A|R|J
Amrit Research Journal December 2024, Vol. 5, Issue 1, 18-29

ISSN: 2738-9553
RESEARCH ARTICLE

Synthesis and Characterization of Copper Oxide Nanoparticles Isolated from *Acmella oleracea* and Study of Antimicrobial and Phytochemical Properties

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(Received: October 5, 2024; Received in Revised form: November 9, 2024; Accepted: November 12, 2024; Available online)

DOI: https://doi.org/10.3126/arj.v5i1.73521

Highlights

- CuO nanoparticles were synthesized by using Acmella oleracea flower extract.
- Synthesized nanoparticles characterized by XRD, FTIR, and UV-vis spectroscopy.
- Nanoparticles exhibit notable phytochemical and antimicrobial activity.

Abstract

This study utilized a green synthesis approach to produce CuO nanoparticles employing flower extract of Acmella oleracea where plant-derived biomolecules served as natural reducing and stabilizing agents. The synthesized nanoparticles were characterized through X-ray diffraction (XRD), UV-Vis spectroscopy, and Fourier Transform Infrared (FTIR) analysis. XRD suggested the monoclinic phase of nanoparticles that belong to the C2/c space group with a crystallite size of 17.35 nm on average. The band gap was calculated using the Tauc model which was 2.27 eV. Phytochemical screening confirmed the presence of alkaloids, essential for nanoparticle formation, through Mayer's, Dragendorff's, Wagner's, and Hager's tests. Antimicrobial tests showed that the CuO nanoparticles had significant activity against microbes such as Escherichia coli, Staphylococcus aureus, and Candida albicans, with inhibition zones of 1.5 to 1.8 cm. These findings highlight the potential of Acmella oleracea mediated CuO nanoparticles for biomedical and environmental applications.

Keywords: *Acmella oleracea*, green synthesis, nanoparticles, antimicrobial, phytochemicals

Introduction

The field of nanotechnology explores materials at the nanoscale, where unique optical, magnetic, and catalytic properties emerge, setting nanoparticles apart from their bulk counterparts [1-4]. Copper oxide nanoparticles have many applications in medicine, agriculture, and environmental remediation and have attracted attention for their antibacterial, antifungal, and photocatalytic properties [5-8] However, traditional synthesis techniques pose challenges, including high costs, environmental hazards, and non-eco-friendly byproducts [9-11]. Pursuing sustainable and environmentally friendly synthesis methods has gained significant

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momentum in the ever-evolving nanotechnology landscape. The use of plant extracts as a green synthesis is a good alternative to chemical and physical methodologies often involving toxic reagents and labor-intensive processes [12-15].

Plant-mediated nanoparticle green synthesis involves three key phases: reduction, growth, and stabilization [16]. During the reduction phase, plant metabolites function as reducing agents, transforming metal ions into their elemental (zero-valent) states and triggering nucleation [17]. The growth phase follows, where reduced metal atoms aggregate to form nanoparticles. Continued reduction and thermodynamic stabilization occur here, though excessive nucleation may cause aggregation, affecting morphology. Finally, the stabilization phase involves capping by plant metabolites, which prevents agglomeration, preserves functional integrity, and ensures stable nanoparticle morphology [18].

Among the many plants used in green synthesis, the genus *Acmella oleracea* has garnered attention for its medicinal and industrial applications [19]. Known for its rich composition of bioactive compounds, *Acmella oleracea* has a long history of use in traditional medicine [20]. Recent studies have demonstrated that *Acmella oleracea* extracts are highly effective in facilitating the green synthesis of nanoparticles, including CuO nanoparticles. The presence of phytochemicals like flavonoids and terpenoids in *Acmella oleracea* not only supports nanoparticle synthesis but also enhances the intrinsic antibacterial activity of the resulting materials [21-22].

