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ORIGINAL ARTICLE

Swertia chirayita as a Green Source for Copper Nanoparticles

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ABSTRACT
Copper's nanoscale dimensions offer significant potential for green synthesis, particularly through the use of plant materials for biological, biotechnological, and medicinal applications. This paper details the green manufacturing of copper oxide nanoparticles (CuONPs) using Swertia chirayita leaf extract. The synthesized CuONPs were comprehensively characterized using FTIR, XRD, UV-Vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). We investigated the antibacterial properties of these green-synthesized CuONPs against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) microorganisms. Our research indicates that CuONPs derived from Swertia chirayita show considerable promise in treating resistant bacterial infections in both Gram-positive and Gram-negative strains.
Keywords: *Copper nanoparticles, green synthesis, bioactivity, Spectral Analysis.*

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INTRODUCTION

Nanotechnology, a rapidly expanding interdisciplinary field, has revolutionized materials engineering, electronics, environmental science, and medicine. It focuses on the creation, production, and utilization of materials at the nanoscale, typically below 100 nm [1]. Among the diverse array of metal-based nanomaterials, copper nanoparticles (CuNPs) have emerged as a promising class due to their unique physicochemical properties [2]. These include high electrical conductivity, significant catalytic activity, and broad-spectrum antibacterial potential, making CuNPs ideal candidates for applications in drug delivery, environmental remediation, healthcare, and diagnostics [1].

Traditional methods for CuNP synthesis, such as chemical precipitation, thermal decomposition, and electrochemical reduction, often involve toxic chemicals and generate hazardous by products. These processes are frequently energy-intensive and pose significant environmental risks [3]. Consequently, there has been a paradigm shift towards more sustainable and environmentally benign nanoparticle synthesis techniques.

Green synthesis leverages biological organisms, including plants, fungi, bacteria, and algae, as natural reducing and stabilizing agents [4]. Plant-mediated synthesis has garnered substantial interest due to its simplicity, cost-effectiveness [5], scalability, and ecological compatibility. Plants are rich in diverse secondary metabolites, such as flavonoids, polyphenols, alkaloids, terpenoids, and proteins [6], which can efficiently reduce metal ions and stabilize the resulting nanoparticles [7]. These phytochemicals not only influence the size and structure of the nanoparticles but can also impart enhanced biological activity [8].

Swertia chirayita, a widely recognized medicinal plant native to the Himalayan region, is extensively used in Tibetan and traditional Ayurvedic medicine. Its well-documented medicinal properties include antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and antidiabetic activities [6, 9]. Phytochemical investigations have revealed that *S. chirayita* is abundant in bioactive compounds such as xanthones (e.g., mangiferin), iridoid glycosides, flavonoids, and terpenoids.

This rich biochemical profile makes *S. chirayita* an excellent candidate for the environmentally friendly synthesis of metal nanoparticles, particularly CuNPs [10, 11]. Specifically, the hydroxyl groups present in flavonoids and polyphenols can act as electron donors, facilitating the reduction of Cu^{2+} ions to metallic

Cu^0 [12]. Concurrently, these substances can stabilize and cap the nascent nanoparticles, thereby preventing agglomeration and enhancing their biocompatibility. Furthermore, the inherent biological activities of these phytoconstituents may synergistically enhance the functional efficacy of the synthesized CuNPs, especially in biomedical applications [13].

The global challenge of antibiotic resistance underscores the critical need for novel antimicrobial agents [14]. CuNPs have demonstrated exceptional antibacterial efficacy against both Gram-positive and Gram-negative bacteria by mechanisms such as bacterial membrane disruption, generation of reactive oxygen species (ROS), and interference with intracellular functions [15]. Therefore, CuNPs synthesized using *S. chirayita* are posited to possess potent antibacterial properties, rendering them suitable for integration into medicinal treatments, antimicrobial coatings, and wound dressings [16]. Beyond antimicrobial applications, CuNPs also show significant promise in cancer therapy. Metallic nanoparticles have been shown to induce cytotoxicity in cancer cells through ROS production, DNA damage, and mitochondrial dysfunction [17]. Evaluating the cytotoxicity of *S. chirayita*-derived CuNPs on human cancer cell lines could open avenues for their application in nano-oncology [18].

