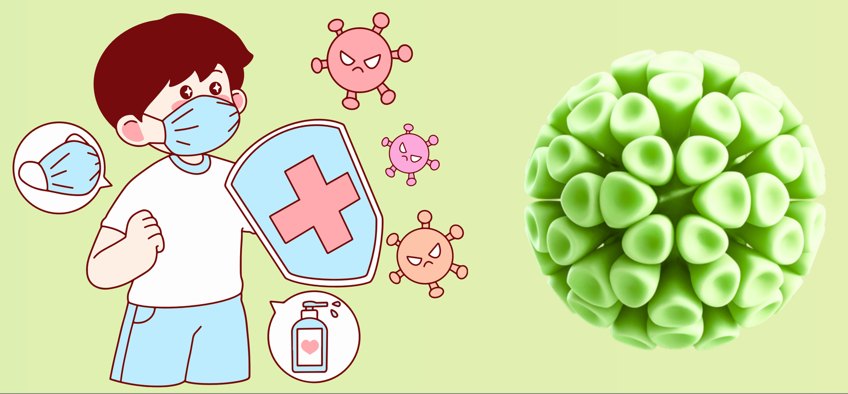
Green Synthesis of Copper Oxide Nanoparticles using *Annona Muricata* Leaf Extract: Optimization, Characterisation and Antimicrobial Activity

Brian Hou Teck Wong1*, Yoon Yee Then2, Yun Khoon Liew3, Chian Ying Teo2*

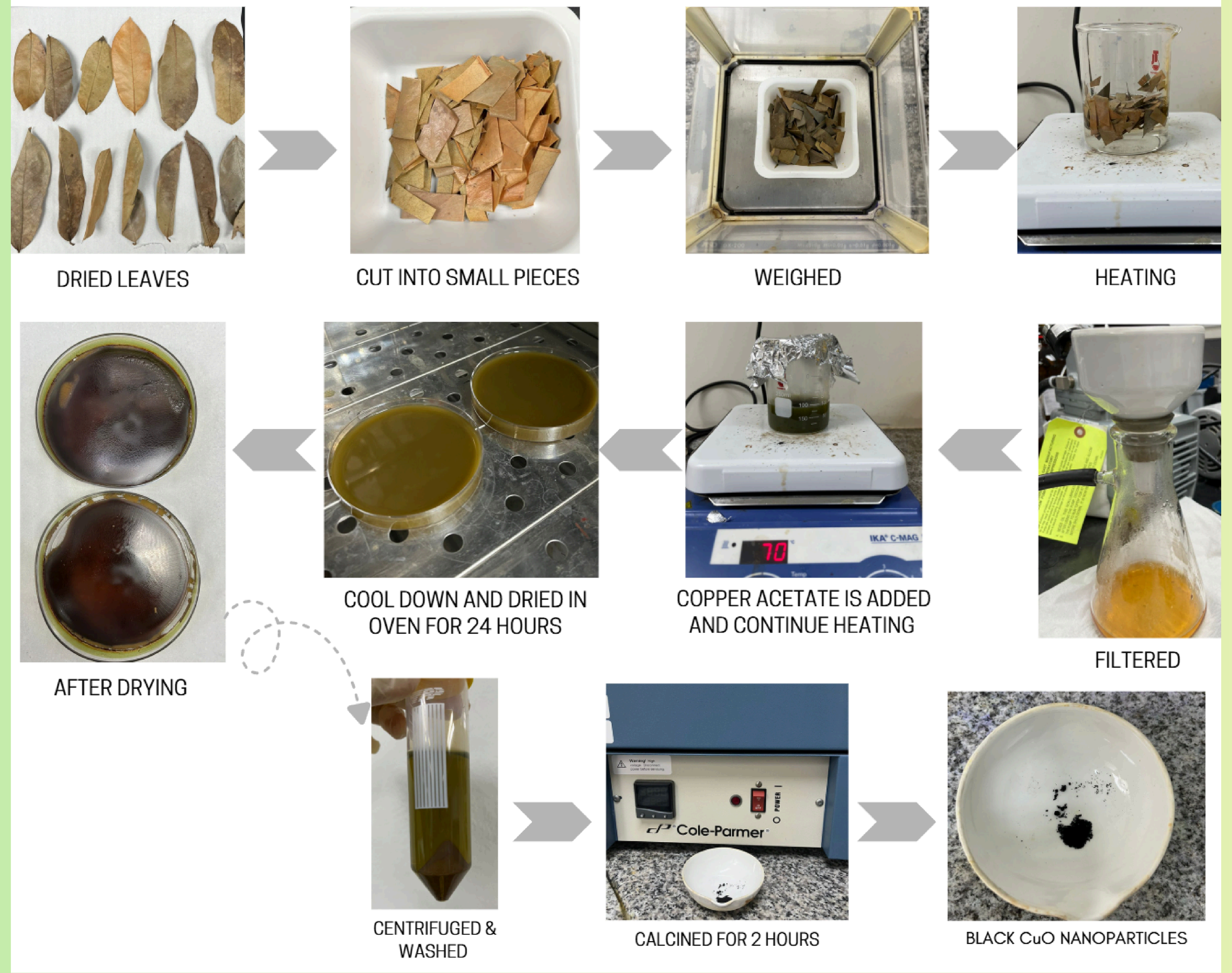
1. School of Postgraduate Studies, IMU University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
2. Department of Pharmaceutical Chemistry, School of Pharmacy, IMU University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
3. Department of Life sciences, School of Pharmacy, IMU University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

