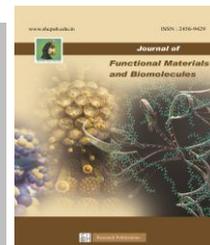




SACRED HEART RESEARCH PUBLICATIONS

Journal of Functional Materials and Biomolecules

Journal homepage: www.shcpub.edu.in



ISSN: 2456-9429

ECO-FRIENDLY SYNTHESIS OF CuO AND ZnO NANOPARTICLES USING TABERNAEMONTANA DIVARICATA LEAF EXTRACT: STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL EVALUATION

A.P. Angeline Mary¹

Received on 15 October 2025, accepted on 25 November 2025,

Published online on December 2025

Abstract

This study explores the green synthesis of copper oxide (CuO) and zinc oxide (ZnO) nanoparticles using aqueous leaf extract of *Tabernaemontana divaricata*. The synthesized nanoparticles were characterized using UV-Vis, FTIR, XRD, SEM, and EDS techniques. Antimicrobial activity was evaluated against *Escherichia coli* and *Salmonella enteritidis* using agar well diffusion. The results confirmed successful nanoparticle formation with distinct crystalline phases and significant antimicrobial efficacy, highlighting the potential of plant-mediated synthesis for biomedical and environmental applications.

Keywords: Green synthesis, CuO nanoparticles, ZnO nanoparticles, *Tabernaemontana divaricata*, antimicrobial activity, SEM, XRD

1. Introduction

Green synthesis offers a sustainable alternative to conventional nanoparticle fabrication, minimizing toxic reagents and energy consumption. *Tabernaemontana divaricata*, rich in flavonoids, alkaloids, and terpenoids, serves as a natural reducing and stabilizing agent. Copper and zinc ox-

ide nanoparticles are known for their catalytic, electronic, and antimicrobial properties [1&2]. This study investigates the synthesis of CuO and ZnO nanoparticles using *T. divaricata* and evaluates their structural and biological properties.

2. Experimental Procedure

Materials and Methods

2.1 Plant Material Processing and Solution Preparation

Fresh and healthy leaves of the *Tabernaemontana divaricata* plant, rich in bioactive compounds, were harvested. The leaves were washed thoroughly with tap water followed by deionized water, and then dried at room temperature for one week. The dried leaves were crushed and ground into fine powder, which was stored in an airtight container. Copper acetate and zinc nitrate were used as precursor salts, and double-distilled water was used for preparing chemical reagents.

*Corresponding author: E-mail: angelinmary@shcpt.edu
¹Department of Chemistry, Sacred Heart College (Autonomous), Tirupattur-635 601, Tamil Nadu, India.

2.2 Plant Extract Preparation

Thirty grams of the powdered leaves were added to 500 mL of deionized water and stirred well. The mixture was boiled for 30 minutes on a heating mantle, cooled for 24 hours, and then filtered using Whatman No. 1 filter paper to obtain a clear solution. The filtrate was stored in a refrigerator and used as a reducing agent for the green synthesis of bimetallic oxide nanoparticles.

2.3 Green Synthesis Procedure

The method involves the simultaneous reduction of copper acetate and zinc nitrate precursor salts using *T. divaricata* extract. A 0.5 M copper acetate solution was prepared by dissolving 3.4938 g of copper acetate in 100 mL of double-distilled water. Similarly, a 0.5 M zinc nitrate solution was prepared by dissolving 5.2060 g of zinc nitrate in 100 mL of double-distilled water.

About 60 mL of leaf extract was boiled for 30–45 minutes at around 60 °C. Then, 35 mL of copper acetate solution was added dropwise into the extract, followed by 35 mL of zinc nitrate solution, also added dropwise. The reaction mixture was maintained at 40 °C for 45 minutes, and the pH was adjusted using NaOH solution. A green-colored precipitate was readily obtained.