This study aims to investigate the environmentally friendly synthesis of CuNPs utilizing *Swertia chirayita* aqueous leaf extract [19]. A primary objective is to thoroughly assess their structural, morphological, and functional characteristics using a suite of advanced characterization techniques. These analyses, including UV-Vis spectroscopy, FTIR, TEM, SEM, EDS, and DLS [3, 20, 21, 22], will elucidate the size, shape, elemental composition, and surface chemistry of the synthesized nanoparticles.

The study will evaluate the antibacterial activity of the CuNPs against *Escherichia coli* and *Staphylococcus aureus*. Additionally, their cytotoxic effects will be assessed on selected human cancer cell lines using the MTT assay [10, 23]. The overarching goal of this research is to establish *S. chirayita* as a sustainable plant source for the production of biocompatible CuNPs. This integrated strategy, with its dual emphasis on antibacterial and anticancer potential, significantly enhances its applicability in addressing pressing issues in biomedical science and public health [24]. Successful implementation of green nanotechnology in this context not only supports environmental sustainability but also elevates the value of locally sourced medicinal plants, fostering innovation in both modern nanomedicine and traditional medicine.

MATERIAL AND METHODS

Materials

The extraction process, copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), purchased from Sigma-Aldrich, was used. The solvent for extraction was a 7:3 (v/v) mixture of ethanol and double-distilled water. *Swertia chirayita* leaves were collected locally in Raipur, Chhattisgarh, India, and their botanical identity was verified by the Raipur IGKV (Indira Gandhi Krishi Vishwavidyalaya).

Preparation of *Swertia chirayita* Leaf Extract

Freshly gathered *Swertia chirayita* leaves were initially cleaned with tap water to remove surface dust, followed by a rinse with double-distilled water to eliminate any residual moisture. The cleaned leaves were then shade-dried for one week. After drying, the leaves were ground into a fine powder using a grinder mixer and stored in a dark place at room temperature until use. To prepare the plant extract, 2 g of the dried leaf powder was added to 100 mL of the ethanol:water (7:3) solvent mixture in a 250 mL flask. The mixture was then thoroughly agitated on a magnetic stirrer with a heated plate, maintaining a temperature between 45 °C and 55°C for 20–30 minutes. The resulting crude extract was first filtered using Whatman filter paper, followed by vacuum filtration through glass wool to obtain a clear filtrate. This filtrate was either used immediately or stored at 4°C for subsequent use [25, 26].

Synthesis of Copper Nanoparticles (CuNPs)

For the synthesis of copper nanoparticles, 5 mL of the prepared aqueous *Swertia chirayita* leaf extract was combined with 50 mL of a 5 mM copper sulfate ($CuSO_4$) solution. The pH of this mixture was adjusted to 7.0 by the dropwise addition of a 1 N NaOH solution, which resulted in a visible color change to greenish, indicating the initiation of nanoparticle formation. Following the reaction, the mixture was centrifuged to collect the precipitated CuNPs as pellets. These pellets were then dried overnight in a hot air oven at 45–55°C. The resulting dark green powder, identified as the synthesized CuNPs, was stored at room temperature for further analysis.

Evaluation of Antibacterial Activity

The antibacterial efficacy of the synthesized CuNPs was assessed against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the disc diffusion method [27]. Seven different treatment groups were established:

(a) Water (Control)
(b) Plant Extract

(c) 0.1% $CuSO_4$ solution
(d) 1% $CuSO_4$ solution
(e) 0.15% (w/v) CuNPs
(f) 0.20% (w/v) CuNPs
(g) 0.25% (w/v) CuNPs

All treatments were performed in triplicates, and the entire experiment was replicated three times for statistical robustness. After incubation, the zones of inhibition around the discs were measured in millimeters (mm). The percentage mycelial inhibition rate was determined by comparing the treated plates to the water control (devoid of CuNPs) using Vincent's formula.

