



# Synthesis of CuO NPs from Vernonia Cinereum Extract: Harnessing Their Exceptional Photophysical Properties and Antibacterial Effects

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## ABSTRACT

Copper oxide nanoparticles (CuO NPs) have garnered substantial interest due to their exceptional properties, which include catalytic, electrical, optical, photonic, textile, nanofluid, and antibacterial characteristics. These properties are significantly affected by the nanoparticles' size, shape, and the surrounding medium. This study explores an environmentally friendly approach to synthesizing CuO NPs utilizing the leaf extract of Vernonia cinerea, a plant recognized for its medicinal properties. This method is not only non-toxic but also cost-effective, providing a viable alternative to conventional chemical synthesis techniques. The synthesis involved preparing the leaf extract and combining it with a copper salt solution, facilitating natural reduction. Characterization through UV-Vis spectroscopy revealed a prominent absorption peak at approximately 296 nm, confirming the successful formation of the nanoparticles. Scanning electron microscopy (SEM) indicated that the mean particle size was approximately 24 nm, and the morphology exhibited a well-defined structure that is important for various applications. Additionally, the antibacterial activity of the synthesized CuO NPs was assessed against several bacterial strains, including Bacillus subtilis, Bacillus cereus, Staphylococcus albus, and Pseudomonas aeruginosa. The results demonstrated that a concentration of 8 µg/ml of the green CuO NPs displayed significant efficacy against these bacterial strains, which can be attributed to the presence of heterocyclic compounds derived from the plant. In summary, the utilization of Vernonia cinerea for the biosynthesis of copper oxide nanoparticles not only provides a sustainable and environmentally friendly production method but also results in nanoparticles with considerable antibacterial potential. This research presents promising opportunities for future applications in both medical and environmental fields.

**Keywords:** Vernonia cinereum, CuONPs, Green synthesis, SEM analysis, Antibacterial agent, Nanoparticle

## 1. Introduction

Metallic nanoparticles are promising systems for drug delivery due to their nanoscale properties. Ranging in size from 1 to 10 nanometers, these nanostructures can be categorized into nano-powders and nanocrystals. Two main synthesis strategies exist: the top-down approach, which breaks down larger structures, and the bottom-up approach, which builds materials from the atomic level [1-4]. The latter often uses green synthesis methods that employ eco-friendly solvents like water and ethanol, reducing toxic waste [5,6]. In particular, plant extracts rich in phytochemicals—such as ketones and flavones—are effective for synthesizing metal/metal oxide nanoparticles [7-9]. This approach counteracts the toxicity issues associated with conventional methods that use hazardous chemicals and solvents, which can limit clinical applications [10]. Biologically synthesized nanoparticles offer an eco-friendly alternative, utilizing microorganisms for assembly and organization at the nanoscale, thereby improving their environmental compatibility [11].

Medicinal plants have been a fundamental source of treatment for human ailments throughout history [12,13]. Approximately one-fourth of the global population, or about 1.42 billion people, relies on traditional medicine, particularly herbal remedies, to address their health issues [14,15]. Herbal medicines are a favorable alternative to modern synthetic drugs, as they typically have minimal or no side effects and are considered safe [16,17]. The majority of herbal formulations utilize either fresh or dried parts of plants. A solid understanding of these crude drugs is crucial for ensuring the safety and effectiveness of herbal products [18-20]. Standardization is essential in guaranteeing that each medicine packet sold contains the correct dosage and can deliver its intended therapeutic effect [21]. Key factors in the standardization of indigenous crude drugs include the determination of extractive values, ash residues, and the content of active components such as saponins, alkaloids, and essential oils [22-24].

One notable plant in this context is Vernonia cinereum, a perennial species belonging to the sunflower family. This plant is native to tropical regions of Africa and Asia (including India, Sri Lanka, Indochina, and Indonesia). It has become established in places like Australia, Mesoamerica, tropical South America, the West Indies, and Florida. Generally classified as an annual herb, Vernonia cinereum can grow up to 120 cm (4 feet) tall and features numerous flower heads that form flat-topped clusters with pinkish or purplish disc florets, lacking ray florets [25-27]. It's important to distinguish this species from Emilia sonchifolia, which can be confused with it due to its similar appearance; however, the former has shorter, vase-shaped flower bracts.



