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Phytochemical Analysis and Synthesis of Copper Nanoparticles Using Aqueous Leaf Extract of Rotheca Serrata (Bharangi)

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Abstract

Background: *Green synthesis of metal nanoparticles using plant extracts has gained significant attention due to its eco-friendly, cost-effective, and sustainable nature. Plant-mediated synthesis avoids the use of toxic chemicals and utilizes naturally occurring phytochemicals as reducing and stabilizing agents. Rotheca serrata (Bharangi) is a medicinal plant rich in bioactive compounds, making it a suitable candidate for the green synthesis of copper nanoparticles (CuNPs).*

Materials and Methods: *In the present study, an aqueous leaf extract of Rotheca serrata was prepared and subjected to preliminary phytochemical screening to identify the presence of bioactive constituents. The extract was then employed for the green synthesis of copper nanoparticles through the bioreduction of copper ions. The synthesized CuNPs were characterized using ultraviolet–visible (UV–Vis) spectroscopy to confirm nanoparticle formation and Fourier transform infrared (FTIR) spectroscopy to identify the functional groups involved in the reduction and stabilization processes.*

Results: *Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, and terpenoids in the Rotheca serrata leaf extract. These phytochemicals are known for their strong reducing and capping abilities and play a vital role in nanoparticle formation. UV–Vis spectral analysis showed a distinct surface plasmon resonance (SPR) absorption peak around 355 nm, confirming the successful synthesis of copper nanoparticles. The position and intensity of the SPR peak provided insights into the optical properties, size and distribution of the CuNPs. FTIR spectra exhibited characteristic absorption bands corresponding to hydroxyl, carbonyl and amino functional groups, indicating the involvement of plant phytochemicals in the reduction and stabilization of the nanoparticles. Additionally, absorption bands observed in the 400–600 cm^{-1} region further confirmed the formation of copper-based nanoparticles.*

Conclusion: *The combined results of phytochemical screening, UV–Vis spectroscopy and FTIR analysis confirm the successful green synthesis of copper nanoparticles using Rotheca serrata leaf extract. This environmentally benign and sustainable approach demonstrates the effective role of plant phytochemicals in nanoparticle synthesis and highlights the potential of plant-mediated methods for the fabrication of metal nanoparticles with diverse applications.*

Keywords: *Rotheca serrata; Copper nanoparticles; Green synthesis; Phytochemical screening.*

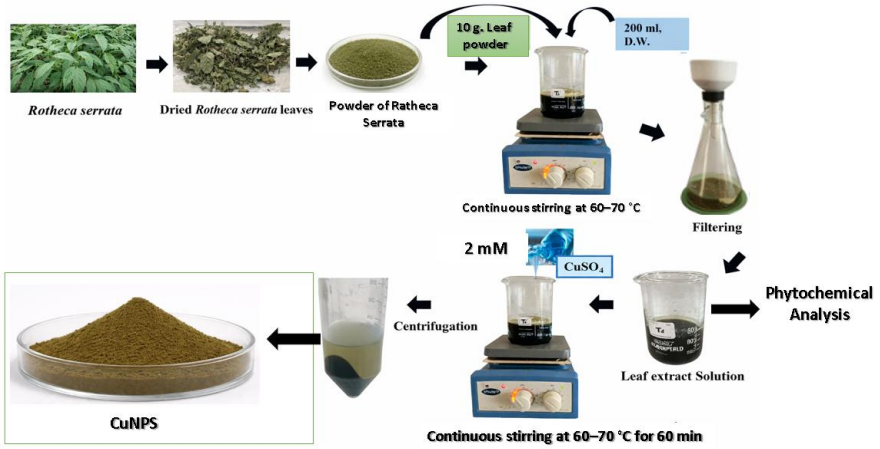

Figure 1. Graphical overview of phytochemical screening and copper nanoparticle synthesis

I. Introduction

Nanotechnology has transformed modern science by enabling controlled manipulation of matter at the nanometer scale (1–100 nm), where materials exhibit unique physical, chemical and biological properties distinct from their bulk counterparts. Metal nanoparticles have emerged as pivotal nanostructures due to their remarkable optical, electrical, catalytic and antimicrobial characteristics, opening new avenues in diverse fields such as biomedicine, environmental remediation, electronics and catalysis [1]. Among various metal nanoparticles, copper nanoparticles (CuNPs) have attracted significant interest because of their cost-effectiveness, abundant availability and versatile properties, especially compared to noble metals like gold and silver. This has spurred research into their synthesis and applications in recent years [2-3].