The antimicrobial potential of CuO nanoparticles is especially significant in the face of rising antibiotic resistance [23]. These nanoparticles exert their antibacterial effects by disrupting bacterial cell membranes, causing leakage of cellular contents and eventual cell death. This mechanism is effective against both Gram-positive and Gram-negative bacteria, positioning CuO nanoparticles as a promising solution to combat multidrug-resistant pathogens [24].

This research emphasizes the eco-friendly synthesis of copper oxide (CuO) nanoparticles utilizing the bioactive compounds found in the flower extract of Acmella oleracea, a plant native to tropical and subtropical regions which are widely recognized for its anti-inflammatory, antibacterial, and antifungal effects [5,25-31].The analysis of synthesized nanoparticles was performed utilizing various analytical techniques, such as XRD, UV-visible spectroscopy, and FTIR, and further studied the antimicrobial potential of CuO nanoparticles, offering a novel approach that integrates traditional medicinal insights with modern nanotechnology to foster sustainable and innovative solutions across diverse scientific fields.The synthesis of CuO nanoparticles involving green methods offers significant environmental benefits, including reduced reliance on toxic chemicals and lower energy consumption. For instance, the biomolecules present in the extract serve a dual purpose in the synthesis process. They function as natural reducing agents to transform metal precursors into nanoparticles and as stabilizing agents to prevent nanoparticle aggregation. This approach not only streamlines the synthesis process but also reduces the reliance on synthetic chemicals, making the method more environmentally sustainable.

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Materials and Methods

Raw Materials

Copper oxide nanoparticles were synthesized using flowers of *Acmella oleracea* collected from Sundarbazar, Lamjung, Nepal. The plant, a member of the Asteraceae family, was authenticated by taxonomists at the National Herbarium and Plant Laboratories in Godavari, Nepal.

Chemicals

All chemicals used in the experiments, including copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), ethanol (C_2H_5OH), sodium hydroxide (NaOH), and other HPLC grade reagents, were obtained from the Department of Chemistry at Tri-Chandra Multiple Campus.

Preparation of *Acmella oleracea* Flower Extract

The collected flowers were carefully cleaned and dried under mild sunlight for 5 days. The dried flowers were pulverized and kept in sealed containers to protect them from contamination. For extraction, the powdered plant material was immersed in ethanol. The mixture was stirred while maintaining a temperature of 60°C for one hour, followed by a standing period of 24 hours at room temperature (25°C). Then the solution was filtered to isolate the plant extract and was stored at 4°C for later use. This extract was used for both nanoparticle synthesis and phytochemical screening [18,32-33].

Synthesis of *Acmella Oleracea* CuO Nanoparticles

Approximately 2 g of the powdered plant material was poured into the beaker containing 100 mL of ethanol. The mixtures were warmed at around 60°C for one hour with continuous stirring. The extract was treated with a 0.1 M copper sulfate pentahydrate solution and stirred at 60°C for one hour in a water bath, cooled, followed by centrifuging at 8500 rpm for 20 minutes. The resulting product was washed with distilled ethanol several times to remove impurities. After washing, the product was dried at 60°C for one hour and then calcined at 400°C for two hours to produce the final material. **Figure 1** shows the flowchart of the process.