After completion of the reaction, the solution was placed on a magnetic stirrer and maintained at 50 °C for 1 hour. The precipitate was then collected in centrifuge tubes and centrifuged at 5000 rpm for 15 minutes. The pellets were washed several times with deionized water and finally with ethanol to remove impurities. The purified precipitate was collected in a 100 mL beaker using acetone

and dried in a hot air oven at 60 °C for 1 hour. The dried product was cooled, finely ground, and stored in an airtight container as the synthesized bimetallic nanoparticles.

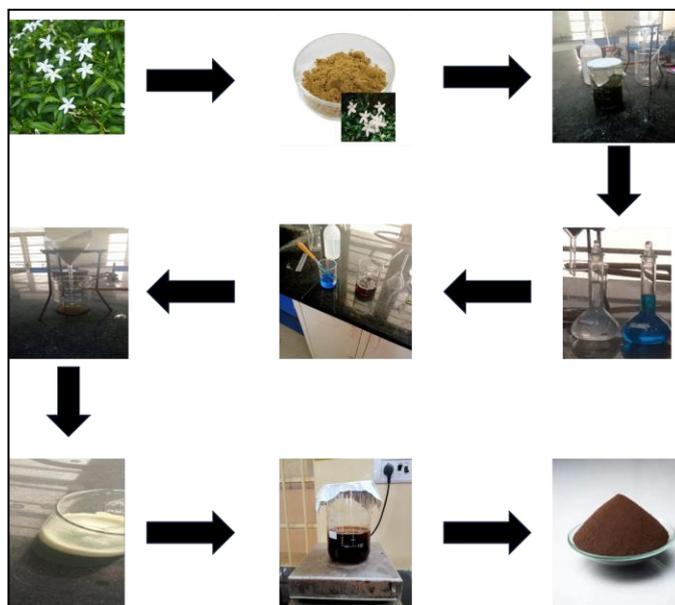


Fig.1. synthesis of CuO nanoparticles and ZnO/CuO nanocomposites.

3. Results and Discussion

3.1 Powder X-ray Diffraction (XRD) Analysis

X-ray Diffraction (XRD) is a powerful technique for analyzing the crystalline structure of CuO and ZnO nanoparticles. CuO nanoparticles generally exhibit characteristic diffraction peaks corresponding to planes such as (111), (200), and (220). The analysis of these peaks provides information on crystal phases and crystallite size. ZnO nanoparticles, in contrast, show distinct peaks associated with the hexagonal wurtzite crystal structure, such as (100), (002), and (101). XRD is crucial for determining the crystalline nature, purity, and phase composition of nanoparticles, as well as estimating lattice parameters [5&6]. These structural details directly

influence the optical and electronic behavior of CuO and ZnO nanoparticles, which is vital for their application in catalysis and optoelectronics.

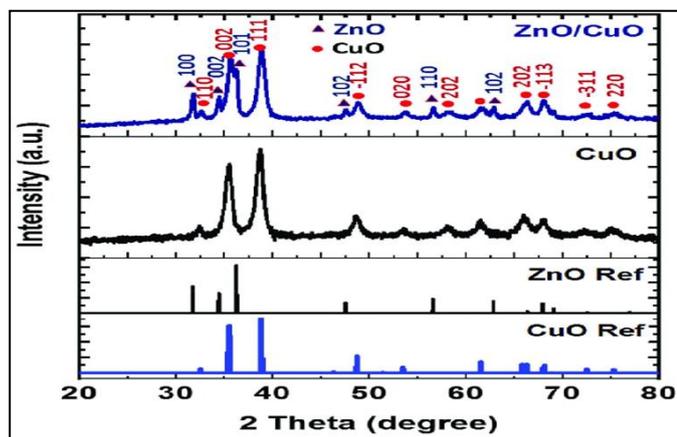


Fig. 2. XRD pattern of CuO nanoparticles and ZnO/CuO nanocomposites.

The XRD patterns of CuO and ZnO/CuO nanoparticles synthesized by pulsed laser ablation in liquid are shown. As observed, all the peaks in the CuO sample could be indexed to the monoclinic structure of CuO. The diffraction peaks located at $2\theta = 31.79^\circ, 34.47^\circ, 36.32^\circ, 47.56^\circ, 56.67^\circ, 62.94^\circ, 66.48^\circ, 68.04^\circ, 69.19^\circ, 72.65^\circ,$ and 77.04° can be perfectly indexed to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes of hexagonal ZnO.

