JNCS Journal of Nepal Chemical Society
DOI: https://doi.org/10.3126/jncs.v45i1.74491
RESEARCH ARTICLE
January 2025, Vol. 45, No. 1, 111-121

Green Synthesis of Copper Oxide Nanoparticles Using *Mentha* (Mint) Leaves Characterization and Its Antimicrobial Properties with Phytochemicals Screening

Janaki Baral[1], Neena Pokharel[1], Sujan Dhungana[1], Laxmi Tiwari[1], Devendra Khadka[2,3], Megh Raj Pokhrel[3], Bhoj Raj Poudel[1*]

[1] *Department of Chemistry, Tri Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal*

[2] *Tribhuvan Multiple Campus, Tribhuvan University, Tansen, Palpa, Nepal*

[3] *Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal*

[*]*Corresponding E-mail: bhoj.poudel@trc.tu.edu.np*

(Received: December17, 2024; revised: January 6, 2025; accepted:January 8, 2025)

Abstract

This study demonstrates the synthesis of copper oxide nanoparticles from the leaf extract of *Mentha* as a natural reducing and stabilizing agent. The synthesized nanoparticles were characterized by UV-visible spectroscopy, Fourier-transform infrared (FTIR) and X-ray diffraction (XRD). UV-vis shows an absorption peak at 275 nm associated with plasmon surface vibrations. FTIR analysis confirmed the reduction and stabilization of CuO nanoparticles, while XRD revealed the monoclinic structure with an average particle size of 19.08 nm, calculated using Debye-Scherrer's equation. Phytochemical analysis of the methanolic extract identified active components biomolecules such as flavonoids, saponins, and terpenoids, which contribute as reducing and capping agents in nanoparticles. The nanoparticles demonstrated strong antimicrobial activity against *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), and *Candida albicans* (fungus).

Keywords: Copper oxide; Nanoparticles; Green synthesis; *Mentha* leaf; Antimicrobial

Introduction

Nanotechnology has emerged as a revolutionary area in modern science that involves the design and manipulation of materials at the nanoscale in the range of 1-100 nm [1-3]. These nano-scale materials exhibit unique physicochemical properties enabling novel applications across medicine, environmental science, and industry areas [4-7]. Among the diverse nanomaterials, nanoparticles—including carbon-based, metallic, polymeric, and semiconductor types-have garnered significant interest because of their high surface-area-to-volume ratio, tunable characteristics, and potential for multifunctionality [8-11].

Metallic nanoparticles have shown immense promise for various applications, with copper oxide (CuO) nanoparticles standing out due to their versatility in antimicrobial treatments, catalysis, energy storage, and sustainable environments.[12-15]. Copper has a critical role in enzymatic functions, antioxidant defences, and the elimination of pathogens in living organisms [16]. The nanoscale form of copper oxide combines these biological benefits with enhanced reactivity and adsorption capacity, enabling unique properties such as antibacterial, antioxidant, and anticancer activities. These attributes make CuO nanoparticles highly attractive for use in healthcare, agriculture, and beyond [17].

The production of nanoparticles has traditionally relied on chemical synthesis methods, which often involve toxic reagents and high-energy inputs leading to negative impacts

on the environment [18, 19]. Green synthesis methods have emerged as a sustainable and practical alternative to produce nanoparticles with minimal environmental impact and enhanced functional properties [20]. Plant-based green synthesis consists of an abundance of phytochemicals such as phenols, flavonoids, and terpenoids that help in reducing and stabilizing nanoparticles during synthesis [21].

The genus *Mentha* (mint) has long been valued in traditional medicine and industry for its rich bioactive composition, offering antimicrobial, antioxidant, and anti-inflammatory benefits [22, 23]. Recent research has shown that mint extracts, rich in phytochemicals such as polyphenols and terpenoids effectively aid in the green synthesis of nanoparticles like CuO and help in increasing their antibacterial properties [24, 25]. The synthesized CuO nanoparticles from the green method hold great promise in addressing the growing challenge of antibiotic resistance [26]. They show activity against both Gram-positive and Gram-negative bacteria by disrupting cell membranes, leading to leakage of intracellular contents and cell death. This mechanism makes them a potential solution against multidrug resistance against microbes [27].

This study explores the synthesis of CuO nanoparticles using mint leaf extracts followed by analysis through analytical methods and evaluation of antimicrobial properties. By employing plant-based green synthesis, this research adheres to the principles of green chemistry, emphasizing environmental sustainability and biocompatibility. Additionally, the study contributes to the main functions of phytochemicals in nanoparticle formation and their uses in biomedicine and environmental protection.

Materials and Methods

Plant Extract and Chemicals

Copper oxide nanoparticles were synthesized using Mint (*Mentha*) plants collected from Siranchok-5, a rural municipality in the Gorkha district of Gandaki Province, Nepal (28.1122° N, 84.6442° E). Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) and sodium hydroxide (NaOH) were obtained from Thermo Fisher Scientific India Pvt. Ltd. (India) while distilled water was sourced from Ace Scientific Trade Concern, Kathmandu, Nepal.