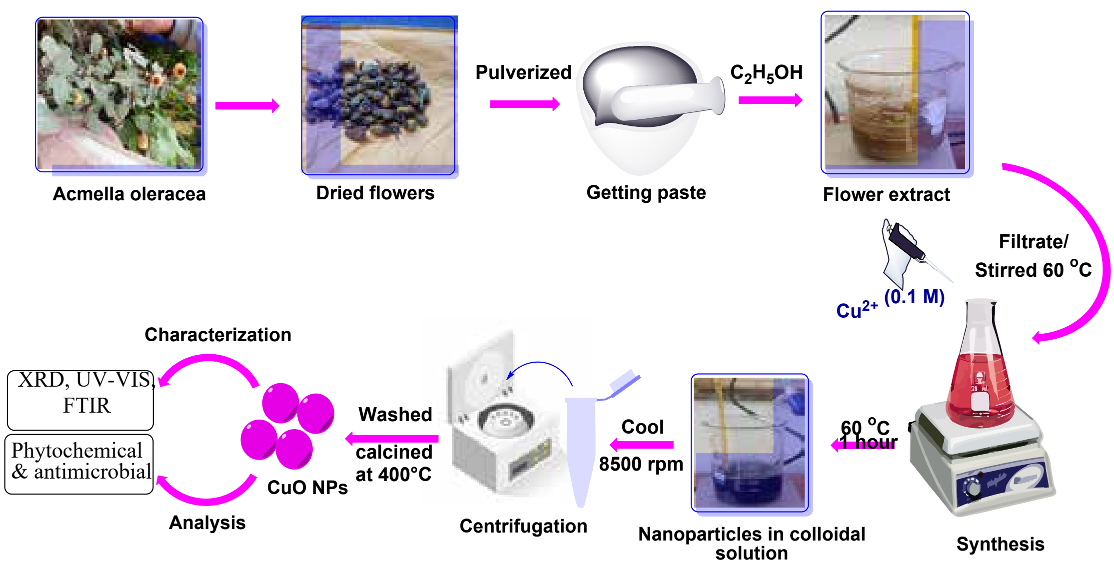

Fig 1. Representation of synthesis of CuO nanoparticles using *Acmella oleracea* flower extract and $CuSO_4$.

Phytochemical Analysis

Acmella oleracea is an interesting plant for culinary and medicinal use. Traditionally, it has been employed to address various health issues, including anemia, body pains, cancer, gastric ulcers, infections, gingivitis, gout, inflammation, wounds, malaria, speech disorders, and parasitic infections [34]. To identify its phytochemical profile, a range of standardized methodologies were employed to detect specific bioactive constituents. The presence of alkaloids was confirmed through Mayer's, Hager's, and Dragendorff's reagent tests, [35] whereas carbohydrate was detected through a series of tests including Wagner's, Molisch's, Fehling's, Barfoed's, and Benedict's methods [36]. Phytosterols were analyzed using the Liebermann-Burchard and Salkowski reactions, and glycosides were detected via Legal and Keller-Killian tests. Tannins were identified through ferric chloride, potassium dichromate, and lead acetate assays. Saponins were evaluated using the foam test. The presence of reducing sugars was assessed using Fehling's and Benedict's tests. Flavonoids were identified through Shinoda and zinc-hydrochloride reduction methods, while amino acids were detected via Biuret and ninhydrin reactions [34].

Antimicrobial Activity of CuO Nanoparticles

A comprehensive antimicrobial assay was conducted to evaluate their effectiveness against microorganisms, involving preparing microbial culture media and Mueller-Hinton agar (MHA) plates. To prepare the liquid broth medium, 13 g of liquid broth powder (Sisco Research Laboratories Pvt. Ltd., India) was dissolved in 1 L of distilled water. The mixture was sterilized at 121°C with a pressure of 15 psi for 25 minutes. Then, the medium was cooled to 40–50°C before being dispensed into sterile 15 mL Falcon vials. Bacterial cultures were then introduced into the medium, and the vials were incubated for 24 hours. For the MHA plates, 39 g of Mueller-Hinton agar powder (Sisco Research Laboratories Pvt. Ltd., India) was dissolved in 1 L of distilled water. The medium was sterilized at 121°C and 15 psi for 25 minutes. Once cooled to 40–50°C, it was poured into sterile 25 mL Petri dishes. The prepared plates were then stored in a refrigerated environment until needed. Antimicrobial assays were performed using these plates, employing sterile cotton swabs for inoculation [37].