Characterization of Synthesized CuNPs

To ascertain their physicochemical characteristics, the produced copper nanoparticles (CuNPs) were thoroughly characterized. In order to verify CuNP production and determine their surface plasmon resonance, absorbance spectra in the 220–540 nm range were recorded using UV-Vis spectroscopy [28]. To determine which particular biomolecules from the *Swertia chirayita* leaf extract were in charge of the reduction and stabilization of the metal ions, Fourier-Transform Infrared (FT-IR) spectroscopy was performed in the 500–4000 cm^{-1} range [20]. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were both used to analyze the morphological features of the CuNPs, such as their size and form [22]. The chemical composition of the nanoparticles was examined using Energy-Dispersive X-ray Spectroscopy (EDS) in the context of SEM. subsequently, a Dynamic Light Scattering (DLS) or Zeta Potential Analysis was used to gauge the stability and particle size distribution. After approximately five minutes of vortexing to break up any aggregates, 1-2 mL of the CuNP suspension was put into a disposable zeta cell.

RESULTS AND DISCUSSION

Antibacterial Activity of CuNPs

According to the disc diffusion method [15], the green-synthesised copper nanoparticles (CuNPs) showed strong antibacterial activity against strains of both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria. Additionally, early studies using the MTT assay demonstrated a significant dose-dependent cytotoxicity of these CuNPs against human cancer cells, indicating that they may have uses in biomedicine beyond antibacterial action [29]. The average zones of inhibition seen under different treatments are compiled in the accompanying table (Table 1), which emphasizes the variations in antibacterial effects:

Table 1: Zone of Inhibition Measurements for Various Treatments against *E. coli* and *S. aureus*

Treatment	*E. coli* Zone of Inhibition (mm)	*S. aureus* Zone of Inhibition (mm)
0.15% CuNPs	11.2	13.4
0.20% CuNPs	13.8	15.6
0.25% CuNPs	15.4	18.2
Plant Extract	5.1	5.8
CuSO4 (1%)	6.3	7.2
Water (Control)	0.0	0.0

The results show that the CuNPs' antibacterial action is dose-dependent, with bigger inhibition zones for both bacterial strains consistently occurring at increasing concentrations. Crucially, in terms of avoiding bacterial growth, the CuNPs performed noticeably better than the plant extract by itself as well as the traditional copper sulfate solution (Figure 1). This emphasizes how copper's nanostructure confers elevated antibacterial capabilities.

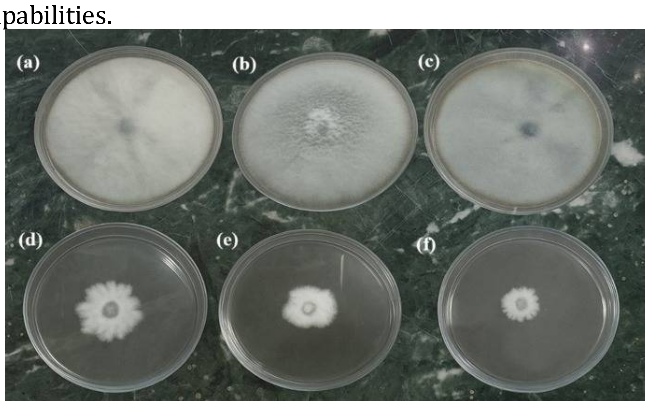

Figure 1: Growth inhibition of (*E. coli*) by *Swertia chirayita* Leaves Extract (a-f).

UV-Visible Spectroscopy Analysis of CuNPs

A noticeable color shift from yellow to green upon adding the *Swertia chirayita* plant extract to the aqueous copper sulfate ($CuSO_4$) solution [19] visually verified the successful production of CuNPs. The surface plasmon resonance (SPR) phenomenon, which results from the collective oscillation of conduction electrons in metal nanoparticles interacting with light photons, is responsible for this color shift. The production of CuNPs was further supported by UV-Vis spectroscopy, which showed a distinctive absorbance signal at 269 nm (Figure 2). The successful reduction of Cu^{2+} ions and the creation of stable CuNPs are indicated by this particular peak. The study also looked into how pH affected the synthesis of nanoparticles which revealed that the ideal pH for reducing Cu^{2+} ions to CuNPs is 7. The prominent absorbance peak seen at neutral pH suggests that this ideal pH promotes the ionization of phenolic groups in the plant extract, increasing their reducing capacity. A rise in particle size was associated with a progressive drop in the peak value, indicating that UV-Vis spectroscopy can also shed light on the dynamics of particle growth.