**Figure 1. Image showing the structure of Vernonia cinereum plant**

*Vernonia cinereum* has recently emerged as a potential source for anticancer drugs, as research has highlighted its therapeutic properties, including anti-inflammatory, antihelminthic, and anticancer effects [28-30]. Rich in biologically active compounds, such as antioxidants, it offers protective benefits against tumors [31-34]. The plant has undergone extensive investigation for various pharmacological activities to substantiate its traditional uses and has been scientifically validated to exhibit antimicrobial, anti-inflammatory, antidiabetic, antitumor, and antiviral properties.

Copper oxide nanoparticles are produced in large amounts for various uses. These nanoparticles are becoming popular in energy and environmental fields due to their potential in photocatalysis, solar cells, and pollution treatment. CuO nanoparticles are easy to obtain, inexpensive, and low toxicity [35-38]. They can also be used as antimicrobial, antibiotic, and antifungal agents in coatings, plastics, and textiles. Copper and its compounds are known for their strong ability to kill bacteria and are commonly found in pesticides and health products. CuO nanoparticles can be prepared by mixing plant extracts with cupric chloride metal. These nanoparticles have special structural, morphological, vibrational, and optical properties [39]. Studies have shown that it can effectively break down methyl blue dye using visible light [40]. No researchers reported that plants like *Vernonia cinereum* are used to make copper oxide nanoparticles through green synthesis. Their antibacterial activity will be tested against both gram-positive and gram-negative bacteria. This approach to making copper oxide nanoparticles will become increasingly important.

## 2. Materials

Copper chloride and sodium hydroxide were purchased from Merck India., Acetone, Acetonitrile, Chloroform, Dichloromethane, diethyl ether, Ethanol, Hexane, Toluene, N, N-Dimethyl formamide, Hydrochloric Acid, Acetic Acid (AR, Merck) were used as received. Double distilled water is obtained by distilling distilled water over alkaline potassium permanganate.

## 3. Methods

### 3.1. Selection and Collection of Plants

The Indian herbal plant *Vernonia cinereum* was collected from our college campus. The dry and waste part of the plant was separated. The collected plants were washed with tap water. The plants were cut into small pieces and air-dried thoroughly under shade (at room temperature) for 7 days to avoid direct loss of phyto-constituents from sunlight. The shade-dried materials were powdered using the pulverizer and sieved up to 50 meshes. It was then homogenized to a fine powder and stored in an air-tight container for further analysis.

### 3.2 Preparation of water extracts of plants

To prepare the plant extracts, 10 grams of freshly dried plant powder were combined with 200 milliliters of distilled water. The mixture was then heated at 80°C for 2 hours while continuously stirring to ensure thorough extraction. After heating, the solution was allowed to cool to room temperature. It was then subjected to centrifugation using an ultra-fast centrifuge to separate the extract from the solid residues. The resulting extract was stored at 4°C for future applications.

### 3.3 UV-visible Absorption Spectrum.

The electronic spectra were recorded in the 200-900 nm regions on a Deep Vision UV/VIS spectrophotometer using a cuvette with a 1 cm path length. The concentration of ligand and metal complexes was kept at  $1.00 \times 10^{-5}$  mol L<sup>-1</sup>, at 310 K.

### 3.4 Antibacterial activity

The disc diffusion methodology was employed to assess the antibacterial potential of plant-mediated metal nanoparticles (NPs) against various bacterial strains, including *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP). Prior to the experiments, the bacterial strains were sub-cultured overnight in nutrient broth and incubated at 37°C for 24 hours. To evaluate the antibacterial effectiveness of the NPs, an overnight culture of the bacterial strains was evenly spread on pre-prepared agar plates and allowed to dry for 5 minutes. Filter discs infused with varying concentrations of the NPs (2–15 µg/ml) were dried and positioned on the surface of the agar plates. The plates were then incubated and subsequently examined for the zone of inhibition (ZOI). Streptomycin was utilized as a positive control, while DMSO served as a negative control.

### 3.5 Solubility test

Approximately 0.1 grams of the substance was placed in a clean 10 ml test tube. To this, 1 ml of a low-polarity solvent, such as benzene or petroleum ether, was added and the mixture was shaken thoroughly. If the substance remained insoluble, the procedure was repeated using solvents with increasing polarity, including acetone, acetonitrile, toluene, ethanol, and others. Finally, the solubility of the substance was assessed using high-polarity double-distilled water. Upon confirming its solubility, an additional 0.1 grams of the nonpolar substance was added until the saturation limit was reached. This process allowed us to determine the saturation or solubility limit of our nanoparticles.