Preparation of Mint Plants Extract

The collected fresh leaves were carefully cleaned and dried in a shaded area or under mild sunlight for 5 days. The dried leaves were pulverized and kept in sealed containers to protect them from contamination. For extraction, the powdered plant material was immersed in distilled water. 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. Then the solution was filtered to isolate the plant extract. This extract was used for both nanoparticle synthesis and phytochemical screening [28, 29].

Synthesis of CuO Nanoparticles

Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) was used as the precursor for synthesizing copper oxide nanoparticles. Approximately 0.1 M solution of $CuSO_4 \cdot 5H_2O$ was prepared by dissolving 25 g of the compound in water and diluting it to 1 L in a volumetric flask. To initiate the synthesis, 50 mL of mint plant extract and 50 mL of the 0.1 M $CuSO_4 \cdot 5H_2O$ solution were combined dropwise in a 1:1 ratio at 60°C. The mixture was stirred continuously for one hour using a magnetic stirrer, with 1 M NaOH being introduced midway through the stirring process to adjust the pH to 10. Initially, the solution exhibited a dark brownish colour. As the reaction progressed and precipitates began to form, the mixture was removed from heat and allowed to cool. After that, the mixture was centrifuged at about 8500 rpm for about 20 minutes which was then washed with distilled water at least three times to remove impurities. The pure

product was dried at 60°C for one hour and then calcined at 400°C for two hours to enhance the crystallinity, remove organic residues, and improve the stability and purity of the nanoparticles. The final material was then characterized for further analysis. The representative diagram of the process is shown in Fig.1.

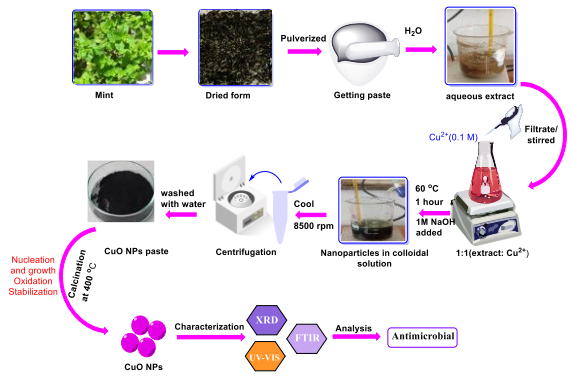

Fig. 1: Representation of CuO nanoparticles synthesis from Mint leaf extract.

Preparation of Mint Extract for Phytochemical Screening

The dried mint leaves were finely ground into a powder and completely submerged in an 80% methanol solution. The mixture was allowed to macerate for 96 hours at room temperature, with intermittent shaking to enhance the extraction. After the maceration period, the mixture was passed through filter paper using a funnel to separate the solid residues from the liquid extract. The collected filtration was then concentrated by evaporating the methanol in a water bath set at 45 °C.

To identify its phytochemical profile, a range of standardized methodologies were employed to detect specific bioactive constituents. Flavonoids were identified through Shinoda and zinc-hydrochloride reduction methods. The presence of alkaloids was confirmed through Mayer's test whereas Tannins were identified through ferric chloride, potassium dichromate, and lead acetate assays. Saponins were evaluated using the foam test. Phytosterols were analyzed using the Liebermann-Burchard and Salkowski reactions, and glycosides were detected via Legal and Keller-Killian tests [30 - 32].

Antimicrobial Activity of CuO Nanoparticles

The antimicrobial activity of the green synthesized CuO-Nps and aqueous extract of *Mentha* (mint) against two bacterial strains *Escherichia coli* (ATCC 8739), Gram-negative, *Staphylococcus aureus* (ATCC 6538P), Gram-positive, and one fungal species- *Candida albicans* (ATCC 2091) was investigated using agar well diffusion method. It involved 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 Petri dishes (25 mL in each Petri dish) and stored in a refrigerated environment until needed. Antimicrobial assays were performed using these plates, employing sterile cotton swabs for inoculation [33]. The prepared media plates were appropriately labelled with sample names. Wells (9 mm diameter, 3 mm depth) were created on the surface of the plates using the back of a sterilized pipette tip to accommodate the samples and standards. All samples were dissolved in DMSO at a concentration of 50 mg/mL. Each prepared well

was loaded with 100 µL of the respective sample solution, a negative control, DMSO (C^-) and a standard kanamycin (Sigma Aldrich-USA, Purity: 95%) solution (5 mg/mL, 10 µL) for bacterial strains and itraconazole (Deurali-Janta Pharmaceuticals, Nepal) (20 mg/mL; 10 µL) for fungal strain as positive controls. The plates were incubated at 37 °C for 24 hours. After the 24-hour incubation period, the diameters of the zones of inhibition were measured.