Traditional physical and chemical methods for CuNPs synthesis often require high energy, hazardous reagents and complex instrumentation and may produce toxic by-products that pose environmental and biological risks. Consequently, there has been a strong movement toward developing sustainable and environmentally benign synthesis strategies that can minimize ecological impact while maintaining nanoparticle functionality. In this context, green synthesis approaches using biological systems are gaining momentum as eco-friendly alternatives, aligning with the principles of green chemistry [4-6].

Plant-mediated synthesis represents one of the most promising green nanotechnological practices due to its simplicity, scalability, cost-effectiveness and the inherent presence of naturally active phytochemicals. Plant extracts contain diverse bioactive molecules such as flavonoids, phenolic compounds, alkaloids, terpenoids and proteins which can serve as reducing, capping and stabilizing agents in the fabrication of nanoparticles without the need for external toxic chemicals. These phytochemicals not only facilitate the reduction of metal ions but also help stabilize the resulting nanoparticles, often enhancing biocompatibility and functional performance [7-8].

Several studies have demonstrated the successful green synthesis of CuNPs using plant extracts. For instance, Jatropha curcas leaf extract has been used to synthesize stable CuNPs with significant photocatalytic activity and DNA binding properties, highlighting the dual functional role of plant phytochemicals in reduction and stabilization [1]. Numerous studies highlight the efficacy of diverse plant sources such as *Catha edulis* leaves, which produce crystalline CuNPs or CuONPs (20-50 nm) with potent antibacterial activity against pathogens like *E. coli* and *S. aureus*, achieving significant zones of inhibition through phytochemical-mediated stabilization and ROS mechanisms. Similarly, extracts from *Hibiscus sabdariffa* yield stable CuNPs exhibiting strong antimicrobial effects against *S. aureus* (13 mm inhibition zone) and *E. coli* (11 mm), alongside antioxidant properties from flavonoids and phenolics. Extracts from *Ephedra alata* and *Aegle marmelos* have produced eco-friendly CuONPs with spherical morphology, demonstrating photocatalytic dye degradation, wastewater remediation and antibacterial activity against *E. coli* (up to 29 mm inhibition) and *S. aureus*, underscoring biocompatibility and multifunctionality enhanced by green protocols [9-12].

The expanding literature indicates that the nature and composition of plant phytochemicals directly influence the size, morphology, stability and functional properties of CuNPs, which is crucial for targeted applications. Advanced reviews have underscored that green synthesized CuNPs typically exhibit particle sizes ranging from ~1.8 nm to 37 nm, possess surface functionalities identifiable by FTIR and demonstrate performance advantages in biomedical and environmental applications compared to conventional synthesis methods. Additionally, eco-friendly CuNPs have been investigated for their catalytic degradation of pollutants, antimicrobial efficacy, antioxidant potential and therapeutic performance, reinforcing the broad applicability of green nanomaterials [13-14].

Despite the considerable research progress, scalability challenges, broader mechanistic understanding of biosynthesis pathways and optimization of synthesis parameters remain areas of active investigation. Specifically, elucidating how individual phytochemical constituents interact with metal ions at the molecular level during nanoparticle formation is essential for precise control over nanoparticle features and functionality.

Rotheca serrata (Bharangi), a medicinal plant with documented anti-inflammatory, antimicrobial and antioxidant properties, is rich in flavonoids, phenolics, terpenoids and other bioactive compounds, making it an excellent candidate for green nanoparticle synthesis. However, reports focusing on its use for CuNPs fabrication are relatively limited, creating a valuable research opportunity to explore its full potential in nanotechnology. The integration of *R. serrata* phytochemicals in CuNPs synthesis could lead to nanoparticles with enhanced biological compatibility and application-specific properties for biomedical or environmental uses [15]. The taxonomic classification of Bharangi (*Rotheca serrata*) is given below:

Taxonomic Classification of Bharangi

- Kingdom: Plantae
- Subkingdom: Tracheobionta (Vascular plants)
- Division (Phylum): Magnoliophyta (Angiosperms)
- Class: Magnoliopsida (Dicotyledons)

- Order: Lamiales
- Family: Lamiaceae
- Genus: *Rotheca*
- Species: *Rotheca serrata* (L.) Steane & Mabb.

