



# Green Grape Seed Extract-Mediated Synthesis of CuO Nanoparticles: Characterization and Enhanced Antibacterial Activity

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## Abstract.

Green grape seed extract was used to create copper oxide (CuO) nanoparticles as an environmentally acceptable stabilising and reducing agent. When copper sulphate pentahydrate was reduced with sodium hydroxide (NaOH), the colour changed from blue to greenish-black, indicating that the pH had been corrected to 9. Functional groups including O-H stretching and metal-oxygen bonding were validated by FTIR analysis, confirming the creation of nanoparticles. Absorbance peaks at 372.75 nm and 390.05 nm were visible in UV-Vis spectroscopy, emphasising electronic transitions and charge transfer. X-ray diffraction (XRD) verified a monoclinic crystal structure with a crystallite size of 30.55 nm, while dynamic light scattering (DLS) showed an average particle size of 181.6 nm with a Polydispersity Index of 0.185. The CuO nanoparticles exhibited notable antibacterial activity, exhibiting 40 mm, 42 mm, and 43 mm inhibitory zones.

**Key Words:** Grape Seed Extract, Antimicrobial Activity, Functional Groups, Spectroscopic Analysis, Bioactive Agents

## 1. INTRODUCTION

Nanomaterial's, with particle dimensions less than 100 nm, and are making significant strides across various industries. Their exceptional qualities such as antimicrobial effects, high diffusivity, electrical conductivity, remarkable hardness, and robustness make them integral to innovative technologies, especially in the medical field. The emergence of phytonanotechnology has introduced an environmentally friendly method for nanoparticle production. By harnessing the unique secondary biomolecules in plants, metals are reduced to oxides, creating nanoparticles with distinctive shapes and sizes [1]. Green synthesis of nanoparticles, a key aspect of Nano biotechnology, is valued for its eco-friendly, cost-effective, and versatile nature [2, 3]. With industrial and population growth escalating chemical waste and environmental damage, sustainable solutions are essential. Green chemistry, which uses natural products for nanoparticle synthesis, provides an eco-friendly, recyclable, and less polluting alternative. This approach leverages plant extracts to create metal nanoparticles, improving stability and morphology without harmful chemicals. Recent research focuses on plant-mediated methods due to natural reducing agents. Factors like extract concentration and reaction conditions affect nanoparticle quality [4]. Biogenic methods, in particular, have proven superior to conventional techniques by producing large volumes of clean, well-defined nanoparticles with minimal environmental impact. In response to health and economic challenges from cancer and infections [5], researchers have turned to nanoparticles (NPs) to enhance treatments. Copper nanoparticles (CuNPs) are notable for their affordability and potential to match the efficacy of metals like silver and gold, with broad antimicrobial and cancer therapeutic potential [6]. CuNPs can be produced through chemical methods such as solvothermal, sonochemical, and microwave techniques [7]. Similarly, copper oxide nanoparticles have gained concern due to their unique properties and wide applications in industry and healthcare [8]. Nanoparticles from MO are used in drug carriers, UV protection, medical fillers, cosmetics, sensors, photo catalysts, and as antibacterial agents. Copper oxide nanoparticles are valued for their low band gap energy, biocompatibility, and antibacterial and anti-inflammatory properties.

They are small, high in surface area, biodegradable, and reduce charge carrier recombination. Green synthesis using plant extracts provides a non-toxic, environmentally friendly method that allows precise control over nanoparticle size and shape. In contrast, traditional methods like electrochemical reduction, laser ablation, UV irradiation, and aerosol processes are effective but costly, involve toxic chemicals, and can lead to contamination and hazardous byproducts [9]. They can be synthesized using hydrothermal, [10] sonochemical, [11] co-precipitation, microwave, and sol-gel methods [12] and are utilized in drug transport, antibacterial [13, 14] anticancer, antifungal, antidiabetic [15] Antifungal, antioxidant applications [16, 17]. CuO nanostructures also show promise for environmental pollution control due to their high specific surface area [18]. Various copper oxide nanoparticles are reported from natural sources like *Hylocereus costaricensis* Peel Extract [19] have antifungal, antibacterial, antitumor, and antioxidant activities, *Macroptilium Lathyroides* [20], *Zizyphus spina* [21], *Heliotropium baciferum* [22], *Zizyphus oenoplia* [23], *Aloe vera* [24], *Azadirachta indica* [25], *Simarouba glauca* leaf extract [26], *Santa Maria feverfew* leaf extract [27], *Clausena anisata* leaf extracts [28] and *Justicia adhatoda* leaf extracts [29].