Background / Introduction

This study delivers an integrated solution to two urgent global challenges: the escalating antimicrobial resistance crisis and the environmental toxicity of conventional nanoparticle manufacturing. By establishing a completely green route to antimicrobial copper oxide nanoparticles, we eliminate the need for toxic reagents, high energy input, and hazardous byproducts that undermine the safety and sustainability of current synthesis methods. Our study is the first reported use of Annona muricata (soursop) leaf extract—a sustainable bioresource rich in phenolic, flavonoid, and alkaloid phytochemicals as both a natural reducing and stabilizing agent for the fabrication of copper oxide nanoparticles (CuO NPs). The resulting CuO NPs retained the material's desirable p-type semiconducting properties, monoclinic crystal structure, high conductivity, thermal stability, and cost-effectiveness, while being produced through a simple, eco-friendly, one-pot process. This breakthrough not only provides a new class of sustainably manufactured antimicrobial agents to help combat drug-resistant pathogens but also opens immediate avenues in antidiabetic therapy, environmental remediation, catalysis, sensing, and energy storage. The results of our study thus demonstrated that green nanotechnology can simultaneously address public health and environmental priorities without compromise.



Methodology



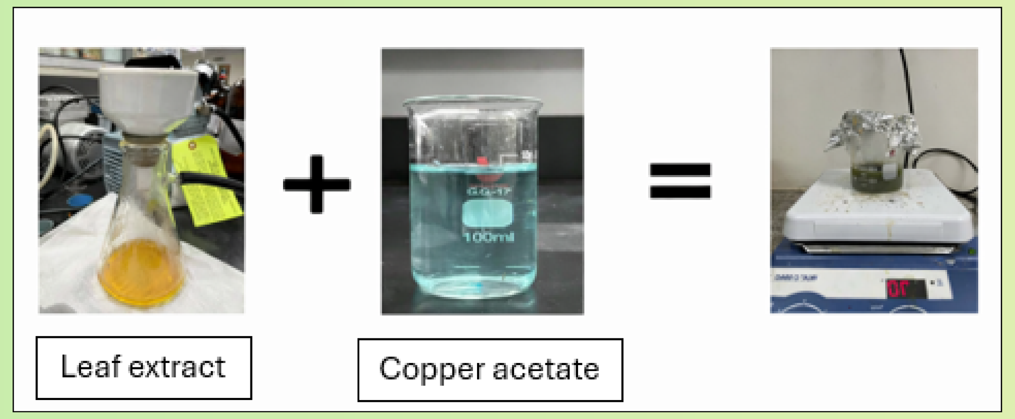
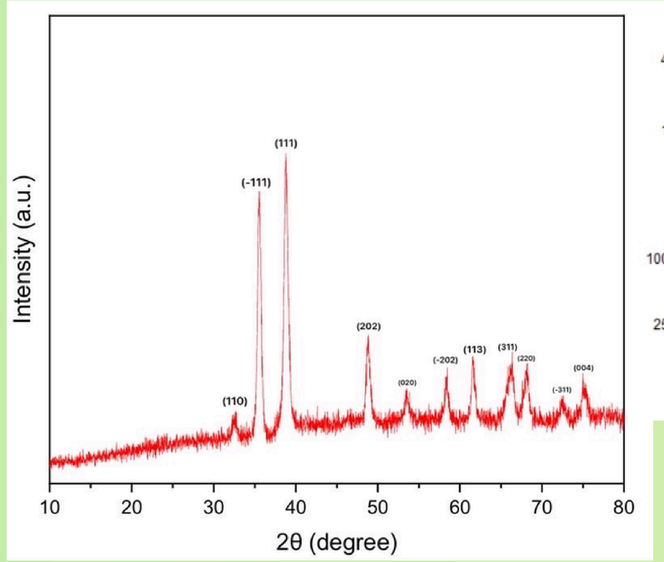


Figure 1: Visual observation of color change during the green synthesis of CuO NPs using *A. muricata* leaf extract. The reaction mixture exhibited a distinct color transition from light blue to dark brown upon addition of the leaf extract to the copper(II) acetate monohydrate solution, indicating the reduction of copper ions and the formation of CuO NPs.