Nanoparticles Analysis

The crystalline and phase composition of the synthesized nanoparticles were studied using X-ray diffraction (XRD). This was performed with an X-ray diffractometer (D2 phaser, Bruker), with Cu Kα radiation in the 2θ range of 20°-80° utilizing Kα radiation with a wavelength of 1.5406 Å. To evaluate the optical properties, a SPECORD 200 Plus UV-is spectrophotometer (Germany) was employed. A UV-Vis spectrum of the sample, prepared as a dilute suspension in distilled water, was recorded by scanning the wavelength range from 200 to 600 nm. Functional groups related to the sample, acting as stabilizing and reducing agents, were identified through Fourier-transform infrared spectroscopy (FTIR) between 4000–400 cm^{-1} wavenumber and 4 cm^{-1} scan resolution. FTIR analysis was carried out using a PerkinElmer Spectrum IR (Version 10.6.2).

Results and Discussion

Phytochemical Study

Phytochemicals present in leaf extract were initially assessed using various chemical tests. As mentioned in published literature, this research also proves the leaf extract does not contain alkaloids, quinones, tannins but possesses positive tests for flavonoids, saponins, and terpenoids, as shown in **Table 1** [34]. These classes of bioactive constituents are believed to act as reducing and stabilizing agents during the synthesis of nanomaterials [35]. Studies suggest that phytochemicals such as tannins and flavonoids exhibit antibacterial properties and play a significant role in scavenging free radicals [36].

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

Phytochemicals	Saponin	Flavonoid	Terpenoid and steroid	Alkaloid	Tannins	Quinone
Remark	+	+	+	-	-	-

Where, the symbol "+" indicates the presence of phytochemicals, and the symbol "–" denotes their absence.

Mechanism of CuO Nanoparticles Synthesis

Plant extracts are abundant in diverse biomolecules, as shown by phytochemical studies, which play a key role in nanoparticle synthesis. However, the process of synthesizing nanoparticles based on natural resources is complex, as the precise mechanisms are not yet fully understood. Studies have emphasized that functional groups such as hydroxyl, carbonyl, amine, and methoxide are critical for the reduction and stabilization of nanoparticles. These groups are commonly found in plant metabolites like flavonoids, phenols, alkaloids, proteins, and quinones [37]. The synthesis of CuO nanoparticles utilizing mint plant extract involves a bio-mediated process where biomolecules interact with Cu^{2+} ions. This interaction results in the reduction of copper ions from Cu(II) to Cu(0) which then undergoes oxidation to form 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 **Fig. 2**. Similar nanoparticle synthesis mechanisms have been proposed in earlier studies [38-39].

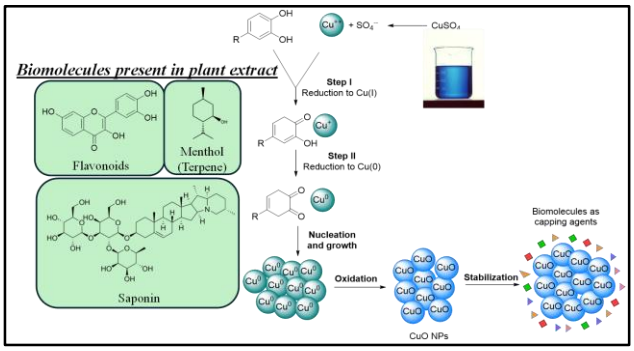

Fig. 2: A possible mechanism of CuO nanoparticles synthesis from *Mint* leaf extract.

Antimicrobial Study

The synthesized CuO nanoparticles were evaluated against three microorganisms, Gram-negative *Escherichia coli* (ATCC 8739), Gram-positive *Staphylococcus aureus* (ATCC 6538P), and the fungus *Candida albicans* (ATCC 2091) using the agar well diffusion method. A clear inhibitory zone around each well on the Petri plates confirmed the antimicrobial potential of the biosynthesized CuO-NPs which is shown in **Figure 3** and the results are summarized in **Table 2**. Kanamycin was employed as a positive control to benchmark the antimicrobial effectiveness of the synthesized nanoparticles.

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

Microbes	Reference culture	Type	Positive control (c+) cm	CuO-Nps (cm)	Plant Extract
Escherichia coli	ATCC 8739	Gram -ve	2.6	1.4	0
Staphylococcus aureus	ATCC 6538P	Gram +ve	2.3	1.3	0
Candida albicans	ATCC 2091	Fungi	2.5	1.5	0

While many mechanisms have been proposed for the antimicrobial properties of CuO nanoparticles, their exact mode of action remains still unclear. It is suggested that CuO-NPs release copper ions that disrupter microbial cell membranes causing denaturing proteins, and ultimately causing cell death. Copper ions are positively charged particles that bind to the negatively charged bacterial cell wall leading to the rupture. Inside the microbial cell, copper ions interact with DNA, inducing cross-linking of nucleic acid strands and causing structural abnormalities. These ions also interfere with key metabolic processes within the microbial cell causing cell death [40].