This article examines the synthesis of CuO NPs using green grape seed extracts, emphasizing eco-friendly and cost-effective benefits. Green grapes in India contain beneficial phytochemicals such as stilbenes, which offer anti-inflammatory and antioxidant properties, and flavonoids like quercetin and kaempferol, known for their anti-inflammatory, and antimicrobial properties [30]. Quercetin, a flavonoid, and resveratrol, a stilbene, are potent antioxidants that may help protect against cardiovascular diseases. Tannins and catechins present in the grapes support cardiovascular health and cancer prevention. Additionally, phenolic acids like as caffeic and gallic acid offer further antioxidant and anti-inflammatory benefits. These phytochemicals make green grapes a valuable addition to a healthy diet. [31, 32].

## 2. Materials and Methods

Fresh green grapes were purchased, and the seeds were dried and powdered. Copper sulfate pentahydrate was sourced from Sigma-Aldrich, and the synthesis was performed in a basic medium with sodium hydroxide (NaOH). All other chemicals used were of high purity.

### 2.1 Preparation of Natural Extract from Grape Seed

The seed extract was made by cleaning, drying, and finely grinding green grape seeds. After combining the powdered seeds with distilled water in a 1:10 ratio, the mixture was left to steep for a number of hours. To get rid of any solid leftovers, the mixture was filtered after steeping. The liquid extract that was produced was then prepared for use in the production of copper oxide nanoparticles.

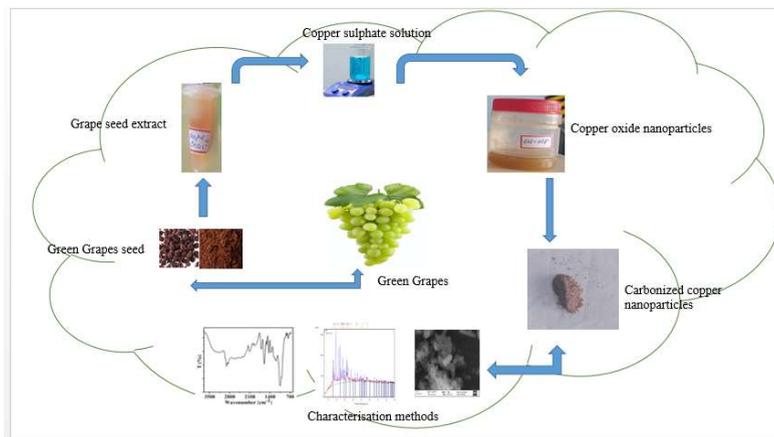
### 2.2 CuO Nanoparticle Formation Using Grape Seed Extract

Green grape seed extract functioned as the stabilising and reducing agent, whereas copper sulphate pentahydrate was the precursor. 50 mL of a 0.2 M  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  aqueous solution and 50 mL of grape seed extract were combined in a 1:1 volume ratio for the synthesis. 0.2 M NaOH was used to bring the mixture's pH down to 9, and the mixture was aggressively agitated at 50°C. A colour shift from blue to greenish-black indicated that copper sulphate had been reduced to copper oxide. Centrifugation at 2000 rpm for 30 minutes was used to separate the resultant CuO nanoparticles, which were then cleaned of contaminants using distilled water and dried for additional examination (Figure 1).

### 2.3 Physical and Chemical Characterization

The CuONPs were analysed using several techniques. Optical absorption was measured with a PerkinElmer Lambda 365 over a wavelength range of 200–800 nm. Molecular vibrations were analysed using a Thermo Nicolet 380 FTIR spectrophotometer and spectra were recorded in the 500–4,000  $\text{cm}^{-1}$  range. X-ray diffraction analysis was performed using a Shimadzu 6100 diffractometer with Copper  $K\alpha$  radiation ( $\lambda = 1.54060$

Å) to verify the crystal structure and estimate the crystalline size of the CuO nanoparticles. Finally, the nanoparticles' surface morphology and structural properties were assessed using a scanning electron microscope (SEM).



