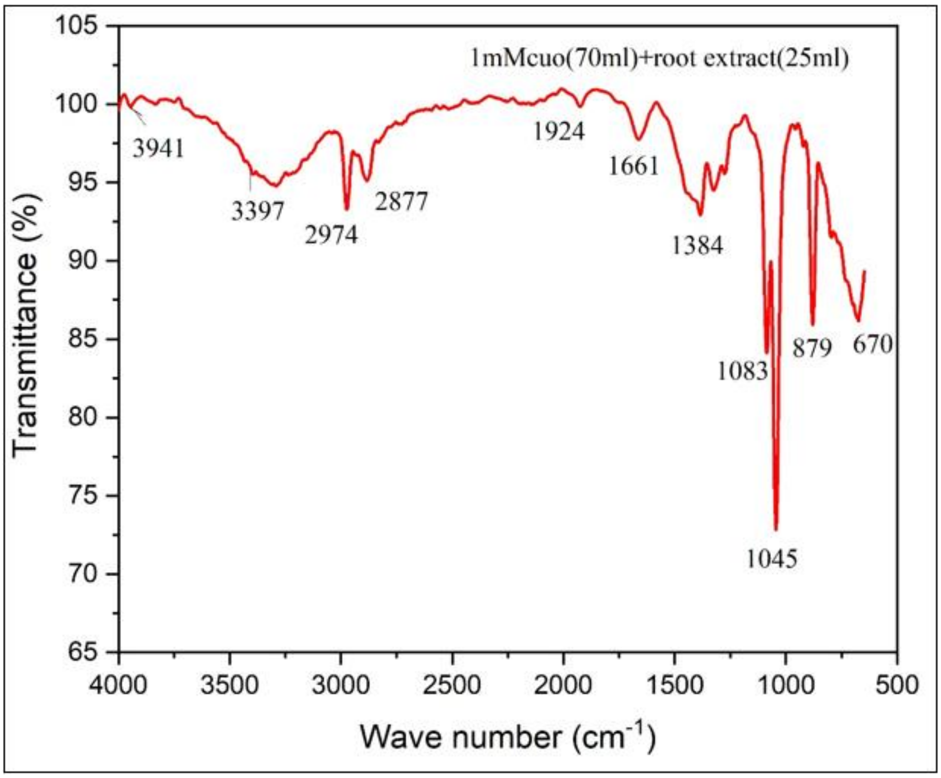

Figure 3: FTIR spectrum of *root* extract of *Rauvolfia serpentina* biosynthesized with CuO Nps

The FTIR spectrum (Fig. 3) was recorded for the interaction between the root extract and Cuo nanoparticles; Broad peaks at 3941 cm⁻¹ & 3397 cm⁻¹ correspond to O–H stretching vibrations (hydroxyl groups) and possibly N–H stretching (amines). These indicate the presence of alcohols, phenols, or amines from *Rauvolfia serpentina* root extract, acting as reducing and stabilizing agents. Peaks at 2974 cm⁻¹ & 2877 cm⁻¹ are due to C–H stretching vibrations of alkanes (methyl and methylene groups), suggesting the presence of organic phytochemicals (alkaloids, terpenoids, etc.). Peaks at 1924 cm⁻¹ & 1661 cm⁻¹ are due to C–H stretching vibrations of alkanes (methyl and methylene groups). The 1924 cm⁻¹ may be due to overtone or combination bands, sometimes associated with aromatic systems. The peak at 1384 cm⁻¹ is characteristic of C–N stretching (amines) or the symmetric stretching of NO_3^- groups, which could originate from plant alkaloids. The peak in the range of 1083 cm⁻¹ & 1045 cm⁻¹ is associated with C–O stretching vibrations of alcohols, ethers, or phenols, indicating that phytochemicals are capping the nanoparticles. The peaks at 879 cm⁻¹ & 670 cm⁻¹ are metal–oxygen (Cu–O) stretching vibrations, confirming the presence of CuO nanoparticles. The sharpness of these peaks indicates good crystallinity. The FTIR spectrum confirms the efficacious green synthesis of CuO nanoparticles, where phytochemicals (alkaloids, terpenoids, etc.) from Rauvolfia serpentina root extract serve as both reducing and stabilising agents.