The research demonstrated that biosynthesized CuO nanoparticles exhibited greater antibacterial activity against Gram-negative bacteria compared to Gram-positive bacteria, with zones of inhibition of 1.4 cm and 1.3 cm, respectively, as shown in **Fig. 3**. The higher activity of CuO-NPs towards Gram-negative bacteria might be attributed to their thin cell membrane, which allows CuO nanoparticles to penetrate bacterial cell membranes more effectively [41]. Interestingly, the highest zone of inhibition (1.5 cm) was seen against *Candida albicans*. This may be due to the larger and more complex structure of fungal cells, offering a greater surface area for nanoparticle interaction [42].

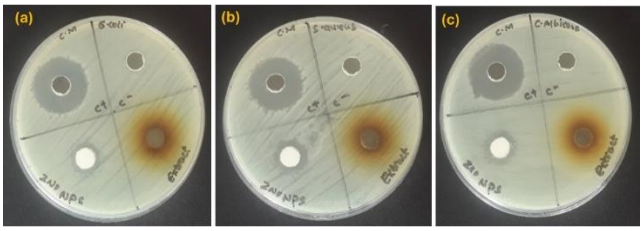

Fig. 3: Antimicrobial test showing zone of inhibition: a) *Escherichia coli, (b) Staphylococcus aureus, and (c) Candida albicians.*

Mentha leaf extract comparatively showed no inhibitory activity against bacterial or fungal strains. This could be because the extract was water-based, potentially leading to incomplete extraction of active compounds. Previous studies have demonstrated the potent antimicrobial activity of Mentha leaf extracts in organic solvents, such as ether, chloroform, ethanol, methanol, and ethyl acetate, suggesting that the active components in Mentha are solvent-dependent [43].

XRD Study

The crystalline structure of CuO-NPs is determined by XRD. The diffraction peaks of nanoparticles were found at 32.52°, 35.45°, 38.73°, 48.76°, 53.41°, 58.31°, 61.57°, 66.23°, 68.14°, 72.41°, and 75.02°, which corresponded to the miller indices (110), (-111), (111), (-202), (020), (202), (-113), (-311), (220), (311) and (004) planes, respectively, shown in **Fig. 4**. XRD suggests a monoclinic crystal structure of nanoparticles that closely matched the standard data from JCPDS card No. 00-000-0661 [44].

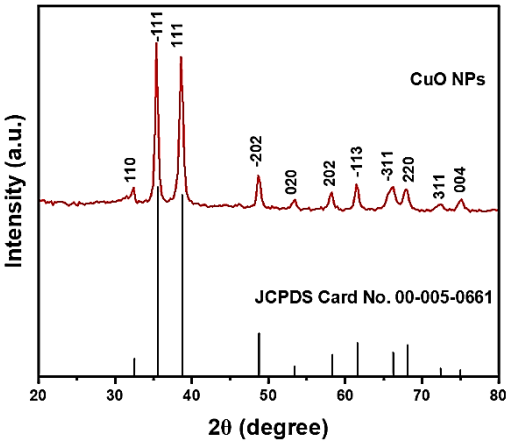

Fig. 4: XRD pattern of CuO nanoparticles synthesized using Mint plant extract.

The crystallite size of CuO nanoparticles was calculated using Debye-Scherrer's **equation** (1), [45, 46] yielding a value of 19.08 nm. This result aligns with previous studies that reported comparable sizes for CuO-NPs, further confirming their successful formation [47].

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

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.

UV-Visible Spectroscopy

The biosynthesized CuO nanoparticles were further validated by UV-vis analysis, which displayed a characteristic peak near 275.33 nm, as illustrated in **Fig. 5(a)**. The observed colour change of the mixture from light brown to brownish black with the addition of copper salts in the extract provided visual evidence for the successful synthesis of CuO-NPs using Mentha leaf extract which can be assigned by the surface plasmon resonance (SPR) phenomenon, as previously reported [48].

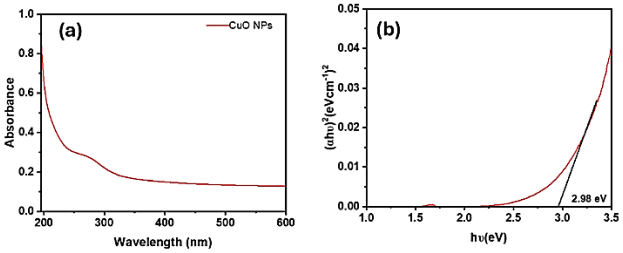

Fig. 5: UV-Vis spectroscopy (**a**) and band energy gap (**b**) of synthesized CuO nanoparticles.

The bandgap energy (Eg) of CuO nanoparticles was determined using the Tauc equation (2) [49].

$$(\alpha h\nu)^m = C(h\nu - Eg) \qquad (2)$$

where, α = absorbance coefficient, C = constant, h = Plank's constant, ν = photon frequency, Eg = optical band gap, m = 2 for indirect band gap semiconductors.