Characterization of Nanoparticles

The crystalline or amorphous structure of CuO nanoparticles was analyzed through XRD. The measurements were conducted on a Bruker D8 Diffractometer (Bruker GmbH, Karlsruhe, Germany) with CuKα radiation (λ = 1.54056 Å) over a 2θ range of 10° to 80° at an operating voltage of 40 kV. The diffraction peaks obtained were analyzed using the Debye-Scherrer formula, which is commonly used to estimate the crystallite size in powder diffraction experiments [38-40]. The isolated CuO nanoparticles were studied using a UV-Vis spectrophotometer (SPECORD 200 Plus), with measurements taken across the 200–700 nm wavelength range. This analysis aimed to verify the successful synthesis of the nanoparticles and assess their optical properties as the band gap energy through the Tauc plot [41-42]. FTIR spectroscopy was utilized to confirm the functional groups in the sample. The specific stretching vibrations associated with CuO were detected, offering important information about the material's chemical structure and bonding. FTIR measurements were conducted on a PerkinElmer 10.6.2 spectrometer, with 4000 - 400 cm^{-1} wavenumber and 4 cm^{-1} scan resolution.

Results and Discussion

Phytochemical Analysis

The aqueous extract of *Acmella oleracea* was analyzed using standard phytochemical screening methods, which identified several bioactive constituents. Alkaloids were confirmed by positive results in Mayer's, Dragendorff's, Wagner's, and Hager's tests. Tannins were specifically detected using the lead acetate test, while the $FeCl_3$ and $K_2Cr_2O_7$ tests showed no evidence of their presence. Additionally, the foam test for saponins produced a negative outcome. Additionally, the plant contained flavonoids, phenolic compounds, terpenoids, steroids, and quinones, as summarized in **Table 1,** where "+" indicates the presence and "–" indicates absence. These findings highlight the phytochemical richness and potential bioactive properties of the *Acmella oleracea* flower.

Table 1. Phytochemical analysis of *Acmella oleracea* extract.

Phytochemicals	Saponin	Flavonoid	Phenolic	Terpenoid & steroid	Alkaloid	Lead acetate	Quinone
Remark	-	+	+	+	+	+	+

Mechanism of CuO Nanoparticles Synthesis

Plant extracts are rich in various biomolecules, as demonstrated by phytochemical analyses. Certain biomolecules play a significant role in the synthesis of nanoparticles. However, the formation of nanoparticles from plant products is intricate, and understanding the exact underlying mechanisms remains a challenge. Research has highlighted that functional groups such as hydroxyl, carbonyl, amine, and methoxide are instrumental in reducing and stabilizing nanoparticles. These groups are typically present in plant-derived metabolites like phenols, flavonoids, alkaloids, quinones, and proteins[43].The synthesis of CuO nanoparticles utilizing Acmella oleracea plant extract involves a bio-mediated process. Metabolites within the extract interact with Cu^{2+} ions, which are released by dissolving $CuSO_4$ in water, resulting in the formation of metal complexes. These complexes are reduced to initiate the formation of CuO seed particles. Over time, these seed particles aggregate and nucleate, eventually forming stable CuO nanoparticles. A graphical representation of this mechanism is shown in Figure 2. Similar nanoparticle synthesis mechanisms have been proposed in earlier studies [44-45].