Similarly, the diffraction peaks observed at $2\theta = 32.65^\circ, 35.72^\circ, 38.85^\circ, 48.84^\circ, 53.69^\circ, 58.17^\circ, 61.56^\circ, 67.95^\circ,$ and 75.35° are indexed to the (110), (002), (111), (202), (020), (202), (113), (113), and (-222) planes of monoclinic CuO. This indicates the successful incorporation of both compounds. The crystallite size of the CuO and ZnO nanoparticles was calculated using the Scherrer equation: 56.65 nm and 52.53 nm.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is used to identify the chemical composition and surface functional groups of nanoparticles. In the FTIR spectra of CuO nanoparticles, absorption bands in the $400\text{--}800\text{ cm}^{-1}$ region confirm metal–oxygen vibrations, validating the oxide phase. Additional peaks above 1000 cm^{-1} may indicate the presence of organic capping agents or impurities. For ZnO nanoparticles, characteristic peaks in the $400\text{--}500\text{ cm}^{-1}$ range correspond to Zn–O stretching vibrations, confirming oxide formation. Peaks beyond 1000 cm^{-1} may be attributed to surface-adsorbed species or residual biomolecules from the plant extract.

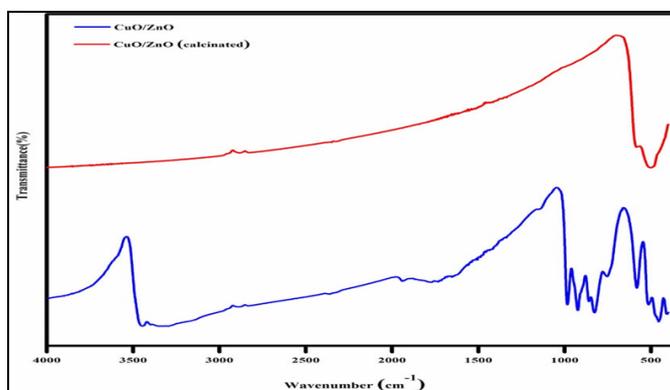


Fig. 3. FTIR spectra of CuO nanoparticles and ZnO/CuO nanocomposites

The as-prepared and calcined CuO/ZnO nanoparticles were recorded using FTIR spectra in the wavenumber range $4000\text{--}400\text{ cm}^{-1}$. The spectra are shown in the figure. From the observations, the band between 600 and 400 cm^{-1} corresponds to metal–oxide vibrations, while a broad O–H stretching vibration was observed at 3490 cm^{-1} . After calcination of CuO/ZnO at 400°C , impurities were removed and the characteristic metal–oxide vibrations

were confirmed. Strong absorption bands were observed at 586 cm^{-1} and 481 cm^{-1} , corresponding to Cu–O and Zn–O stretching vibrations, respectively. The calcined CuO/ZnO nanoparticles clearly demonstrate the purity of the sample.

3.3 UV-Visible Spectroscopy

Ultraviolet (UV) characterization of copper oxide (CuO) and zinc oxide (ZnO) nanoparticles is essential for understanding their unique optical properties. Both materials exhibit distinct UV absorption features due to their bandgap structures. CuO nanoparticles typically absorb UV light in the range of 300–400 nm, corresponding to transitions between the valence and conduction bands. This absorption is significant for applications such as photocatalysis and sensors. [3&4] ZnO nanoparticles, on the other hand, display a UV absorption edge around 370 nm, associated with their wide bandgap. Thus, UV characterization highlights the electronic transitions and optical properties of these nanoparticles within the ultraviolet region.

The UV-visible spectra of CuO/ZnO nanoparticles and ZnO nanoparticles are shown in the figure. The CuO/ZnO nanoparticles exhibited stronger UV absorption compared to ZnO nanoparticles in both UV and visible ranges. This indicates that the absorption edge of CuO/ZnO nanoparticles is red-shifted into the visible region.