**Fig. 1. Copper Oxide Nanoparticle Synthesis**

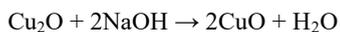
### 3. Result and Discussion

#### 3.1 Mechanism of formation of CuONPs

In synthesizing copper oxide (CuO) nanoparticles, copper sulphate ( $\text{CuSO}_4$ ) reacts with Gallic acid, which reduces copper ions ( $\text{Cu}^{2+}$ ) to copper (I) ions ( $\text{Cu}^+$ ). The reaction is:



Sodium hydroxide (NaOH) is then added, which reacts with the copper ions to form copper oxide nanoparticles:

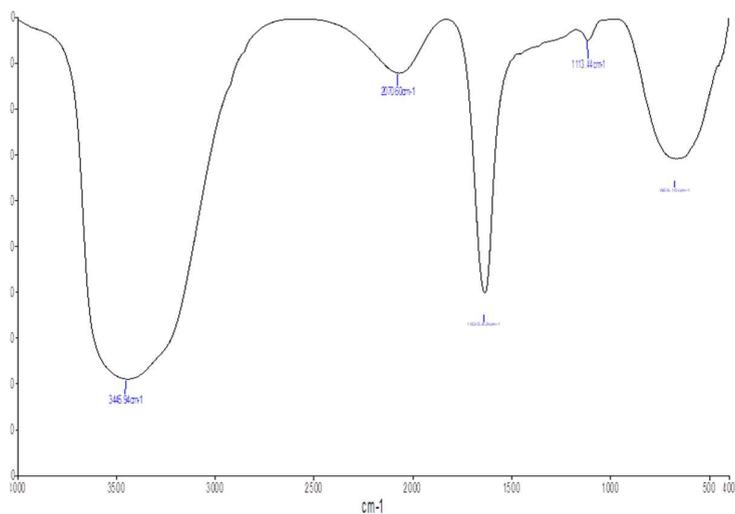


If  $\text{Cu}^{2+}$  is present the reaction is,



### 3.2 FTIR

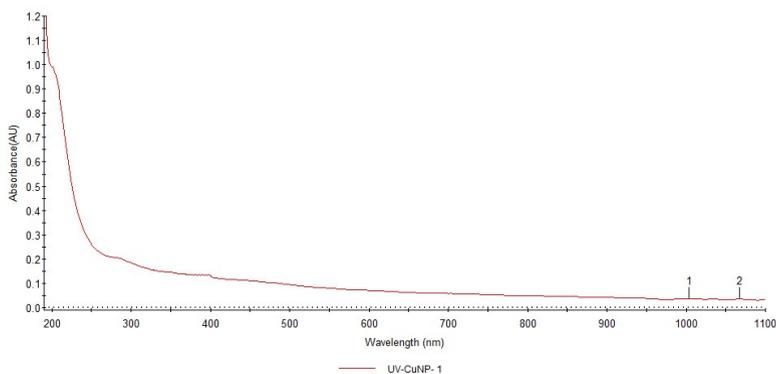
The FTIR analysis of CuONPs synthesized using green grape seed powder reveals (Figure 2) various functional groups present in the sample. The peaks at  $3446.9\text{ cm}^{-1}$  and  $3469.4\text{ cm}^{-1}$  correspond to O-H stretching vibrations, indicating the presence of hydroxyl groups from adsorbed water or alcohols. A notable peak at  $2070.6\text{ cm}^{-1}$  suggests the existence of  $\text{C}\equiv\text{C}$  or  $\text{C}\equiv\text{N}$  stretching vibrations, potentially resulting from organic residues or specific complexations with the nanoparticles. The peak at  $1113.4\text{ cm}^{-1}$  reflects carbon-oxygen stretching from organic compounds in the grape seed extract, while the peak at  $606.15\text{ cm}^{-1}$  confirms the Cu-O bond and successful synthesis of copper oxide nanoparticles [33].