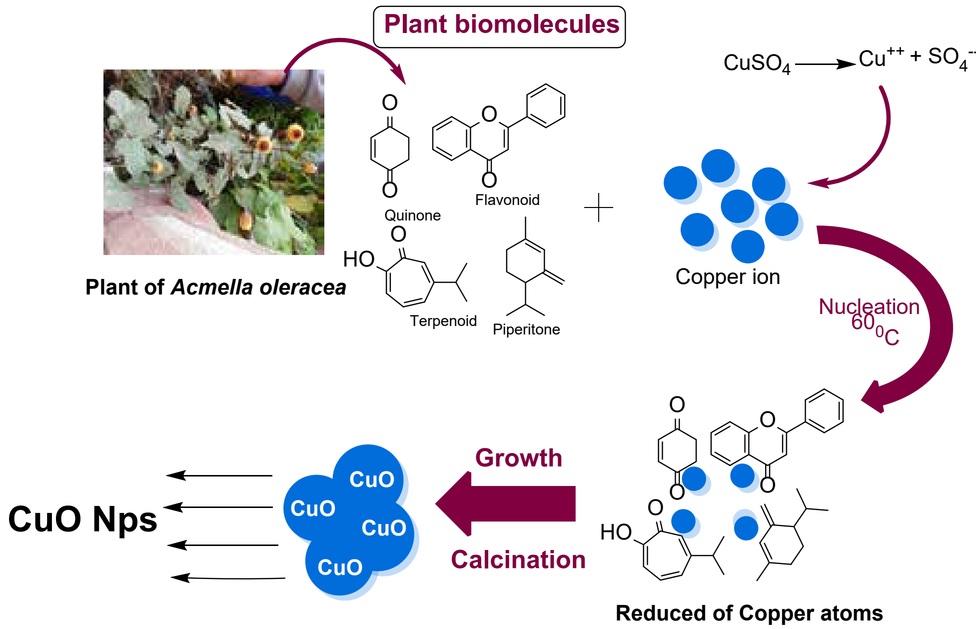

Fig 2. A possible mechanism for the synthesis of CuO nanoparticles utilizing the extract of *Acmella oleracea* flower.

Antimicrobial Activity

The synthesized CuO nanoparticles exhibited significant antimicrobial properties against the tested microbial strains, as demonstrated by the zones of inhibition depicted in **Figure 3**. This is likely due to its small size, which enhances its penetration ability and causes rapid cell damage. The measured zones were 1.8 cm for **Figure 3 (a)** *Escherichia coli* (Gram-negative), **Figure 3 (b)** 1.7 cm for *Staphylococcus aureus* (Gram-positive), and **Figure 3(c)** 1.5 cm for *Candida albicans* (fungal strain).

The study revealed that biosynthesized CuO nanoparticles exhibited lower efficacy against Gram-positive bacteria and fungal strains in comparison to their activity against Gram-negative bacteria. This difference is attributed to electrostatic forces, as the opposite charges of bacterial surfaces and copper ions released by the nanoparticles facilitate adhesion and enhance bioactivity. Negatively charged peptidoglycans are thought to interact with Cu^{2+} ions released by nanoparticles in a solution form. Also, it is suggested that these Cu^{2+} ions attach to the bacterial cell wall which is negatively charged disrupting its integrity which leads to cell wall rupture, protein denaturation, and ultimately bacterial cell death [46]. This result correlates with earlier research, emphasizing enhanced interaction of CuO nanoparticles with Gram-negative bacteria. This is attributed to their thinner cell membranes, which contrast with the thicker membranes and greater surface area observed in Gram-positive bacteria [4,46]. Similarly, the response in fungi such as *C. albicans* could be influenced by the complexity of the fungal cell wall and its biochemical composition.

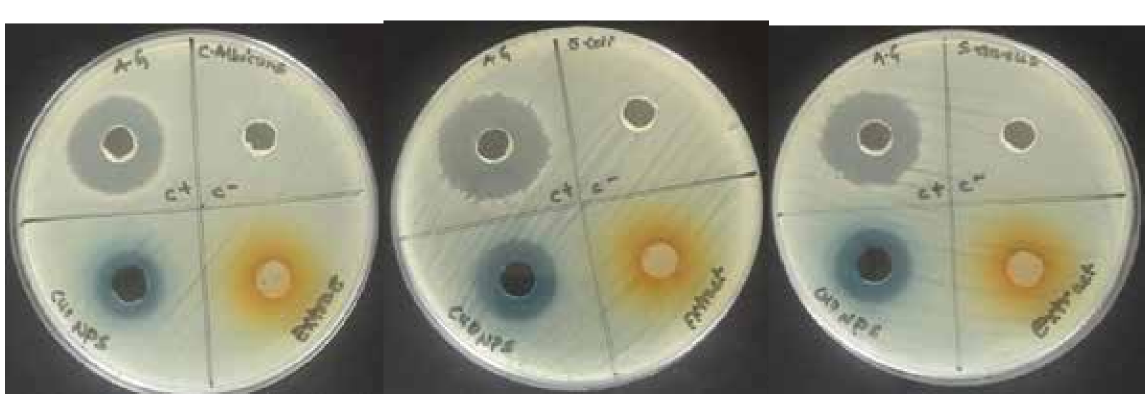

Fig 3. Antimicrobial test showing zone of inhibition (a) Gram +ve bacteria (*S. aureus*) (b) Gram -ve bacteria (*E. coli*) (c) fungi (*C. albicans*)