X-ray Diffraction (XRD):

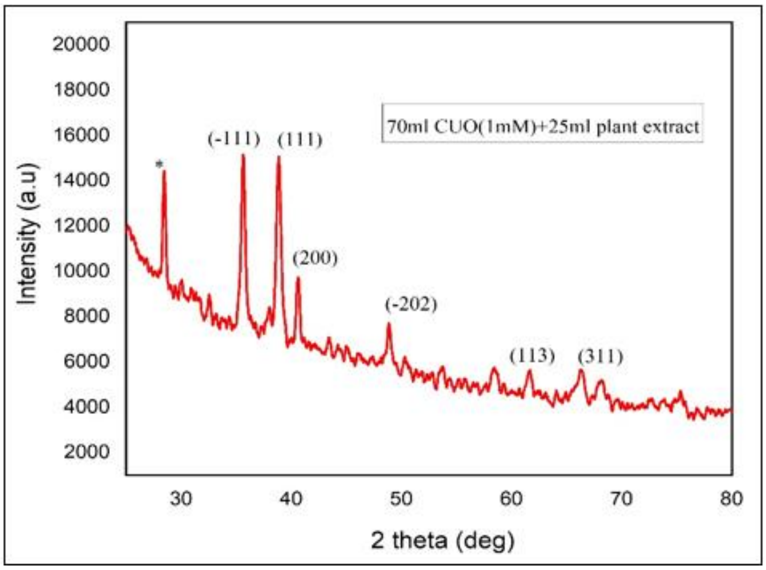

Figure 4: XRD pattern of *root* extract of *Rauvolfia serpentina* biosynthesized with CuO Nps

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The XRD diffraction pattern of CuO NPs is presented in Fig. 4. X-ray diffraction analysis reveals the presence of peaks at 2θ values ranging from 20° to 80°. The marked (-111), (111), (200), (-202), (113), and (311) diffraction peaks in the XRD patterns are well matched with the standard diffraction peaks JCPDS: card no. 48-1548 [21]. The intensity of the (-111) and (111) peaks is much stronger than that of other peaks, which indicates the preferred orientation of the formed nanocrystals along these directions. The size of the Cu nanoparticles is assessed by applying Debye-Scherrer's equation.: $D = 0.9\lambda/\beta\cos\theta$, where D is the average crystallite size. λ is the X-ray wavelength (1.54 nm); β is the (FWHM), and θ is the Bragg angle. The average crystallite size of CuO Nps was found to be 23.8 nm. Other researchers have confirmed the green synthesis of CuO NPs in this size range. 22.4 nm of CuO NPs were synthesized using *Bacopa monnieri* leaf extract [22]. The average size of CuO NPs was calculated as 29.7 nm in a green synthesis using *Curcuma longa* root extracts [23].

Scanning Electron Microscope (SEM):