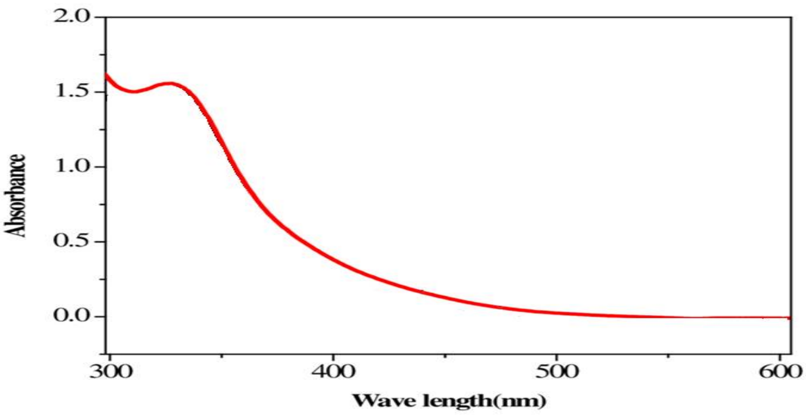

Figure 2: UV Graph of *Swertia chirayita* Leaves Extract

FT-IR Characterization of CuNPs and Involved Biomolecules

The main biomolecules found in the leaf extract of *Swertia chirayita* that are in charge of stabilizing and reducing the CuNPs [21, 30] were identified using Fourier-Transform Infrared (FT-IR) spectroscopy. The broad bands at 3315.28 cm^{-1} in the FT-IR spectra of the *S. chirayita* leaf extract (Figure 3) are indicative of the hydroxyl (OH) functional groups present in phenolic and alcoholic chemicals. While a significant band at 1635.50 cm^{-1} most likely corresponds to the aromatic bending of alkene-type C=C bonds, minor peaks detected between 526.98 and 452.95 cm^{-1} were attributed to the aromatic bending vibrations of alkane C-H groups. Significantly, the synthesized CuNPs' FT-IR spectrum showed characteristic bands at roughly 3264.52 cm^{-1} and 1636.62 cm^{-1}, which are comparable to those in the plant extract [31, 32]. The presence of flavonoids and other phenolic compounds adsorbed onto the surface of the nanoparticle is strongly indicated by these distinctive peaks.

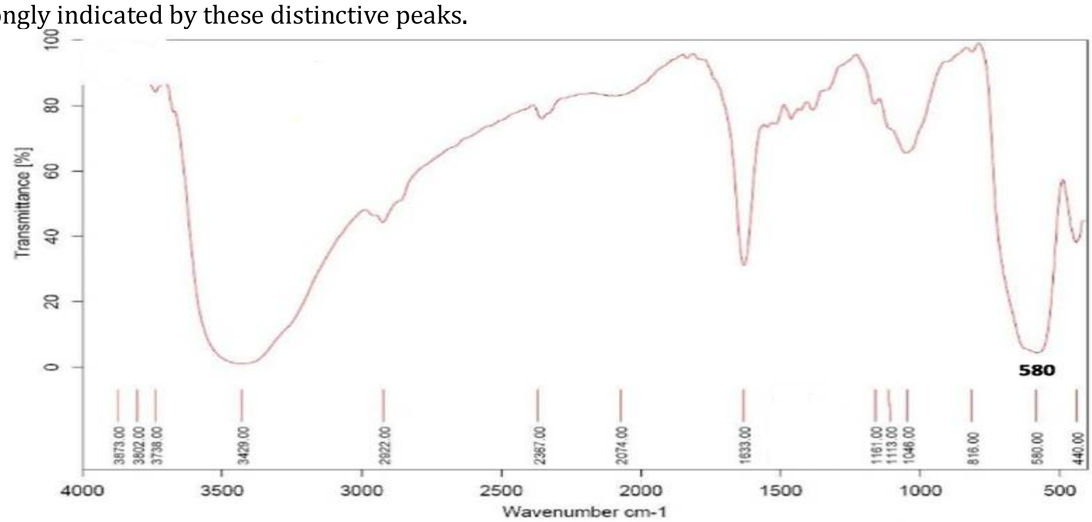

Figure 3: FTIR Graph of *Swertia chirayita* Leaves Extract

It is well known that flavonoids, in particular, can reduce Cu^{2+} ions to CuNPs by releasing a reactive hydrogen atom via an enol-keto tautomerism [10]. By chelating metal ions through their carbonyl groups or π-electrons, these biomolecules also help to stabilize and cap the nanoparticles, reducing agglomeration and improving the overall stability of the produced CuNPs [33].