Common Names

- English: Blue fountain bush, Bharangi
- Hindi / Marathi / Sanskrit: Bharangi

Figure 1 Plant of *Rotheca serrata* (Bharangi)

The present study addresses this gap by investigating the eco-friendly green synthesis of CuNPs using aqueous leaf extract of *Rotheca serrata*, performing comprehensive phytochemical screening and characterizing the synthesized nanoparticles using techniques such as UV–Vis and FTIR spectroscopy to confirm formation and identify the functional groups involved in reduction and stabilization. This work contributes to the growing body of green nanotechnology literature and underscores the prospective utility of plant-mediated CuNPs across diverse scientific domains.

II. Materials and Methods

Plant collection

Fresh leaves of *Rotheca serrata* (L.) were collected from Sadavali village, Sangameshwar Taluka, Ratnagiri district, Maharashtra, India. The identity of the plant material was confirmed by Assistant Professor Dr. Ranjit Bansode and a voucher specimen was deposited at the Department of Botany, A.S.P. College, Devrukh, Sangameshwar, Ratnagiri, India, to ensure reproducibility and facilitate future reference.

Material used for Phytochemical Screening

The following chemicals are used in various phytochemical tests to detect the presence of specific compounds in plant extracts. Hager's reagent, which is a saturated solution of picric acid is used for detecting alkaloids. Zinc powder and concentrated hydrochloric acid (HCl) are used in the Pew's test for flavonoids, while distilled water and 10% sodium hydroxide (NaOH) are used in the Legal's test for glycosides. The foam test for saponins requires distilled water and plant extract, while the Salkowski test for terpenoids and steroids uses chloroform and concentrated sulfuric acid (H2SO4). For detecting tannins, lead acetate and 5% ferric chloride ($FeCl_3$) are used in the lead acetate and Braymer's tests, respectively. Alcoholic alpha-naphthol and concentrated sulfuric acid (H_2SO_4) are needed for the Molisch test to detect carbohydrates. The biuret test for proteins requires 4% sodium hydroxide (NaOH) solution and 1% copper sulfate ($CuSO_4$), while the stain test for fats and oils involves filter paper and the plant extract. These chemicals are essential for performing specific phytochemical tests to identify the presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, steroids, tannins, phenols, carbohydrates, proteins and fats in plant materials.

Preparation of Aqueous *Rotheca serrata* Leaf Extract

Fresh leaves of *Rotheca serrata* (L.) were collected and thoroughly washed with tap water, followed by washing with distilled water at least twice to remove surface contaminants. The cleaned leaves were cut into small pieces and processed in the laboratory. The samples were then dried in a hot air oven at 40 °C for 24 h. The dried leaves

were finely powdered using a mortar and pestle and stored in airtight polyethylene zipper bags until further use. For the preparation of the aqueous leaf extract, 10 g of the powdered leaf sample was transferred into a clean and dry 500 mL beaker containing 200 mL of distilled water. The mixture was heated at 60–70 °C for 30 min and then allowed to cool to room temperature. The solution was subsequently filtered through Whatman No. 41 filter paper to obtain a clear filtrate. The resulting leaf extract was stored at 4 °C for further use in the preparation of copper oxide nanoparticles [16-17].