### 3.6. Green Synthesis of Copper Oxide Nanoparticle

To 50 ml of aqueous green extract of *Vernonia cinereum*, approximately 0.6 g of copper chloride dihydrate ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) was added and stirred magnetically at room temperature until the light blue color transitioned to a light green hue. The mixture was then heated to 80 °C for 2 hours, during which the pH was maintained at 14 by adding a few drops of 0.01 M NaOH solution. Upon contact with the sodium hydroxide, the green mixture spontaneously transformed into a brown solution, indicating the formation of water-soluble copper oxide nanoparticles. The resulting brown solution was subsequently collected and washed several times with low-polar solvents to eliminate impurities, yielding the final product. After drying at 60°C in a vacuum oven overnight, a brown powder was obtained. This product was then subjected to further heating in a hot air oven at 110°C for 3 hours, followed by calcination at 400°C in a muffle furnace for 2 hours. The final nanoparticles were obtained as brown crystals with a yield of 94%. The saturation limit of the compound was determined to be 0.8 g in 1 ml of water. The reaction is illustrated in scheme 1.



Scheme 1. Green synthesis of CuO NPs using *Vernonia Cinereum* extract

## 4. Result and Discussion

### 4.1. UV-visible spectroscopy.

A green method for synthesizing copper oxide nanoparticles using *Vernonia Cinereum* extract has been reported. UV-Vis spectroscopy can be employed to investigate the size and shape of these nanoparticles in aqueous solution. The absorption spectrum was recorded for the sample across the wavelength range of 200 to 800 nm. Typically, the absorption peak of CuO falls between 280 nm and 360 nm, often showing a prominent peak around 296 nanometers (nm). This peak arises from the inter-band transition of copper's core electrons, along with the bandgap variation linked to the quantum size effect. Figure 2 illustrates the UV-Vis absorption spectrum of the copper oxide nanoparticles.

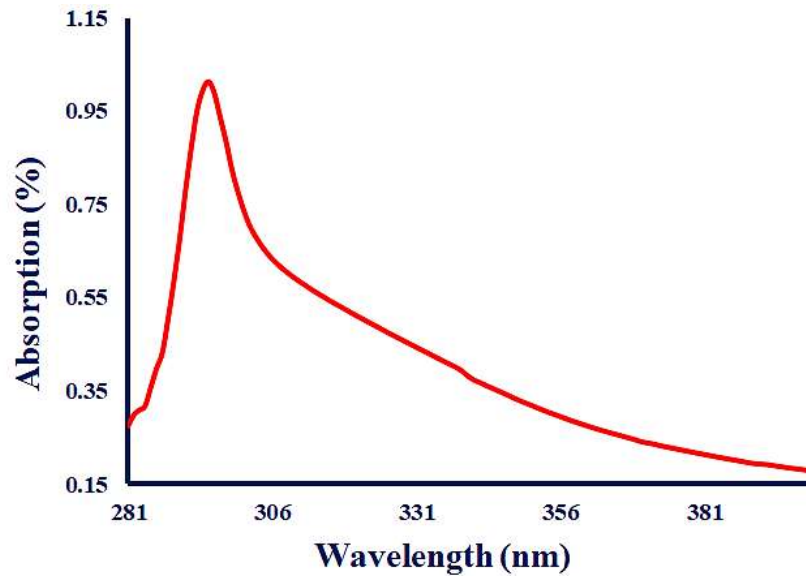


Figure 2. UV-VIS spectrum of CuO nanoparticle

#### 4.2. Surface morphological study

The surface morphology of the synthesized CuO nanoparticles was extensively analyzed utilizing Scanning Electron Microscopy (SEM), a technique recognized for its effectiveness in examining the microstructures of materials. The SEM images indicated that the samples predominantly presented spherical shapes, with an average diameter of 24 nanometers. This finding suggests that the extract obtained from Vernonia Cinereum significantly impacts the synthesis of CuO nanoparticles, resulting in diameters that range from 21 to 29 nanometers. Beyond their distinctive morphology, the CuO nanoparticles exhibit excellent dispersibility, an essential characteristic that enhances their applicability across various fields. Furthermore, the distinct coating layer observed in the SEM imagery strongly implies that the phytochemicals extracted from Vernonia Cinereum serve as effective capping agents. These phytochemicals are likely pivotal in stabilizing the nanoparticles during the synthesis process and minimizing their agglomeration.