Further analysis demonstrated that the plant extract exhibited no antimicrobial activity, underscoring the enhanced properties imparted by the CuO nanoparticles. When compared to positive controls, the CuO nanoparticles achieved inhibition zones moderately smaller but significant enough to confirm their efficacy (e.g., 1.8 cm for *E. coli* versus 2.6 cm for the control), shown in **Table 2**. This reinforces the role of nanoparticles as an important antimicrobial agent. This finding demonstrates the effectiveness of CuO nanoparticles, synthesized through eco-friendly methods, as promising antimicrobial agents. The given data also provides a basis for further exploration of their mechanisms of action and applications in antimicrobial formulations.

Table 2. Antimicrobial test results of *Escherichia coli, Staphylococcus aureus* and *Candida albicans*

Strain	Reference culture	Type	Positive control (cm)	CuO NPs (cm)	Plant extract (cm)
Escherichia coli	ATCC8739	Gram +ve	2.6	1.8	0
Staphylococcus aureus	ATCC6538P	Gram -ve	2.3	1.7	0
Candida albicans	ATCC2091	Fungi	2.5	1.5	0

XRD Analysis

The crystal structure and average crystallite size of the biosynthesized CuO nanoparticles were analyzed using XRD. As depicted in **Figure 4**, the XRD shows well-defined diffraction peaks within the 2θ range of 20º to 80º, measured with a step size of 0.020º and a scanning speed of 20° per minute. The observed peaks correspond to specific Miller indices, namely (110), (002), (111), (-202), (020), (202), (-113), (022), (220), (311), and (004), with 2θ values recorded at 32.20º, 35.36º, 38.59º, 48.65º, 53.38º, 58.07º, 61.45º, 65.96º, 67.97º, 72.25º, and 74.90º, respectively.

The XRD analysis reveals two significant peaks at 2θ values of 35.36º (Intensity = 100%, d-spacing = 2.53) and 38.59º (Intensity = 93.33%, d-spacing = 2.33), which are significant to the (002) and (111) planes, respectively that are the most pronounced in the XRD pattern which shows a high level of crystallinity and structural stability within CuO nanoparticles. The crystal structure was identified as monoclinic, following the JCPDS card number 89-5899, which is consistent with previous studies on CuO nanoparticles synthesized from plant extracts such as *Pyrus pyrifolia* leaves, *Urtica dioica* leaves, and *Ephedra alata* [47-49]. The monoclinic phase of CuO is characterized by its distinct crystal structure, which belongs to the space group C2/c. The distinct and sharp diffraction peaks suggest a successful synthesis of crystalline CuO nanoparticles, confirming that the biosynthetic route used in this study resulted in well-ordered nanostructures. The CuO nanoparticles size was evaluated from the Debye-Scherrer formula (equation 1), which provides a method to estimate the average size based on XRD data [38-40]

$$D = \frac{k\lambda}{\beta cos\,\theta} \qquad (1)$$

where D is the crystallite size (Å), λ is the wavelength of the incident X-rays equal to 1.54 Å, k is a constant of 0.9, β is the full width at half maximum (FWHM) and θ is Bragg's angle. The crystallite size of the CuO nanoparticles was 17.35 nm on average, which aligns with previously reported values in the literature for CuO nanoparticles synthesized through biosynthesis methods.