This behavior can be explained as follows: (i) the rough surface of CuO/ZnO nanoparticles allows more light reflection and absorption inside the structure, unlike the smooth surface of ZnO nanoparticles, and (ii) CuO has the

potential to red-shift the absorption spectrum into the visible region.

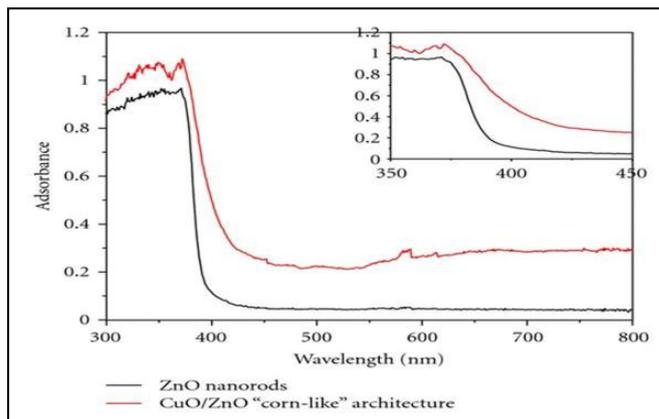


Fig. 4. UV-Vis spectra of ZnO and CuO/ZnO nanoparticles.

The N_2 adsorption/desorption isotherm curve of CuO/ZnO nanoparticles was also measured (figure). The specific surface area of the CuO/ZnO nanoparticles was found to be $11.24\text{ m}^2/\text{g}$, which is much higher than that of ZnO nanoparticles ($4.81\text{ m}^2/\text{g}$) and commercial ZnO powder ($3\text{ m}^2/\text{g}$).

3.4 Morphological and Elemental Analysis (SEM-EDX)

Scanning Electron Microscopy (SEM) provides high-resolution images that reveal the morphology and surface features of nanoparticles. SEM analysis of CuO nanoparticles may display structures such as nanorods or nearly spherical particles, helping to understand their growth and aggregation. [7&8] For ZnO nanoparticles, SEM images often show hexagonal or rod-like morphologies, along with particle size distribution and surface texture. By magnifying from a few nanometers to several micrometers, SEM offers detailed insights into nanoparticle shape and aggregation behavior.

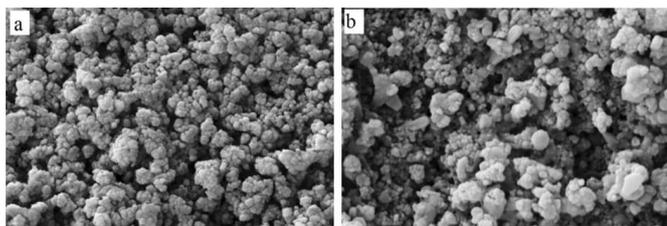


Fig. 5. SEM images of CuO/ZnO nanoparticles.

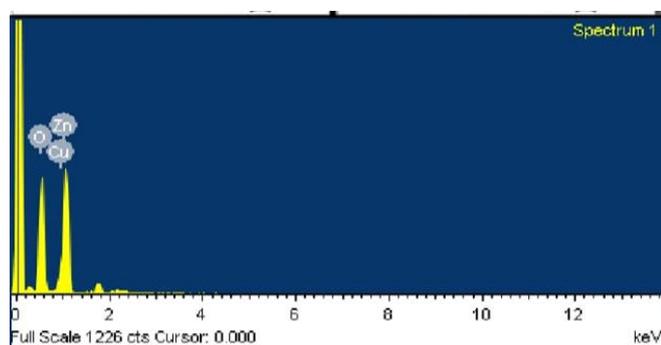


Fig. 6. EDX spectra of CuO/ZnO nanoparticles.

The surface morphologies and particle sizes are shown in the figure. Figures a and b reveal that polygonal ZnO nanoparticles were arranged along with adjoining spherical nanoparticles. The formation of polygonal shapes was observed in all directions.