The calculated band gap energy was 2.98 eV of synthesized CuO nanoparticles. **Fig. 5(b)** illustrates the relationship between $(\alpha h\nu)^{1/m}$ and $h\nu$ for the CuO nanoparticles (CuO-NPs), with m=2. The calculated optical band gap of the synthesized CuO-NPs is higher than the range reported in previous studies, which typically falls between 1.2 and 2.5 eV [50]. The high energy band gap could be by various factors such as the quantum confinement effect or variations in synthesis conditions, including pH, temperature, and concentration [51].

FTIR Analysis

In this study, FTIR was employed to examine the Mentha leaf extract and the synthesized CuO nanoparticles, aiming to detect the biomolecules responsible for the reduction of CuO NPs. The leaf extract shows significant

peaks at 3285 cm^{-1} (O-H stretching), 1591 cm^{-1} (C=C stretching), 1387 cm^{-1} (C-H bending), 1258 cm^{-1} (C-O stretching), and 1046 cm^{-1} (C-O stretching) which indicated the functional groups of biomolecules. The peaks at 615.48, 587.63, and 548.55 cm^{-1} in the CuO nanoparticles spectra are indicative of metal-oxide vibrations [52]. The broad peak at 3352 cm^{-1} for nanoparticles corresponds to hydroxyl (O-H) functional groups indicating the association of biomolecules or water in nanoparticles. The shifting of functional group peaks and changes in peak intensity indicate the interaction between plant extract components and metal ions, leading to the synthesis of nanoparticles[53,54].

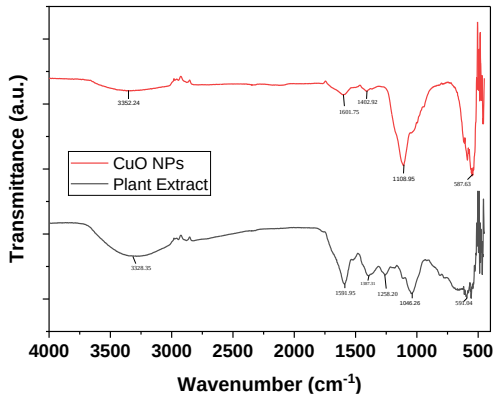

Fig. 6. FTIR Spectroscopy of synthesized CuO nanoparticles. (red line spectra) and mint extract (black line spectra).

Conclusions

The synthesis of copper oxide nanoparticles (CuO-NPs) from mint leaf extract represents a sustainable, environmentally friendly, and cost-efficient approach. Phytochemicals present in mints, such as flavonoids, saponins, and terpenoids, help in the reduction and stability of nanoparticle synthesis. XRD confirmed their monoclinic crystalline structure with a crystallite size of 19.08 nm of nanoparticles. The UV-vis displayed a surface plasmon resonance peak at 270 nm and FTIR analysis revealed a CuO vibration at 587 cm^{-1}, verifying the successful formation of CuO-NPs. These nanoparticles demonstrated significant antimicrobial activity, showing zones of inhibition of 1.4 cm against Staphylococcus aureus, 1.3 cm against Escherichia coli, and 1.5 cm against Candida albicans. This study underscores the potential of plant-based methods for synthesizing antimicrobial nanoparticles, offering promising prospects for applications in medicine, agriculture, and sustainable nanotechnology. Further investigations can broaden the scope of mint in green chemistry applications.

Acknowledgements

The authors would like to thank the Department of Chemistry at Tri-Chandra Multiple Campus for providing laboratory facilities, the Nepal Academy of Science and Technology in Khumaltar for conducting the XRD analysis, and the Amrit Campus in Lainchaur for their support with FTIR analysis.

Author's Contribution Statement

J. Baral; S. Dhungana: Experimental, Methodology, Data curation, Validation, Writing-original draft, **N. Pokhrel; L. Tiiwari**: Data curation, Validation, Formal analysis, Writing-original draft preparation, **D. Khadka**: Conceptualization, Writing-review and editing, **M. R. Pokhrel; B. R. Poudel**: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing-review and editing, Supervision