Morphological and Elemental Characterization of CuNPs
SEM, TEM, and EDS Analysis

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) in tandem with energy-dispersive X-ray spectroscopy (EDS) [22] were used to extensively examine the morphological properties of the green-synthesised CuNPs. Although some agglomeration was seen, most likely as a result of sampling preparation, SEM analysis showed that the majority of the particles were spherical (Figure 4a-b). When combined with SEM, TEM analysis offered comprehensive information on the particle diameters [34, 35]. These investigations produced a size distribution histogram (Figure 4a), which showed that the CuNPs had a narrow size range, usually between 2 and 10 nm, and an average particle diameter of 5 nm.

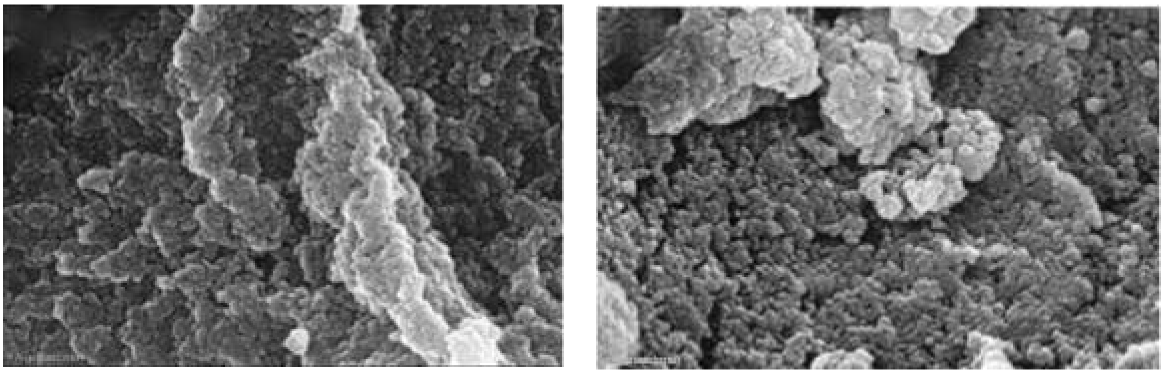

Figure 4a: SEM Images of *Swertia chirayita* Leaves Extract

The elemental composition and stability of the synthesized CuNPs were confirmed by EDS analysis (Figure 4b) [22]. Quantitative investigations demonstrated that the *S. chirayita*-mediated CuNPs comprised 79.87% copper (Cu). The detection of weaker signals for other elements in the EDS spectrum is likely attributed to the presence of various biomacromolecules from the plant extract, such as flavonoids, phenolic chemicals, sugars, glycosides, steroids, and tannins, which serve as capping and stabilizing agents on the nanoparticle surface [36]