Figure 2b shows isometric nanoparticles, with sizes ranging from 20 to 130 nm due to the different shapes of ZnO nanoparticles with CuO. In some regions, the diameter of the ZnO nanoparticles was around 98 nm, while in other regions aggregated spherical particles reached diameters of about 130 nm.

Energy Dispersive Spectroscopy (EDS) complements SEM by providing elemental composition data. For CuO and ZnO nanoparticles synthesized using *Tabernaemontana divaricata* leaf extract, EDS confirms the presence of copper, zinc, and oxygen. Additionally, traces of carbon or other elements may appear, originating from organic biomolecules present in the plant extract. Proper sample

preparation and optimized analysis conditions are essential for obtaining accurate compositional results.

The elemental composition of the nanoparticles was determined using EDX spectra. The presence of Zinc (Zn), Copper (Cu), and Oxygen (O) elements in the ZnO/CuO nanoparticles was confirmed, as shown in the figure. The respective elemental percentages were: Zn – 23.24%, Cu – 22.39%, and O – 55.37%. These values support the successful formation of ZnO/CuO nanoparticles.

The elemental composition of the nanoparticles was determined using EDX spectra. The presence of Zinc (Zn), Copper (Cu), and Oxygen (O) elements in the ZnO/CuO nanoparticles was confirmed, as shown in the figure. The respective elemental percentages were: Zn – 23.24%, Cu – 22.39%, and O – 55.37%. These values support the successful formation of ZnO/CuO nanoparticles.

3.5 Antimicrobial Activity

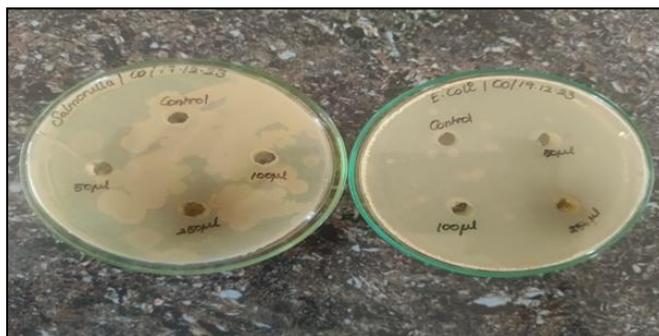


Fig.7. Inhibition zone for microbial growth

Antibacterial activity was assessed using the disc diffusion method against *E. coli* (gram-negative) and *S. aureus* (gram-positive). Nanoparticles were dispersed in DMSO and tested on Mueller–Hinton agar plates.

Both Fe_2O_3 and NiO nanoparticles exhibited inhibitory zones, indicating effective antimicrobial action.

Preparation of Inoculum

The antimicrobial properties of plant extracts were tested against Gram-negative bacteria, *Escherichia coli* GIM1.708 (EC) and *Salmonella enteritidis* 10982 (SE). The dried CuO nanoparticles were dissolved in DMSO at a concentration of 1 mg/mL and further diluted to 50 µg/mL, 100 µg/mL, and 250 µg/mL. Pure bacterial and fungal cultures were obtained and identified using culture and microscopic analysis.

Before susceptibility testing, the bacteria were cultured on nutrient agar, and fresh cultures were sub-cultured on Mueller–Hinton agar (MHA) for testing the antibacterial activity of CuO and ZnO nanoparticles. Antimicrobial activity was evaluated using the cup plate/well diffusion method against different bacterial cultures.

Gram-positive and Gram-negative bacteria were pre-cultured in Mueller–Hinton broth (MHB) overnight in a rotary shaker at 37 °C. The bacterial concentration was adjusted to 10^8 cells/mL using the 0.5 McFarland standard. The fungal inoculum was prepared from 48-hour cultures in potato dextrose broth (PDB). A spectrophotometer (A595 nm) was used to adjust the spore density to 10^6 spores/mL.

The enhanced activity may be due to the generation of reactive oxygen species (ROS) and interaction of nanoparticles with bacterial cell walls.