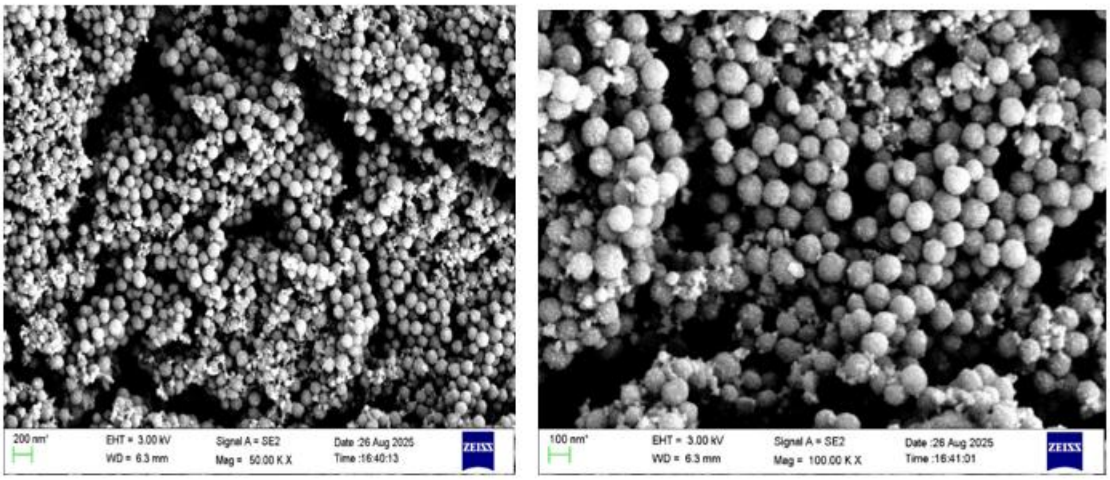

Figure 5: SEM images of the synthesized CuO Nps

The morphology and surface structure of the synthesized CuO NPs were analyzed using SEM, and the obtained micrograph is shown in Fig. 6. The SEM images confirm that the shape of the nanoparticles was spherical with a 40–80 nm size range, aggregated into dense clusters. Previous studies have confirmed the CuO NPs' spherical shape [24]. The particles are relatively uniform, with good sphericity, suggesting controlled nucleation during synthesis. Some agglomeration is visible, which is common in green-synthesized metal oxides due to the presence of phytochemical capping agents and van der Waals forces. The surface texture appears smooth to slightly rough, typical of crystalline oxide nanoparticles. No sharp edges are visible, which again confirms spherical/near-spherical growth habit. The particle size distribution for CuO NPs obtained in this study agreed with previous studies [25].

4. Future Prospective

The present study demonstrates that *Rauvolfia serpentina* root extract can act as a sustainable, eco-friendly reducing and stabilizing agent for the synthesis of spherical CuO nanoparticles with sizes in the nanometer regime. Moving forward, several avenues can be explored to enhance the scope and applicability of this work, such as Optimization of synthesis parameters (pH, extract concentration, temperature) to control nanoparticle size, shape, and stability, and Advanced characterization (TEM, XPS, BET) to understand crystallinity, surface chemistry, and interaction with phytochemicals, and electrode materials in supercapacitors/batteries e.t.c. Overall, the integration of green chemistry principles with nanotechnology using medicinal plants like *Rauvolfia serpentina* not only reduces environmental impact but also opens pathways for scalable, multifunctional nanomaterial production suitable for biomedical, environmental, and energy-related applications.

5. Conclusions

In this work, copper oxide nanoparticles were successfully synthesized using *Rauvolfia serpentina* root extract through an eco-friendly and cost-effective green chemistry route. UV–Vis spectroscopy confirmed nanoparticle formation with an absorption peak at ~334 nm, while FTIR analysis revealed the presence of phytochemicals responsible for the reduction and capping of CuO nanoparticles. XRD patterns matched well with the standard JCPDS card (48-1548), confirming monoclinic CuO with crystallite sizes in the 20–30 nm range as calculated by the Scherrer equation. SEM micrographs further demonstrated predominantly spherical nanoparticles with an average particle size distribution in the range of 40–80 nm, consistent with the XRD crystallite data. The combined results clearly validate that *Rauvolfia serpentina* root extract can serve as a reliable biogenic agent for the synthesis and stabilization of nanoscale CuO.

The present study not only highlights a sustainable pathway for nanoparticle production but also provides insights into structural and morphological features that can be tailored for specific applications. These findings open avenues for employing such plant-mediated CuO nanoparticles in diverse fields, including catalysis, antimicrobial coatings, sensors, energy storage, and environmental remediation. Overall, this research reinforces the potential of integrating green synthesis with nanotechnology for developing multifunctional and environmentally benign nanomaterials.

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