**Fig. 2.** FTIR spectrum of CuONPs

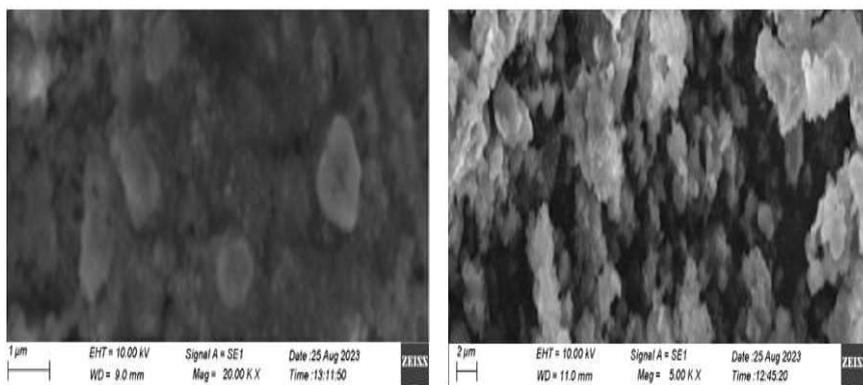
### 3.3 UV-Visible analysis

The UV-Vis spectroscopy analysis of the CuO NPs (Figure 3) produced using green grape seed powder reveals significant information about their optical properties and electronic transitions. The absorbance peaks at 1004 nm and 1066.65 nm, both with an absorbance of 0.036 AU, are in the near-infrared (NIR) region and likely indicate d-d transitions of copper ions ( $\text{Cu}^{2+}$ ) within the nanoparticles. These transitions are typically weak, consistent with the observed low absorbance. The peak at 372.75 nm with an absorbance of 0.049 AU falls in the UV region and is associated with charge transfer transitions, specifically ligand to metal charge transfer (LMCT), where electrons move from the organic compounds in the grape seed extract to the copper ions. A key compound responsible for this LMCT is gallic acid (3, 4, 5-trihydroxybenzoic acid), a prominent phenolic acid in grape seeds. Additionally, the peak at 390.05 nm, with a significantly higher absorbance of 0.479 AU, suggests strong electronic transitions within the copper oxide nanoparticles. This is due to surface plasmon resonance (SPR), a common feature in metallic and semiconductor nanoparticles, or other strong electronic transitions within the material. These findings indicate a combination of weak d-d transitions in the NIR region and stronger charge transfer and electronic transitions in the UV region [34].



**Fig. 3.** UV absorbance peak of CuO nanoparticles

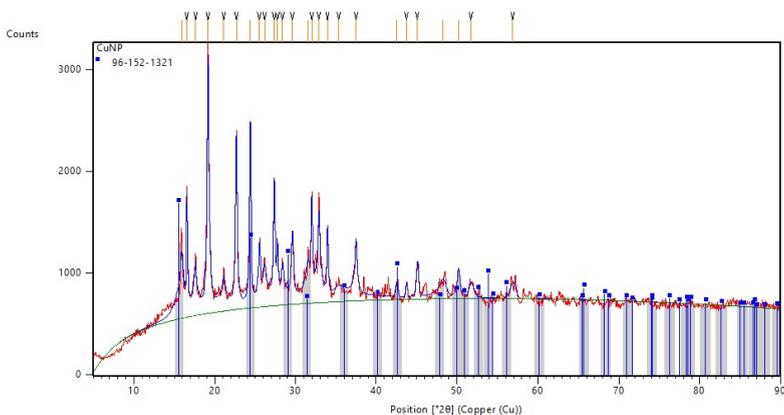




**Fig. 5.** SEM Images of CuONPs of 1  $\mu\text{m}$  and 2  $\mu\text{m}$

### 3.6 XRD

Based on the X-ray diffraction (XRD) results, which include  $2\theta$  values of  $19.1602^\circ$ ,  $24.3658^\circ$ ,  $27.3453^\circ$ ,  $31.9810^\circ$ , and  $32.8885^\circ$ , the copper oxide nanoparticles (CuONPs) exhibit a monoclinic crystal structure. The d-spacing values associated with these angles correspond to characteristic planes of monoclinic CuO: specifically, the peak at  $19.1602^\circ$  corresponds to the (110) plane, the peak at  $24.3658^\circ$  to the (101) plane, the peak at  $27.3453^\circ$  to the (102) plane, the peak at  $31.9810^\circ$  to the (111) plane, and the peak at  $32.8885^\circ$  to the (200) plane. These observations (Figure 6) confirm that the nanoparticles are predominantly CuO with a monoclinic structure. The variation in Full Width at Half Maximum values suggests differences in crystallite size, with narrower FWHM indicating more well-defined crystallites. According to Scherrer's equation  $d = k\lambda/\beta\cos\Theta$  the crystallite size has been estimated to be 30.55 nm. This size, being less than 100 nm, indicates the nanocrystalline nature of the biosynthesized CuO NPs. Additionally, the XRD pattern aligns with the standards (JCPDS No: 96-152-1321), confirming both the crystalline nature and the monoclinic phase of the CuO NPs [35].