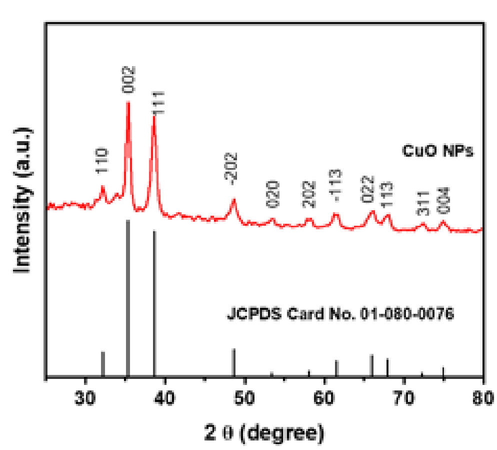

Fig 4. XRD of CuO nanoparticles synthesized using *Acmella oleracea* flower.

UV-Visible Spectroscopy

Researchers are increasingly turning to plant-derived phytochemicals for the biosynthesis of nanoparticles, leveraging their ability to control nanoparticle size and morphology. These natural compounds act as reducing agents and stabilizers during metal ion reduction to form nanoparticles. The reaction's progress can be tracked using UV–Vis spectroscopy, where absorption peaks are linked to surface plasmon resonance (SPR) that helps in the oscillation of conduction band electrons when exposed to electromagnetic radiation that provides insight into the metal ions reduction and formation of nanoparticles [18]. The formation of CuO nanoparticles from the *Acmella oleracea* flower demonstrated a noticeable color shift from pale yellow to green. UV–Vis spectrum presented in **Figure 5(a)** exhibited a broad absorption peak at 280–410 nm, which is likely due to the presence of cuprous oxide impurities indicating the presence of intermediate species during CuO nanoparticle synthesis [50]. This Cu_2O is formed under reducing or mildly oxidative conditions, often due to incomplete oxidation of copper precursors that lower the yield of the CuO nanoparticles. The presence of Cu_2O can influence particle growth and agglomeration, altering the material's overall surface area and porosity. The complete oxidation of Cu_2O to CuO can be obtained by optimizing reaction conditions, such as prolonged reaction times or increased oxidizing agents [51-52].

The *Acmella oleracea* flower extract contains a distinct peak at 320 nm, likely due to the presence of tannins and flavonoids within the extract [53]. These phytochemicals, recognized for their antioxidant activity and non-toxic nature, are essential in metal ions reduction and stability of nanoparticles with the ability to affect the structural properties of the nanoparticles [54]. The band gap of the synthesized CuO nanoparticles was determined using the Tauc model, as described in **equation 2** [41-42].

$$(\alpha h\nu)^n = k(h\nu - Eg) \qquad (2)$$

The calculation involves parameters such as the light energy (hν), absorption coefficient (α), direct band gap energy (E), a constant (k), and the transition index (n). The band gap energies were determined to be 2.27 eV for CuO nanoparticles, **Figure 5(b)**. This value agrees with prior studies documented in the literature [55].