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

References

1. S. Mahendra Kumar, Y. Rajni, and T. Sandeep Prasad, Recent advances in nanotechnology, *Int J Nanomater Nanotechnol Nanomed*, 2023, **9**(2), 015–023. (DOI: 10.17352/2455-3492.000053)
2. M. Nasrollahzadeh, S. M. Sajadi, M. Sajjadi, and Z. Issaabadi, An Introduction to Nanotechnology, *Interface Science and Technology*, 2019, **28**(Elsevier), 1–27. (DOI: 10.1016/B978-0-12-813586-0.00001-8)
3. Y. Khan *et al.*, Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in Various Fields of Nanotechnology: A Review, *Catalysts*, 2022, **12**(11), 1386. (DOI10.3390/catal12111386)
4. N. Baig, I. Kammakakam, and W. Falath, Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges, *Mater. Adv.*, 2021, **2**(6), 1821–1871. (DOI: 10.1039/D0MA00807A)
5. T. Joseph *et al.*, Nanoparticles: Taking a Unique Position in Medicine, *Nanomaterials*, 2023, **13**(3), 574. (DOI: 10.3390/nano13030574)
6. S. A. Afolalu, O. Okwilagwe, A. Abdulkareem, M. E. Emetere, S. O. Ongbali, and O. O. Yusuf, Overview Of Physicochemical And Surface Properties Of Nanoparticles For Engineering Applications, *IOP Conf. Ser.: Earth Environ. Sci.*, 2021, **665**(1), 012049. (DOI: 10.1088/1755-1315/665/1/012049)
7. R. Biswas, M. Alam, A. Sarkar, M. I. Haque, Md. M. Hasan, and M. Hoque, Application of nanotechnology in food: processing, preservation, packaging and safety assessment, *Heliyon*, 2022, **8**(11), e11795. (DOI: 10.1016/j.heliyon.2022.e11795)
8. C. Sudharsana, N. Anvarsha, and P. Kalyani, Carbon-Based Nanocomposites: A Comprehensive Review of Their Multifunctional Applications, in *Nanotechnology and Nanomaterials*, 2024, **5**, IntechOpen. (DOI: 10.5772/intechopen.114402)
9. J. Li, H. Zeng, Z. Zeng, Y. Zeng, and T. Xie, Promising Graphene-Based Nanomaterials and Their Biomedical Applications and Potential Risks: A Comprehensive Review, *ACS Biomater. Sci. Eng.*, 2021, **7**(12), 5363–5396. (DOI: 10.1021/acsbiomaterials.1c00875)
10. M. S. Chavali and M. P. Nikolova, Metal oxide nanoparticles and their applications in nanotechnology, *SN Appl. Sci.*, 2019, **1**(6), 607. (DOI: 10.1007/s42452-019-0592-3)
11. N. Hossain *et al.*, Advances and significances of nanoparticles in semiconductor applications – A review, *Results in Engineering*, 2023, **19**, 101347. (DOI: 10.1016/j.rineng.2023.101347)
12. A. Arun Kumar, R. Subramaniyan@Raja, G. Padmasree, K. Veerabhadra Rao, K. Anuradha, and A. Rathika, Copper Oxide Nanoparticles for Energy Storage Applications, in *Materials for Sustainable Energy Storage at the Nanoscale*, 1st ed., Boca Raton: CRC Press, 2023, 233–240. (DOI:10.1201/9781003355755-20)
13. J. O. Ighalo *et al.*, CuO nanoparticles (CuO NPs) for water treatment: A review of recent advances, *Environmental Nanotechnology, Monitoring & Management*, 2021, **15**, 100443. (DOI: 10.1016/j.enmm.2021.100443)
14. S. Naz, A. Gul, M. Zia, and R. Javed, Synthesis, biomedical applications, and toxicity of CuO nanoparticles, *Appl Microbiol Biotechnol*, 2023, **107**(4), 1039–1061. (DOI: 10.1007/s00253-023-12364-z)
15. A. F. Zedan, A. T. Mohamed, M. S. El-Shall, S. Y. AlQaradawi, and A. S. AlJaber, Tailoring the reducibility and catalytic activity of CuO nanoparticles for low temperature CO oxidation, *RSC Adv.*, 2018, **8**(35), 19499–19511. (DOI:10.1039/C8RA03623C)
16. M. P. J. V. K. K. D. Vetchý, Biological role of copper as an essential trace element in the human organism, *Ceska Slov Farm*, 2018, **67**(4), 143–153.