X-Ray Diffraction



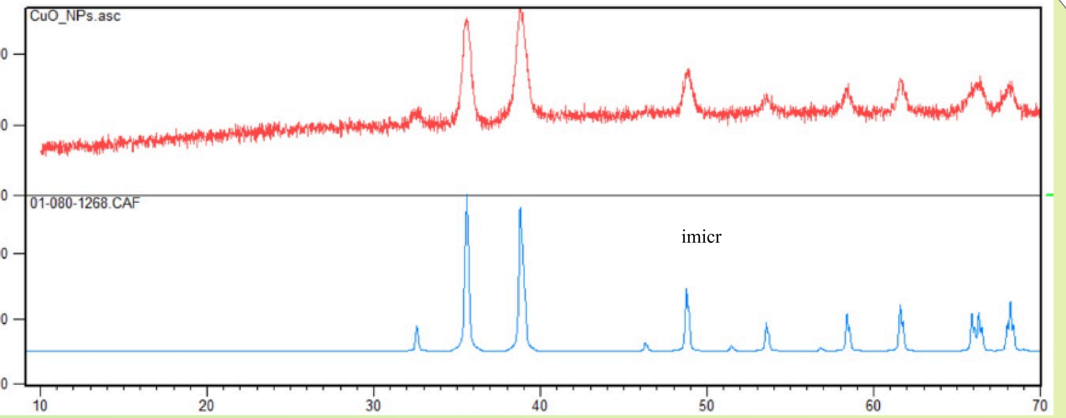
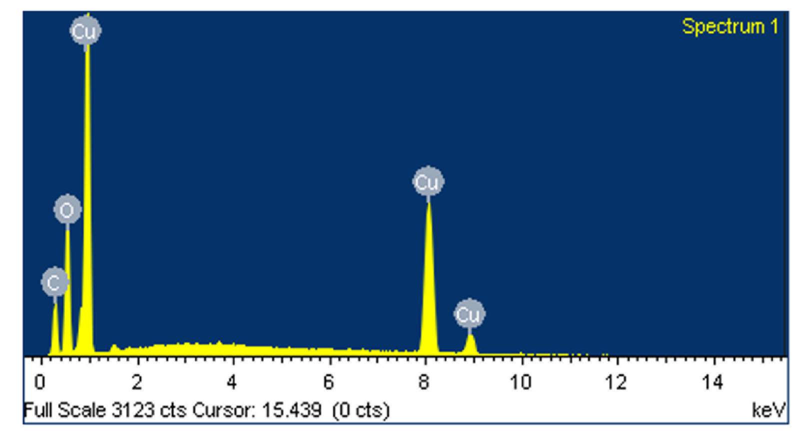


Figure 2: (a) XRD of synthesized CuO NPs using *A.muricata* leaf extract and (b) XRD analysis of CuO NPs compared to JCPDS reference pattern. The diffraction peaks confirm the formation of crystalline monoclinic CuO NPs.

The average crystallite size [$D = K \lambda / \beta \cos(\theta)$] was found to be approximately **29.43 nm.**

FESEM-EDX



Element	Weight %	Atomic %
C	10.59	25.33
O	25.52	45.81
Cu	63.89	28.87

Figure 4: EDX graph of CuO NPs. The spectrum confirms the elemental composition of the CuO NPs, primarily consisting of copper and oxygen, with a minor carbon signal attributed to plant-derived organic residues.