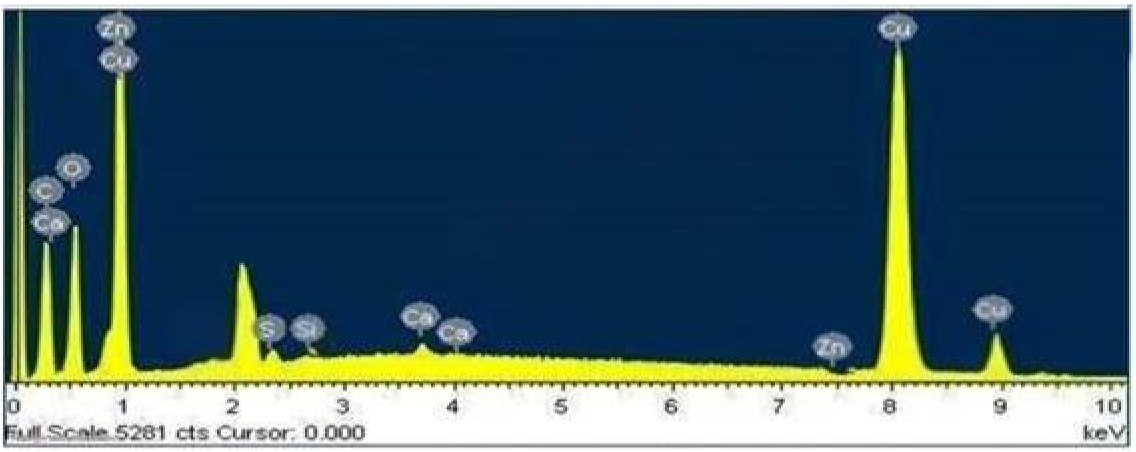

Figure 4b: EDS Spectrum of *Swertia chirayita* Leaves Extract

CONCLUSION AND SOCIETAL IMPACT

This study successfully demonstrates the green synthesis of copper nanoparticles (CuNPs) using *Swertia chirayita* leaf extract, highlighting its significant potential as a sustainable and versatile bioresource [37, 38]. The synthesized CuNPs were thoroughly characterized, confirming their formation, size (average 5 nm), and the crucial role of plant biomolecules in their stabilization. Importantly, the CuNPs exhibited potent dose-dependent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, along with promising cytotoxicity against human cancer cells [39]. This work not only advances the field of green nanotechnology but also underscores the multifaceted relevance of *Swertia chirayita* for pharmaceutical science and public health [40, 41]. To further strengthen the translational impact of these findings, future research should focus on detailed formulation development, in-depth mechanistic studies to elucidate their precise biological actions, and comprehensive *in vivo* toxicity assessments.

REGIONAL RELEVANCE AND SOCIETAL IMPACT OF GREEN NANOTECHNOLOGY IN CHHATTISGARH

The successful application of green nanotechnology in this context holds substantial regional relevance and societal impact for Chhattisgarh, aligning with local environmental, economic, and public health priorities. This research directly contributes to the promotion and scientific validation of indigenous medicinal plants prevalent in Chhattisgarh, particularly in biodiverse tribal areas like Dantewada and Bastar. By substantiating the traditional medicinal knowledge, this effort can foster the commercial utilization, preservation, and sustainable cultivation of these invaluable botanical resources. Furthermore, this low-cost, eco-friendly green synthesis method for CuNPs is particularly advantageous for rural and semi-urban laboratories across Chhattisgarh, where infrastructure might be limited, as it eliminates the need for hazardous chemicals and complex equipment. Local production of such nanoparticles could significantly benefit long-term government health programs by providing affordable therapeutic options for combating infections and potentially aiding in cancer treatment.

Regarding public health applications, the demonstrated antibacterial activity of the CuNPs offers a critical strategy to address antibiotic-resistant illnesses, a growing public health concern within both rural and urban healthcare systems of Chhattisgarh. Economically, this study opens up significant opportunities for rural livelihoods and herbal entrepreneurship. It encourages active participation from tribal farmers, self-help organizations, and herbal cooperatives in value-addition activities, including leaf collection, processing, ethanol-based extraction, and collaborative ventures with startups or universities for green synthesis enterprises. These initiatives are directly in line with and can be supported by existing government programs such as Ayushman Bharat, Startup India, and MSME support.

In terms of education, research, and skill development, this straightforward and inexpensive approach makes it an ideal subject for project work among students in Chhattisgarh universities, thereby promoting practical instruction in green nanotechnology, biotechnology, botany, and chemistry. Its accessibility encourages local laboratories to replicate the technique, fostering a culture of locally based innovation. Lastly, this green synthesis method embodies sustainable and clean technology, directly supporting Chhattisgarh's eco-friendly objectives, particularly those championed by the CG State Medicinal Plant Board and the Chhattisgarh Environment Conservation Board (CECB). By minimizing chemical emissions, this technique actively promotes environmentally responsible laboratory practices and contributes to a greener, healthier future for the state.

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