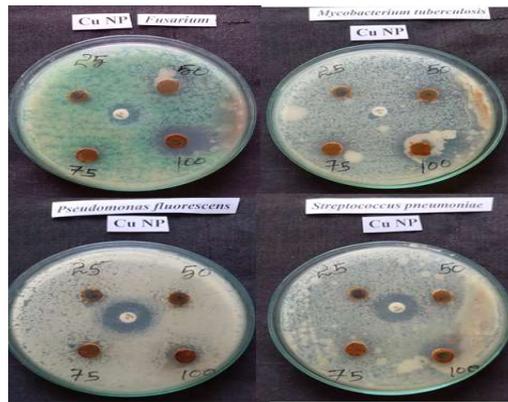


**Fig. 6.** XRD peak of CuO Nps

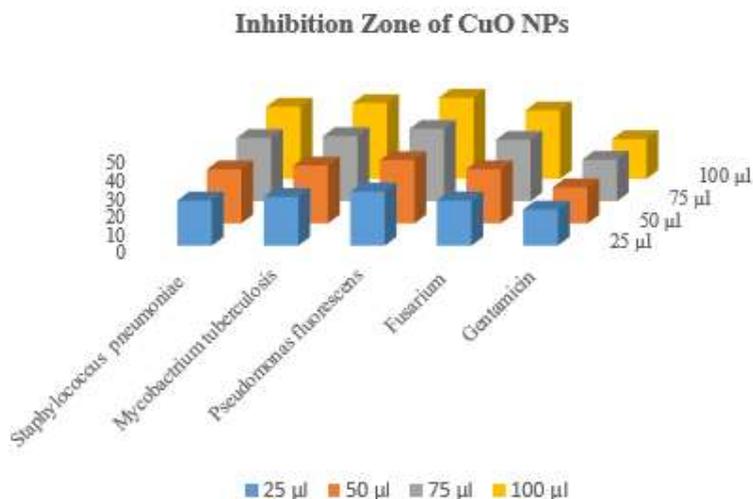
### 3.7 Antimicrobial assay

The nanoscale size of the synthesised CuO nanoparticles (CuONPs) derived from grape seed extract gives them a high surface-to-volume ratio and enhances their capacity to interact with bacterial membranes, giving them a noticeable bactericidal functions and eventually causing cell death. Reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals, are produced by the CuONPs. These ROS damage bacterial membranes, proteins, and DNA and induce oxidative stress. When the cell wall and membrane integrity are compromised by the nanoparticles, bacterial cell lysis and intracellular contents leaking occur. Because of this intricate mechanism, CuONPs exhibit superior antibacterial activity [36] in comparison to conventional antibiotics such as gentamicin. The CuONPs were tested at a concentration of 100  $\mu\text{g}/\text{mL}$ , while gentamicin was used at the same concentration as a standard antibiotic control. Compared to gentamicin, the CuONPs created inhibition zones that were significantly larger and showed considerable antibacterial efficacy against pathogens including *Staphylococcus pneumoniae*, *Mycobacterium tuberculosis*, *Pseudomonas fluorescens*, and *Fusarium*. The inhibition zone of *Staphylococcus pneumoniae* was 40 mm at 100  $\mu\text{L}$ , while that of gentamicin was 20 mm. In a similar manner, the zones of *Mycobacterium tuberculosis*, *Pseudomonas fluorescens*, and *Fusarium* were 42 mm (gentamicin: 20 mm), 43 mm (gentamicin: 23 mm), and 38 mm (gentamicin: 22 mm), respectively (Figure 8).

Grape seed extract's natural constituents, such as polyphenols, flavonoids, and tannins, serve as stabilising agents and are essential to the synthesis of CuONPs. These substances improve dispersion and inhibit the agglomeration of nanoparticles (Figure 7). Bacterial cell death results from the disruption of bacterial cell walls and membranes, disruption of enzymatic processes, and damage to nucleic acids caused by polyphenols and flavonoids [37]. Through the precipitation of proteins and the inhibition of vital microbial functions, tannins further suppress bacterial development. When coupled with CuONPs, these bioactive substances increase the antibacterial activity of the nanoparticles, making them especially effective, especially at higher concentration.



**Fig. 7.** Inhibition Zones of microbes



**Fig. 8.** Effect of Concentration on the Antibacterial Efficacy of CuO Nanoparticles

#### 4. CONCLUSION

Extract's natural bioactive ingredients, including polyphenols, flavonoids, and tannins, are essential for stabilising Eco-friendly grape seed distillation is a sustainable process for creating copper oxide (CuO) nanoparticles. The grape seed and lowering copper ions, which makes it easier to create nanoparticles. These CuO nanoparticles demonstrate their potential for use in medical therapies as well as environmental remediation by demonstrating robust antibacterial activity against a range of pathogens. The grape seed extract's bioactive ingredients provide their own antimicrobial qualities and stabilise the nanoparticles, increasing their antibacterial effectiveness. Effective antibacterial action at lower concentrations is made possible by this synergy. Furthermore, compared to conventional nanoparticle production, this environmentally friendly technology uses fewer harmful chemicals and energy-intensive procedures.

**5. Declaration:** The authors declare no competing interest.

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