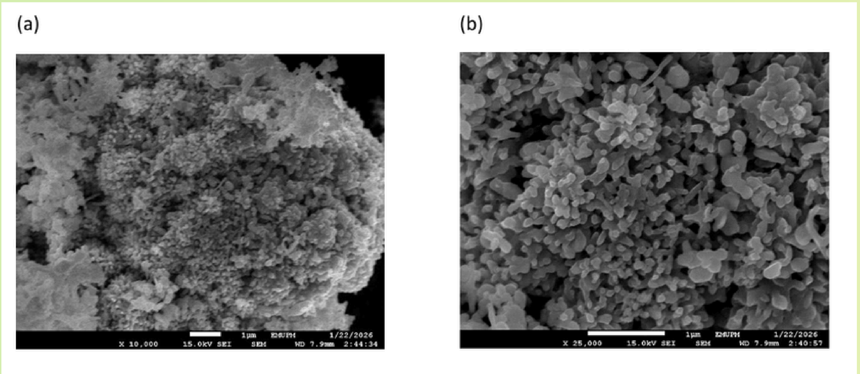
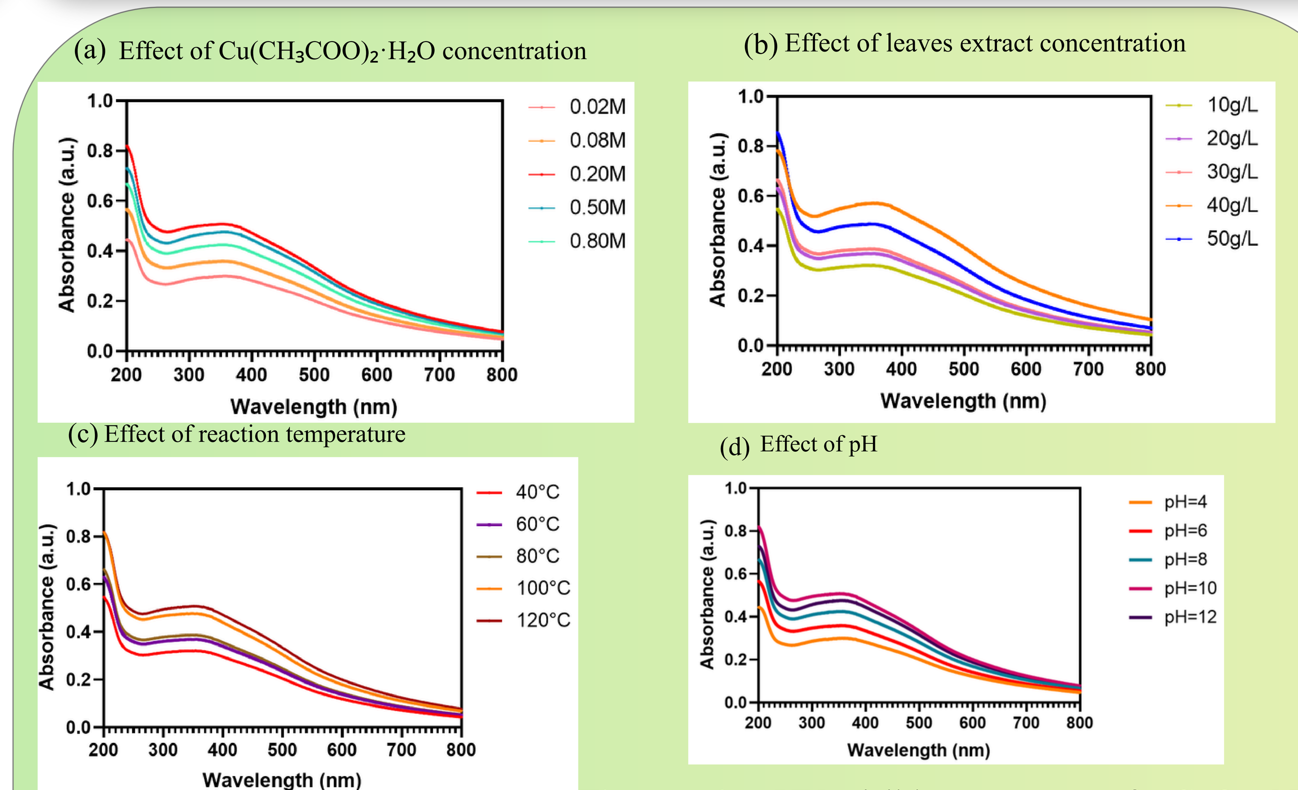


Figure 5: FESEM images of A. muricata-derived CuO NPs (a) with magnification of ×10,000, (b) with magnification of 25,000, The micrographs show the surface morphology and agglomeration of the synthesized CuO NPs.