Figure 4. SEM images of CuO nanoparticle

#### 4.3. Antibacterial activity

The antibacterial activity of copper oxide nanoparticles was assessed against Gram-negative and Gram-positive bacteria, determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition. Bacterial strains, including Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), Pseudomonas aeruginosa (PA), E. coli (EC), and Klebsiella pneumoniae (KP), were added to samples in physiological serum, achieving a concentration of 100,000 bacteria per mL, and incubated at 37 °C. The disk diffusion method was used to measure the zone of inhibition, with varying sample concentrations applied to the agar medium. After 24 hours of incubation at 37 °C, the zones were measured. Results showed that Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), and Pseudomonas aeruginosa (PA) exhibited significant antibacterial activity against copper oxide nanoparticles (see Figure 3 and Table 1).

Table 1 Shows the results of zone inhibition of copper oxide with Bacteria

S.No.	Bacteria	Copper Oxide Nanoparticle 8 µg/ml	Control (STREPTOMYCIN)
1.	BS	14	22
2.	BC	9	20
3.	PA	12	12
4.	SA	9	12
5.	KP	Nil	13
6.	E.coli	Nil	11

**Figure 3. Antibacterial activity of copper oxide nanoparticle**

## 5. Discussion

Medicinal plants have served as a crucial source for treating human illnesses throughout history. To apply plant parts effectively in modern medicine, it is essential to conduct physicochemical and phytochemical standardization, ensuring that the therapeutic benefits of these plants can be harnessed scientifically for the benefit of larger populations worldwide. Laboratories globally have discovered numerous phytochemicals with inhibitory effects on various microorganisms *in vitro*. Further research involving these compounds in animal and human studies is necessary to assess their efficacy within whole-organism systems, particularly concerning toxicity studies and their influence on beneficial normal microbiota. Copper oxide nanoparticles were synthesized using an aqueous green extract of *Vernonia Cinereum*, resulting in brown crystalline particles with a 94% yield. The generation of copper oxide nanoparticles was confirmed through UV-VIS spectrophotometry and SEM analysis. The absorption spectrum displayed a peak at 296 nm, corresponding to the characteristic band of copper oxide nanoparticles. Furthermore, the stability of these nanoparticles during drying ensures that their size and shape are retained, which enhances their functional properties. This is supported by the hydrodynamic diameter ( $D_h$ ) of the CuO nanoparticles, averaging 29 nanometers in aqueous dispersion. Such a small size indicates a high surface-to-volume ratio, which significantly boosts their reactivity and enhances their applicability in various fields, including catalysis, electronics, and drug delivery. The antibacterial properties of copper oxide nanoparticles were tested, showing effectiveness against *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Staphylococcus albus* (SA), and *Pseudomonas aeruginosa* (PA) bacteria. With the rise of bacterial and viral resistance to available antibiotics and in creating new drugs with improved efficacy. Since metals and ligands interact at various stages of the pathogen life cycle, they hold the potential for developing new therapeutic drugs. Nonetheless, this area remains relatively unexplored and uncertain, as the biosynthesis of these nanostructures from fungi, bacteria, and plants often leads to undesirable sizes and shapes, making them less useful when compared to their chemically synthesized versions.

## 6. Conclusions

We have successfully demonstrated the synthesis of copper oxide (CuO) nanoparticles mediated by *Vernonia Cinereum* green plants through a calcination method. This environmentally friendly reduction approach for nanoparticle synthesis is characterized by its simplicity, cost-effectiveness, and sustainability. The phase and morphology of the nanocrystals were thoroughly characterized using UV-visible spectroscopy and scanning electron microscopy (SEM), which revealed distinct bands associated with the identified phases. Furthermore, antimicrobial activity tests conducted against standard bacterial species indicate that the calcination method significantly enhances antibacterial efficacy compared to alternative preparation methods. This enhancement is attributed to the small particle size, the production of reactive oxygen species (ROS), and the increased surface area. These attributes suggest promising opportunities for future applications that prioritize minimal harm and toxicity to human health, thereby ensuring enhanced safety.

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