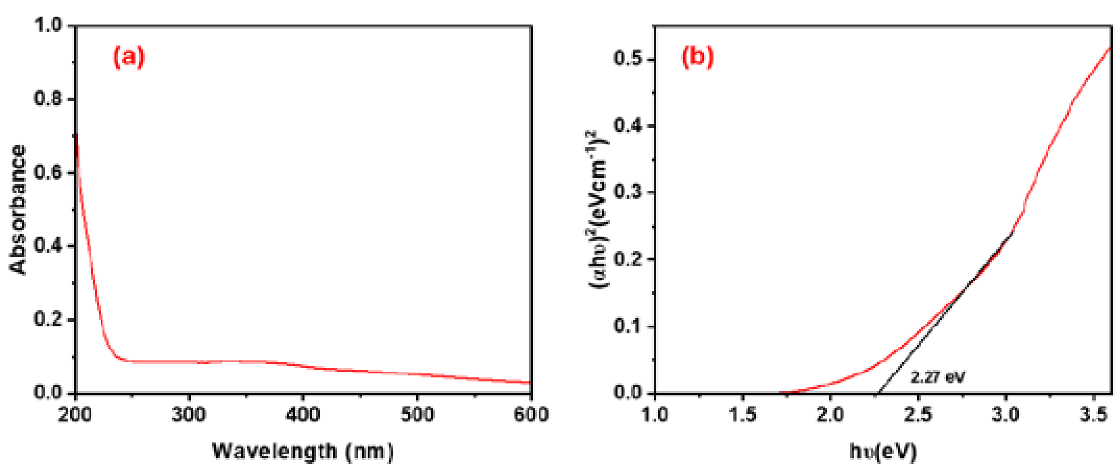

Fig 5: UV-Vis spectroscopy (**a**) and band energy gap (**b**) of synthesized CuO nanoparticles.

FTIR Analysis

FTIR spectroscopy was conducted on the flower extract and CuO nanoparticles, as depicted in Figure 6. The broad band at 3320 cm^{-1} represents O-H stretching on plant extract which corresponds to the phenolic or alcoholic group. The weak O-H stretching band observed in the spectra of CuO nanoparticles suggested either the transformation of the biomolecules into another form, such as oxidation of the alcoholic group to the carbonyl group or the removal of such biomolecules during the synthesis of nanoparticles. The various functional groups C-H (2900 cm^{-1}), C=O (1720 cm^{-1}), N-O (1453 cm^{-1}), and C-O (1236 cm^{-1}) in the plant extract correspond to the biomolecules that are also present in the synthesized nanoparticles. Key peaks at 561 cm^{-1} and 608 cm^{-1} in nanoparticles correspond to Cu-O stretching that validated the formation of CuO nanoparticles. A very weak stretching band of the Cu–O bond at 2347 cm^{-1} was observed, which matches the published literature [56]. The presence of all these bands in the CuO nanoparticles spectra, confirms the successful green synthesis and stabilization of CuO nanoparticles through plant-derived constituents [57-60]

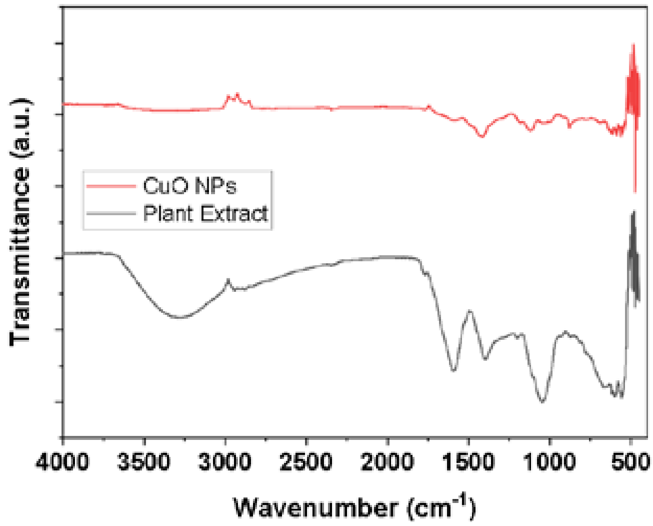

Fig 6. FTIR Spectroscopy of synthesized CuO nanoparticles. (red line spectra) and flower extract (black line spectra).

Conclusions

This study effectively showed the synthesis of copper oxide nanoparticles utilizing flower extract of *Acmella oleracea*. Phytochemical analysis identified the presence of bioactive compounds such as flavonoids, phenolics, alkaloids, terpenoids, and steroids that played a crucial role as reduction and stabilizing during the synthesis process. The characterization of nanoparticles revealed a crystalline monoclinic structure from XRD analysis. Functional groups were identified through FTIR, and UV-visible spectroscopy indicated a 2.27 eV band energy gap. The nanoparticles demonstrated strong antimicrobial effects against Gram-positive, and Gram-negative bacteria, as well as fungi, underscoring potential for applications in both biomedical and environmental fields. This eco-friendly method reduces the use of harmful chemicals, providing a cheaper and more sustainable option compared to traditional methods.

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