UV-Visible Spectroscopy



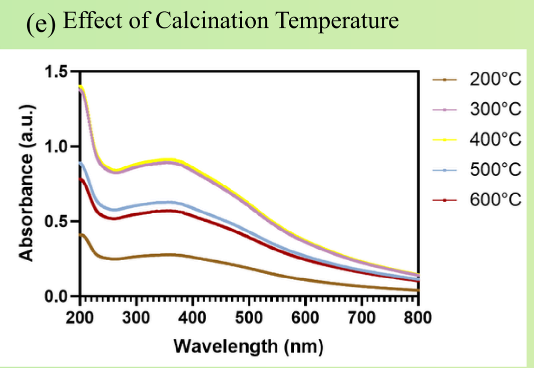


Figure 3. UV–visible spectra of CuO NPs synthesized under: (a) varying metal precursor concentrations, with 0.20 M identified as optimal (λmax = 362 nm); (b) varying concentrations of *A. muricata* extract, with 40 g/L producing well-dispersed CuO NPs; (c) different reaction temperatures, with 80 °C yielding smaller and stable nanoparticles; (d) different pH conditions, with pH 10 showing optimal nanoparticle stability and dispersion; and (e) different calcination temperatures, with 300 °C selected as the optimal condition due to maintained nanoparticle quality and better energy efficiency.

FTIR

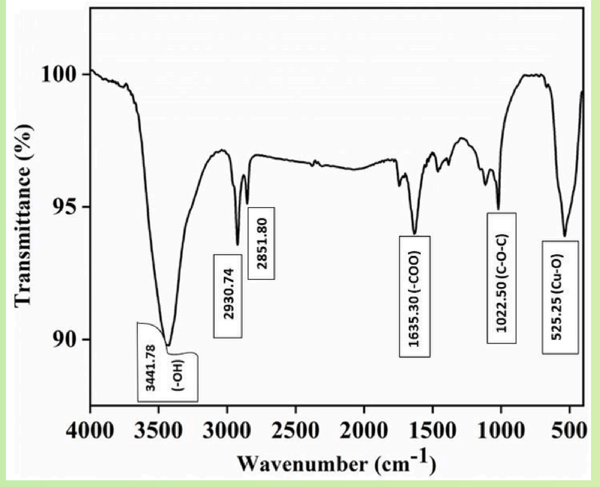


Figure 6: FTIR spectrum of the synthesized CuO NPs. The spectrum confirms the presence of phytochemical functional groups from *A. muricata* extract involved in nanoparticle capping and stabilization, as well as characteristic Cu–O bonding indicating successful formation of CuO NPs. at 525.25 cm^{-1}.

Broth Microdilution (Antibacterial Assay)

Bacterial strain (n=3)	Minimum inhibitory concentration (µg/mL)
Gram-positive	
S. aureus (Susceptible strain) ATCC 35923	125
S. aureus (Methicillin resistant strain) ATCC 43300	500
Gram-negative	
P. aeruginosa (Susceptible strain) ATCC 27853	250
P. aeruginosa (Resistant strain) ATCC BAA-2108	500
E. coli (Susceptible strain) ATCC 25922	125
E. coli (Resistant strain) ATCC BAA-196	250

Table 1: Broth microdilution assay of CuO NPs from *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Conclusion

- Green synthesis using *Annona muricata* leaf extract successfully produced stable and relatively uniform CuO nanoparticles, with optimized synthesis conditions significantly influencing particle size, surface charge, and colloidal stability.
- Characterization analyses confirmed the successful formation of high-purity monoclinic CuO nanoparticles with predominantly spherical morphology, supported by FESEM, EDX, XRD, zeta potential, and particle size distribution results.
- The synthesized CuO nanoparticles demonstrated moderate antibacterial activity, showing better efficacy against susceptible bacterial strains than antimicrobial-resistant strains, indicating the need for further enhancement strategies such as surface modification, metal ion doping, or synergistic antibiotic combinations.

References

1. N. Chakraborty, et al. Nanobiotics against antimicrobial resistance: harnessing the power of nanoscale materials and technologies. J Nanobiotechnol. 2022;20:375. doi:10.1186/s12951-022-01573-9.
2. S.O. Ogungbesan, et al. Transition metal oxide nanohybrid materials: a review of their structures, properties, and applications. J Mol Struct. 2025;1337:142209. doi:10.1016/j.molstruc.2025.142209.
3. C. Gherasim, et al. Copper oxide nanostructures: preparation, structural, dielectric and catalytic properties. Ceram Int. 2022;48(17):25556–25568. doi:10.1016/j.ceramint.2022.05.235.
4. A.R. Maheo, B.S.M. Vithiya, T.A.A. Prasad, P. Tamizhdurai, V.L. Mangesh. Biosynthesis, characterization, biological and photocatalytic investigations of Elsholtzia blanda and chitosan mediated copper oxide nanoparticles. Arab J Chem. 2022;15(3):103661. doi:10.1016/j.arabjc.2021.103661.
5. A. Atri, et al. Green synthesis of copper oxide nanoparticles using Ephedra alata plant extract and study of their antifungal, antibacterial activity and photocatalytic performance under sunlight. Heliyon. 2023;9(2):e13484. doi:10.1016/j.heliyon.2023.e13484.