Screening of Bioactive Phytochemicals

Test for Alkaloids
Hager's Test: 1.0 ml of plant extract was taken and then add few drops of Hager's reagent (1.0ml of saturated solution of picric acid) was added and observe the formation yellow colour indicates the presence of alkaloids [18-19].
Test for Flavonoids
Pew's Test: 1.0 ml of plant extract was taken and then add zinc powder in a test tube, followed by drop wise addition of concentrate HCl observe the formation the formation of purple red or cherry colour indicates the presence of Flavonoids [20-21].
Tests for Glycosides
Legal's Test: 1.0 ml of plant extract was taken and few drops of conc. HCl added and was boiled for 4-5 hours. To this 1 ml of distilled water added followed by the addition of 10% NaOH. Observe the formation of yellow colour indicates the presence of Glycosides [22-26].
Test for Saponin
Foam Test:1.0 ml of plant extract was taken and 5 ml of distilled water mixed vigorously for 15 sec. and observe for persistent froth appearance indicates the presence of Saponin [23,26].
Test for Terpenoids
Salkowski Test: 1.0 ml of plant extract was treated with chloroform and filtered. Filtrates were treated with conc. H2SO4, shaken vigorously and allowed to stand. Observed for the formation of reddish-brown colour at the lower layer which indicates presence of sterols and yellow color at the lower layer indicates the presence of terpenoids [24-26].
Test for Steroids
Salkowski Test: Mix 1.0 ml of plant extract with chloroform. Add 2ml concentrated H2SO4 and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow florescence indicated the presence of steroids [26].
Test for Tannins
Lead acetate Test-1.0 ml of plant extract was taken then add lead acetate observe the formation white precipitate indicates the presence of tannins.
Braymer's Test- Mix 1.0 ml of plant extract with 2 ml water, then add 2 to 3 drops of 5% FeCl3. The solution turned green precipitate indicates the presence of tannins [22-26].
Test for Phenols
Ferric Chloride test: 1.0 ml of plant extract was taken then add 3-4 drops of 5% FeCl3 added and observe for the bluish black coloration indicates the presence of Phenols [26-27].
Test for Carbohydrates
Molisch test:1.0 ml of plant extract was taken then few drops of alcoholic alpha-naphthol was added followed by the Addition of 0.2 ml concentrated sulphuric acid slowly along the sides of test tube, purple to violet colour ring appears at junction [28].
Test for Proteins
Biuret test: 1.0 ml of plant extract was treated with 4% of NaOH solution. Then add 1% CuSo4 solution, violet colour not appears indicates absence of proteins [26, 28].
Test for Fats and Oils
Stain test: Small quantity of extract has to be pressed between two filter paper an oily stain on filter paper indicates that presence of fix oil [26].

Biosynthesis of Copper Nanoparticles (CuNPs)

The biosynthesis of CuNPs was carried out using 2 mM $CuSO_4 \cdot 5H_2O$ as the precursor. Freshly prepared aqueous leaf extract of *Rotheca serrata* (Bharangi) was added to the copper solution, and the mixture was continuously stirred at 60–70 °C for 60 min. A distinct dark brownish-green colour change, typically observed within the first 30 min of stirring, indicated the successful formation of nanoparticles.

The reaction mixture was centrifuged at 10,000 rpm for 15 min to isolate the nanoparticles from unreacted chemicals. The supernatant was carefully discarded, and the nanoparticle pellet was collected. The

pellet was washed several times with double-distilled water to remove any residual chemicals or by-products. After washing, the nanoparticles were dried at room temperature in an air oven to remove moisture. Once completely dried, the sample was finely ground using a mortar and pestle to ensure a uniform particle size distribution, which facilitates homogeneous dispersion for further analysis. The resulting CuONPs were stored in a sealed container to preserve their integrity and stability, making them ready for subsequent characterization [29-30].

III. Characterization of the Nanoparticles:

UV-Vis Spectroscopy: To confirm the formation of nanoparticles, the UV-Vis spectra of the copper nanoparticle solutions were recorded in the range of 200–800 nm. The presence of a characteristic surface plasmon resonance (SPR) peak was used to confirm the synthesis and size distribution of the nanoparticles.

FTIR Spectroscopy: The functional groups involved in the reduction and stabilization of the nanoparticles were identified using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectra of both the leaf extract and the nanoparticles were recorded in the range of 4000–400 cm^{-1} to identify the bioactive compounds that facilitated the nanoparticle synthesis [16-17].

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical comparisons were carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among groups. A p-value < 0.05 was considered statistically significant. Data analysis was performed using Microsoft excel and Origin Software.