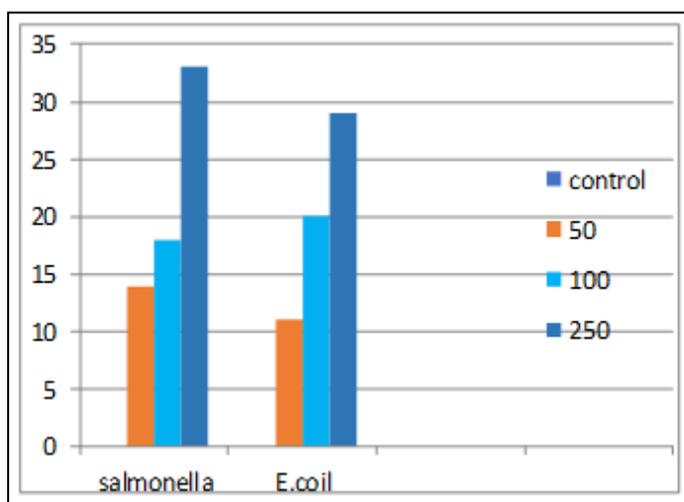
Culture	Control	50µ	100µ	250µ
Salmonella	0mm	14mm	18mm	33mm
E. coil	0mm	11mm	20mm	29mm

Culture	Control	50µ	100µ	250µ
Salmonella	0mm	18mm	28mm	36mm
E. coil	0mm	15mm	23mm	32mm

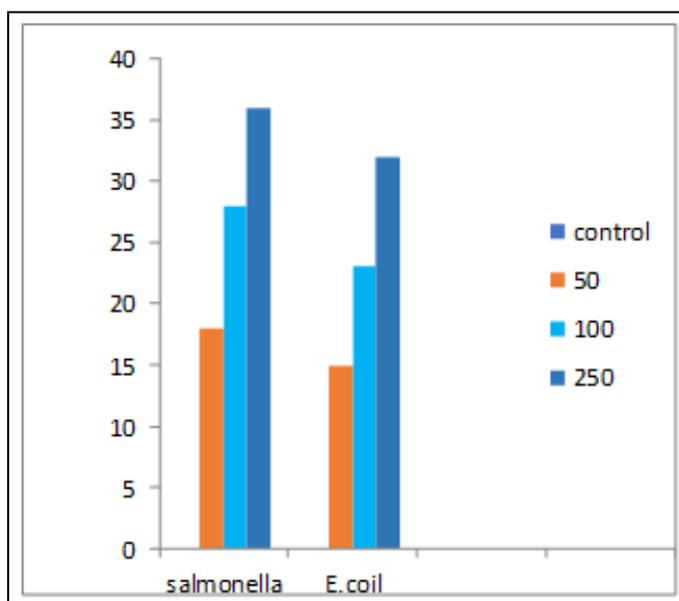
Antimicrobial Screening

Bar graph of the bacterial pathogens for copper oxide and zinc oxide nanoparticles:

CuO and ZnO [24hrs]:



CuO and ZnO [48 hrs] :



The agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts. One milliliter of fresh bacterial or fungal culture was pipetted into the center of a sterile Petri dish. Molten, cooled Mueller–Hinton agar (for bacteria) or potato dextrose agar (PDA, for fungi) was poured into the Petri dish containing the inoculum and mixed well.

After solidification, wells (6 mm diameter) were made using a sterile cork borer. Then, 100 μ L of each extract (20% w/v) was added to the respective wells. The extract concentration (20% w/v) was chosen based on preliminary experiments and literature reports. The plates were kept in a refrigerator for 30 minutes to allow diffusion of the extract into the agar, and then incubated at 37 °C for 24 hours.

Antimicrobial activity was determined by measuring the zone of inhibition (including the well diameter) after incubation. DMSO (10%) was used as a negative control.