17. M. J. Woźniak-Budych, K. Staszak, and M. Staszak, Copper and Copper-Based Nanoparticles in Medicine—Perspectives and Challenges, *Molecules*, 2023, **28**(18), 6687. (DOI:10.3390/molecules28186687)
18. E. M. Modan, A. G. Plăiaşu, Advantages and Disadvantages of Chemical Methods in the Elaboration of Nanomaterials, *MMS*, 2020, **43**(1), 53–60. (DOI: 10.35219/mms.2020.1.08)
19. R. Vishwanath and B. Negi, Conventional and green methods of synthesis of silver nanoparticles and their antimicrobial properties, *Current Research in Green and Sustainable Chemistry*, 2021, **4**, 100205. (DOI: 10.1016/j.crgsc.2021.100205)
20. C. Wu, J. Peng, T. Song, and M. Fu, Green synthesis approach: Utilizing chrysanthemum extract as reducing and stabilizing agent for the fabrication of silver nanoparticles and their antimicrobial properties study, *Alexandria Engineering Journal*, 2024, 149–155. (DOI: 10.1016/j.aej.2024.04.005)
21. M. Thatyana, N. P. Dube, D. Kemboi, A.-L. E. Manicum, N. S. Mokgalaka-Fleischmann, and J. V. Tembu, Advances in Phytonanotechnology: A Plant-Mediated Green Synthesis of Metal Nanoparticles Using Phyllanthus Plant Extracts and Their Antimicrobial and Anticancer Applications, *Nanomaterials*, 2023, **13**(19), 2616. (DOI: 10.3390/nano13192616)
22. M. Nazim, Q.-U.-A. Sadiq, A. Nawaz, S. Anjum, M. Ali, and H. Maryam, Mentha arvensis, a medicinal and aromatic plant, has high nutritional value and several uses: A review, *Bul. Agroteknologi*, 2020, **1**(2), 37. (DOI: 10.32663/ba.v1i2.1180)
23. M. Tafrihi *et al.*, The Wonderful Activities of the Genus Mentha: Not Only Antioxidant Properties, *Molecules*, 2021, **26**(4), 1118. (DOI: 10.3390/molecules26041118)
24. B. Khameneh, N. A. M. Eskin, M. Iranshahy, and B. S. Fazly Bazzaz, Phytochemicals: A Promising Weapon in the Arsenal against Antibiotic-Resistant Bacteria, *Antibiotics*, 2021, **10**(9), 1044. (DOI:10.3390/antibiotics10091044)
25. T. Siddiqui, M. U. Khan, V. Sharma, and K. Gupta, Terpenoids in essential oils: Chemistry, classification, and potential impact on human health and industry, *Phytomedicine Plus*, 2024, **4**(2), 100549. (DOI: 10.1016/j.phyplu.2024.100549)
26. M. Devaraji, P. V. Thanikachalam, and K. Elumalai, The potential of copper oxide nanoparticles in nanomedicine: A comprehensive review, *Biotechnology Notes*, 2024, **5**, 80–99. (DOI: 10.1016/j.biotno.2024.06.001)
27. S. Meghana, P. Kabra, S. Chakraborty, and N. Padmavathy, Understanding the pathway of antibacterial activity of copper oxide nanoparticles, *RSC Adv.*, 2015, **5**(16), 12293–12299. (DOI:10.1039/C4RA12163E)
28. A. A. Barzinjy and H. H. Azeez, Green synthesis and characterization of zinc oxide nanoparticles using Eucalyptus globulus Labill. leaf extract and zinc nitrate hexahydrate salt, *SN Appl. Sci.*, 2020, **2**(5), 991. (DOI:10.1007/s42452-020-2813-1)
29. P. Abhimanyu, M. Arvind, and N. Kishor, Biosynthesis of CuO Nanoparticles Using Plant Extract as a Precursor: Characterization, Antibacterial, and Antioxidant Activity, *Nano Biomedicine and Engineering*, 2023, **15**(4), 369–377. (DOI:10.26599/NBE.2023.9290027)
30. P. B. Lalthanpuii, N. Sailo, B. Lalruatfela, H. T. Lalremsanga, and K. Lalchhandama, Some Phytochemical, Antimicrobial and Anticancer Tests for an Aqueous extract of *Acmella oleracea*, *Rese. Jour. of Pharm. and Technol.*, 2019, **12**(6), 3033. (DOI:10.5958/0974-360X.2019.00513.4)
31 N. Kancherla, A. Dhakshinamoothi, K. Chitra, and R. B. Komaram, Preliminary Analysis of Phytoconstituents and Evaluation of

Anthelminthic Property of Cayratia auriculata (In Vitro), *Maedica*, 2019, **14**(4). (DOI:10.26574/maedica.2019.14.4.350)