IV.Result and Discussion

Phytochemical Analysis

The phytochemical analysis of *Rotheca serrata* (Bharangi) leaf extract, as detailed in Table [1], revealed the presence of several bioactive compounds, each playing a vital role in the reduction and stabilization of copper nanoparticles. These bioactive compounds, including alkaloids, flavonoids, phenolic compounds, terpenoids and saponins, are well-known for their strong antioxidant, reducing and capping properties, making them ideal candidates for the green synthesis of metal nanoparticles [18-28].

Table 1. Major phytoconstituents present in *Rotheca serrata* (L.) leaf extract

Phytochemical Constituents	Aqueous
Alkaloids	+
Flavonoids	+
Carbohydrate	+
Phenolic Compounds	+
Saponins	+
Terpenoids	+
Steroids	+
Tannins	–
Glycosides	+

Key: Present phytochemicals are represented by (+) sign, absent phytochemicals are represented by (-) sign

V. Characterization of Copper Nanoparticles

UV-Visible Spectroscopy

UV–Vis spectroscopy was employed to monitor the green synthesis of copper (Cu) nanoparticles using the aqueous leaf extract of *Rotheca serrata* (Bharangi). The UV–Vis spectrum of the plant extract (Figure 2) exhibits a broad absorption band around 400 nm, which is attributed to the presence of bioactive phytochemicals such as flavonoids, phenolic compounds, and other organic constituents capable of absorbing in the UV–visible region. These biomolecules are known for their antioxidant and reducing properties, which facilitate metal ion reduction during nanoparticle synthesis. Notably, the absence of a distinct surface plasmon resonance (SPR) band in the plant extract confirms that no nanoparticles were present prior to synthesis. In contrast, the UV–Vis spectrum of the synthesized copper nanoparticles (Figure 2) displays a prominent SPR band centered at approximately 355 nm, which is characteristic of CuNPs and confirms their successful formation. The increased intensity and sharpness of the SPR peak indicate the formation of well-dispersed, stable, and uniformly sized nanoparticles, demonstrating the effective reduction of copper ions by the *Rotheca serrata* leaf extract [16-17].

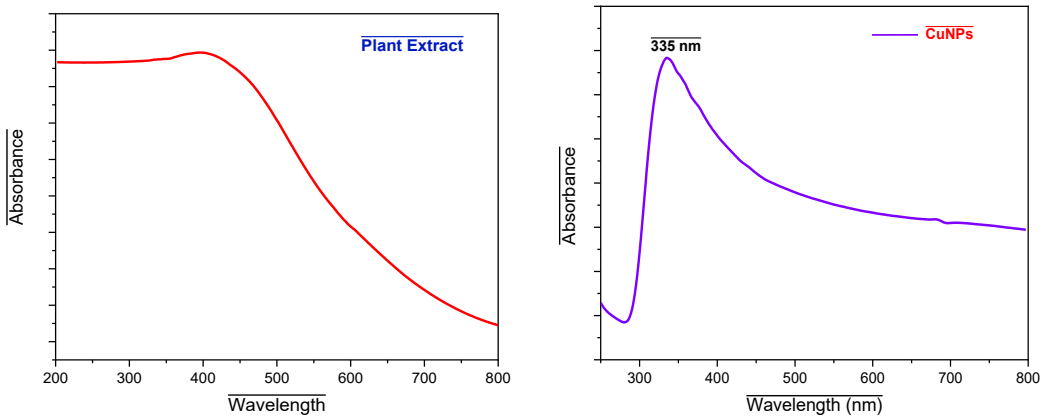

Figure 2. UV–Visible absorption spectra of *Rotheca serrata* leaf extract and green-synthesized copper nanoparticles