4. Conclusion

The present study demonstrates the successful green synthesis of CuO and ZnO nanoparticles using *Tabernaemontana divaricata* leaf extract, which acted as an effective reducing and stabilizing agent. Characterization confirmed their crystalline structure, optical features, and elemental composition, while antimicrobial evaluation revealed significant inhibition of *E. coli* and *Salmonella enteritidis* in a dose- and time-dependent manner. These findings highlight the potential of eco-friendly synthesis as a sustainable alternative to conventional methods. Future research may focus on

elucidating the phytochemical–metal interaction mechanisms, assessing cytotoxicity and biocompatibility, expanding antimicrobial screening to other pathogens, and exploring applications in photocatalysis, water treatment, and biomedical devices. With further optimization and scale-up, such plant-mediated nanoparticles could pave the way for safer and cost-effective nanotechnology solutions.

Conflict of interest: Nil

References

- [1] Khaldari, I., Naghavi, M. R., & Motamedi, E. (2021). Synthesis of green and pure copper oxide nanoparticles using two plant resources via solid-state route and their phytotoxicity assessment. *RSC Advances*, 11, 3346–3353. This study used green tea and lavender leaf tissues to synthesize CuO NPs and evaluated their low phytotoxicity in plant assays. (RSC Publishing)
- [2] Araya-Castro, K., Chao, T.-C., Durán-Vinet, B., Cisternas, C., Ciudad, G., & Rubilar, O. (2021). Green synthesis of copper oxide nanoparticles using protein fractions from an aqueous extract of brown algae *Macrocystis pyrifera*. *Processes*, 9(1), 78. Demonstrates CuO NP synthesis via brown algae protein fractions. (MDPI).
- [3] Aljedaani, R. O., Kosa, S. A., & Abdel Salam, M. (2022). Ecofriendly green synthesis of copper (II) oxide nanoparticles using *Corchorus olitorus* leaves (Molokhaia) extract and their application for environmental remediation of Direct Violet dye via

advanced oxidation process. *Molecules*, 28(1), Article 16.

[4] Focuses on Molokhaia leaf extract for CuO NP synthesis and catalytic degradation of dye pollutants. (MDPI)

[5] Dauthal, P., & Mukhopadhyay, M. (2021). Green synthesis of copper oxide nanoparticles using *Aerva javanica* leaf extract; characterization and investigation of in vitro antimicrobial potential and cytotoxic activities. *Evidence-Based Complementary and Alternative Medicine*, 2021, 5589703. Details CuO NP synthesis using *Aerva javanica*, with antimicrobial and cytotoxicity evaluations. (MDPI)

[6] Nzilu, D. M., Madivoli, E. S., Makhanu, D. S., Wanakai, S. I., Kiprono, G. K., & Kareru, P. G. (2023). Green synthesis of copper oxide nanoparticles and their efficiency in degradation of rifampicin antibiotic. *Scientific Reports*, 13, 14030. Reports on CuO NPs used in antibiotic degradation applications. (MDPI).

[7] Singh, P., B. R. Singh, K. R., Singh, J., Das, S. N., & Singh, R. P. (2021). Tunable electrochemistry and efficient antibacterial activity of plant-mediated copper

oxide nanoparticles synthesized by *Annona squamosa* seed extract for agricultural utility. *RSC Advances*, 11, 18050–18060. Highlights biosynthesized CuO NPs with electrochemical and antibacterial capabilities. (RSC Publishing).

[8] Ramasubbu, K., Padmanabhan, S., Al-Ghanim, K. A., Nicoletti, M., Govindarajan, M., Sachivkina, N., & Rajeswari, V. D. (2023). Green synthesis of copper oxide nanoparticles using *Sesbania grandiflora* leaf extract and their evaluation of anti-diabetic, cytotoxic, anti-microbial, and anti-inflammatory properties in an in-vitro approach. *Fermentation*, 9(4), 332. Comprehensive evaluation of CuO NPs derived from *Sesbania grandiflora* with biomedical activities. (MDPI)

[9] Priya, M., Venkatesan, R., Deepa, S., Sana, S. S., Arumugam, S., Karami, A. M., ... Kim, S.-C. (2023). Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from *Morinda citrifolia* leaf extract. *Scientific Reports*, 13, 18838. Demonstrates broad-spectrum antimicrobial activity of CuO NPs synthesized from *Morinda citrifolia*. (PMC)