32. S. H. Alotaibi, Biophysical properties and fingerprint of Boswellia Sp. Burseraceae, *Saudi Journal of Biological Sciences*, 2019, **26**(7), 1450–1457. (DOI: 10.1016/j.sjbs.2019.09.019)
33. P. Sutradhar, M. Saha, and D. Maiti, Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity, *J Nanostruct Chem*, 2014, **4**(1), 86. (DOI: 10.1007/s40097-014-0086-1)
34. Pramila D. M., Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: Lamiaceae), *J. Med. Plants Res.*, 2012, **6**(3). (DOI:10.5897/JMPR11.1232)
35. Z. Villagrán *et al.*, Plant-Based Extracts as Reducing, Capping, and Stabilizing Agents for the Green Synthesis of Inorganic Nanoparticles, *Resources*, 2024, **13**(6), 70. (DOI:10.3390/resources13060070)
36. Y.-J. Zhang *et al.*, Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases, *Molecules*, 2015, **20**(12), 21138–21156. (DOI: 10.3390/molecules201219753)
37. H. R. El-Seedi *et al.*, Metal nanoparticles fabricated by green chemistry using natural extracts: biosynthesis, mechanisms, and applications, *RSC Adv.*, 2019, **9**(42), 24539–24559. (DOI:10.1039/C9RA02225B)
38. M. Priya *et al.*, Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from Morinda citrifolia leaf extract, *Sci Rep*, 2023,**13**(1), 18838. (DOI: 10.1038/s41598-023-46002-5)
39. S. Nouren *et al.*, Green synthesis of CuO nanoparticles using Jasmin sambac extract: Conditions optimization and photocatalytic degradation of Methylene Blue dye, *Journal of King Saud University - Science*, 2024, **36**(3), 103089. (DOI: 10.1016/j.jksus.2024.103089)
40. S. Stohs, Oxidative mechanisms in the toxicity of metal ions, *Free Radical Biology and Medicine*, 1995, **18**(2), 321–336. (DOI: 10.1016/0891-5849(94)00159-H)
41. M. Ahamed, H. A. Alhadlaq, M. A. M. Khan, P. Karuppiah, and N. A. Al-Dhabi, Synthesis, Characterization, and Antimicrobial Activity of Copper Oxide Nanoparticles, *Journal of Nanomaterials*, 2014, **2014**(1), 637858. (DOI:10.1155/2014/637858)
42. R. Garcia-Rubio, H. C. De Oliveira, J. Rivera, and N. Trevijano-Contador, The Fungal Cell Wall: Candida, Cryptococcus, and Aspergillus Species, *Front. Microbiol.*, 2020, **10**, 2993. (DOI:10.3389/fmicb.2019.02993)
43. G. Bupesh, C. Amutha, S. Nandagopal, A. Ganeshkumar, P. Sureshkumar, and K. Saravana Murali, Antibacterial activity of Mentha piperita L. (peppermint) from leaf extracts – a medicinal plant, *aas*, 2007, **89**(1). (DOI: 10.14720/aas.2007.89.1.14981)
44. S. Rajeshkumar *et al.*, Environment-friendly synthesis copper oxide nanoparticles and its antioxidant, antibacterial activities using Seaweed (Sargassum longifolium) extract, *Journal of Molecular Structure*, 2021, **1242**, 130724. (DOI: 10.1016/j.molstruc.2021.130724)
45. S. Dhungana, B. R. Paudel, and S. K. Gautam, Synthesis and characterization of ZnTe nanoparticles, *Nepal Journal of Science and Technology*, 2016, **17**(1), 1–3. (DOI: 10.3126/njst.v17i1.25054)
46. D. Khadka *et al.*, Evaluating the Photocatalytic Activity of Green Synthesized Iron Oxide Nanoparticles, *Catalysts*, 2024, **14**(11), 751. (DOI:10.3390/catal14110751)
47. A. Rahnama and M. Gharagozlou, Preparation and properties of semiconductor CuO nanoparticles via a simple precipitation method at different reaction temperatures, *Opt Quant*

Electron, 2012, **44**(6–7), 313–322. (DOI:10.1007/s11082-011-9540-1)

48. T. M. D. Dang, T. T. T. Le, E. Fribourg-Blanc, and M. C. Dang, Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method, *Adv. Nat. Sci: Nanosci. Nanotechnol.*, 2011, **2**(1), 015009. (DOI: 10.1088/2043-6262/2/1/015009.
49. P. Sharma *et al.*, Temperature Dependent Synthesis of Zinc Sulphide (ZnS) Nanoparticles and Its Characterization, *Amrit Res. J.*, 2022, **3**(01), 67–74. (DOI:10.3126/arj.v3i01.50498)
50. W. W. Andualem, F. K. Sabir, E. T. Mohammed, H. H. Belay, and B. A. Gonfa, Synthesis of Copper Oxide Nanoparticles Using Plant Leaf Extract of Catha edulis and Its Antibacterial Activity, *Journal of Nanotechnology*, 2020, **2020**, 1–10. (DOI:10.1155/2020/2932434)
51. M. Picollo, M. Aceto, and T. Vitorino, UV-Vis spectroscopy, *Physical Sciences Reviews*, 2019, **4**(4), 20180008. (DOI: 10.1515/psr-2018-0008)
52. R. Sankar, P. Manikandan, V. Malarvizhi, T. Fathima, K. S. Shivashangari, and V. Ravikumar, Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2014, **121**, 746–750. (DOI: 10.1016/j.saa.2013.12.020)
53. S. Saif, A. Tahir, T. Asim, and Y. Chen, Plant Mediated Green Synthesis of CuO Nanoparticles: Comparison of Toxicity of Engineered and Plant Mediated CuO Nanoparticles towards Daphnia magna, *Nanomaterials*, 2016, **6**(11), 205. (DOI:10.3390/nano6110205)
54. S. Dhungana *et al.*, Synthesis and Characterization of Copper Oxide Nanoparticles Isolated from *Acmella oleracea* and Study of Antimicrobial and Phytochemical Properties *Amrit Res. J.*, 2024, **5**(1), 18–29. (DOI: 10.3126/arj.v5i1.73521)