Fourier Transform Infrared Spectroscopy

The FTIR spectrum of green-synthesized copper nanoparticles typically exhibits characteristic absorption bands corresponding to functional groups derived from plant biomolecules as shown in figure 3. A broad band observed in the region of 3200–3500 cm^{-1} is generally assigned to O–H stretching vibrations of hydroxyl groups from phenolic compounds, alcohols and adsorbed water molecules, which play a crucial role in the reduction and stabilization of nanoparticles. The absorption band around 2920–2950 cm^{-1} corresponds to C–H stretching vibrations of aliphatic hydrocarbons, indicating the presence of lipid or fatty acid components that contribute to nanoparticle stabilization. Bands appearing in the range of 2100–2400 cm^{-1} are attributed to C≡C or C≡N stretching vibrations associated with alkynes, nitriles, or conjugated biomolecules involved in metal ion reduction. The prominent peak observed between 1600 and 1650 cm^{-1} is assigned to C=O stretching vibrations of carbonyl or amide groups, suggesting the involvement of proteins and carboxylic acids as effective capping agents. The band in the region of 1380–1450 cm^{-1} corresponds to C–O bending or symmetric stretching of carboxylate groups, further supporting metal–ligand interactions. Additionally, absorption bands between 1020 and 1150 cm^{-1} are attributed to C–N or C–O stretching vibrations of amino acids and polysaccharides, while the low-frequency bands observed between 600 and 400 cm^{-1} are characteristic of Cu–O stretching vibrations, confirming the formation of copper oxide nanoparticles [16-17].The analysis is represented in table 1.

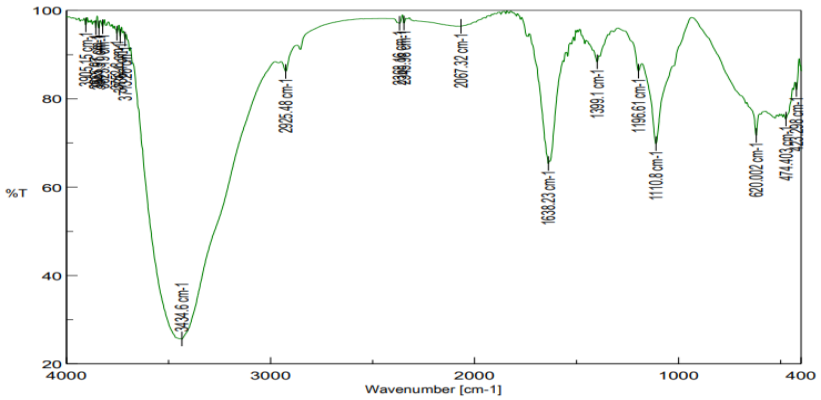

Figure 3. FTIR spectrum showing functional groups involved in CuNPs formation

Table No. 2: FTIR Spectral Analysis of Copper Nanoparticles (CuNPs) Synthesized from *Rotheca serrata* (Bharangi)

Wavenumber (cm^{-1})	Functional Group / Vibration	Typical Source in Plant Extract	Role in CuNPs Synthesis
3200–3500	O–H stretching	Phenols, alcohols, water	Reduction and stabilization
2920–2950	C–H stretching (aliphatic)	Fatty acids, lipids	Surface stabilization
2100–2400	C≡C / C≡N stretching	Alkynes, nitriles, conjugated compounds	Reduction and coordination
1600–1650	C=O stretching (amide I / carbonyl)	Proteins, carboxylic acids	Capping and stabilization
1380–1450	C–O bending / COO^- symmetric stretch	Alcohols, carboxylates	Metal–ligand interaction
1020–1150	C–N stretching / C–O stretching	Amino acids, proteins, polysaccharides	Capping agent
600–400	Cu–O stretching	Copper oxide lattice	Confirmation of CuO formation

VI. Conclusion

The present study successfully demonstrated the green synthesis of copper nanoparticles using the aqueous leaf extract of *Rotheca serrata* (Linn.). The phytochemical screening confirmed the presence of various bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids and steroids, which acted as natural reducing and stabilizing agents in the nanoparticle synthesis process. The change in colour of the reaction mixture provided preliminary evidence of nanoparticle formation, while UV-Visible spectroscopy confirmed the presence of surface plasmon resonance (SPR) peaks characteristic of copper nanoparticles, indicating successful reduction of the metal ions.

Further, FTIR analysis revealed the involvement of functional groups such as hydroxyl, carbonyl and amine groups, confirming their role in both the reduction and stabilization of the nanoparticles. The characteristic metal oxygen vibrations observed in the lower wavenumber region further validated the presence of metallic nanoparticles with oxide surface layers.

This eco-friendly and cost-effective green synthesis approach not only eliminates the need for toxic chemical reducing agents but also provides a sustainable method for nanoparticle production. The results demonstrate that *Rotheca serrata* can serve as a promising plant source for the green synthesis of metal nanoparticles, offering a valuable alternative to conventional chemical methods.

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