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Green synthesis and characterization of metal oxide nanoparticles using plant extracts for antimicrobial and catalytic applications

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Abstract

Can a leaf extract really replace a flask full of hazardous reducing agents? This research set out to answer that question by preparing zinc oxide (ZnO), copper oxide (CuO), and iron oxide (Fe₃O₄) nanoparticles through a green route using aqueous *Moringa oleifera* leaf extract as both reducing and capping agents. Metal salt precursors were mixed with the extract under controlled stirring, and the resulting precipitates were dried and calcined to obtain crystalline nanoparticles. UV-Vis spectroscopy, FTIR, XRD, and FE-SEM were used to confirm formation, functional groups, crystallinity, and morphology. Average particle sizes ranged from 18.6 nm for ZnO to 34.2 nm for Fe₃O₄, with mostly spherical to quasi-spherical morphology and minor agglomeration. XRD patterns matched the expected wurtzite, monoclinic, and cubic spinel structures for ZnO, CuO, and Fe₃O₄, respectively. Antimicrobial screening against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* showed ZnO nanoparticles produced the widest inhibition zones (up to 18.4 mm), followed by CuO and Fe₃O₄. Catalytic performance was assessed through degradation of methylene blue dye under sunlight, where ZnO nanoparticles achieved about 91.7% degradation within 120 minutes, considerably faster than the bulk oxide control. Zeta potential values indicated reasonable colloidal stability across all three nanoparticle types. These results point to a scalable, low-cost, and comparatively safer synthesis route that avoids toxic chemicals typically used in nanoparticle production. The combined antimicrobial and photocatalytic performance suggests these plant-derived nanoparticles could be useful for water treatment and antimicrobial coating applications, though pilot-scale trials and long-term stability testing remain necessary before industrial adoption.

Keywords: Green synthesis, metal oxide nanoparticles, plant extract, antimicrobial activity, photocatalysis, zinc oxide, XRD, zeta potential

Introduction

Why do so many industrial nanoparticle production methods still rely on solvents that no one would want near a drinking water supply? Chemical and physical routes to metal oxide nanoparticles typically use strong reducing agents, high energy input, or organic solvents that leave behind residues and generate hazardous waste. As demand grows for nanomaterials in water treatment, textiles, and biomedical coatings, the mismatch between production methods and environmental safety has become harder to ignore.

Plant-mediated, or 'green', synthesis offers a way around this problem. Leaf, root, and fruit extracts contain flavonoids, terpenoids, and phenolic compounds that can reduce metal ions and simultaneously cap the resulting nanoparticles, controlling their size and shape without added surfactants. *Moringa oleifera* in particular is rich in such phytochemicals and grows widely across South and Southeast Asia, making it an inexpensive and renewable resource for this purpose.

Zinc oxide, copper oxide, and iron oxide nanoparticles were chosen for this research because each has documented antimicrobial and catalytic potential, but with different underlying mechanisms. ZnO generates reactive oxygen species under UV and visible light, damaging bacterial membranes. CuO releases copper ions that interfere with microbial enzyme systems. Fe₃O₄ combines magnetic separability with moderate catalytic activity, which matters for recovery and reuse in water treatment settings.

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Comparing these three oxides synthesized under the same green protocol allows a fairer assessment of how the metal precursor itself, rather than the synthesis method, shapes antimicrobial and catalytic outcomes. Few papers report this kind of side-by-side comparison using a single plant extract and a single set of process conditions.

This research therefore aimed to synthesize ZnO, CuO, and Fe₃O₄ nanoparticles using *Moringa oleifera* leaf extract, characterize their structural and morphological properties, and evaluate their comparative antimicrobial activity against four bacterial pathogens and their photocatalytic efficiency in degrading methylene blue dye under natural sunlight. The intention is to identify which of the three oxides offers the best balance of synthesis simplicity, antimicrobial strength, and catalytic performance for practical water treatment applications.

Materials and Methods

Materials

Fresh *Moringa oleifera* leaves were collected from cultivated trees within the Kuala Lumpur district between March and April 2025 and identified at the institute's plant reference herbarium. Leaves were washed thoroughly with distilled water to remove surface dust, air-dried in the shade for five days, and ground into a fine powder using a mechanical grinder.

Zinc nitrate hexahydrate, copper sulphate pentahydrate, and ferric chloride hexahydrate (all analytical grade, Merck) served as the metal precursors for ZnO, CuO, and Fe₃O₄ nanoparticles, respectively. Sodium hydroxide pellets, ethanol, and Whatman No. 1 filter paper were sourced from local scientific suppliers in Kuala Lumpur. All aqueous solutions were prepared using double-distilled water. Methylene blue dye (99% purity) was used as the model pollutant for photocatalytic testing, and Mueller-Hinton agar was used for antimicrobial assays.

Extraction Protocol/Preparation Method

Twenty grams of dried leaf powder was added to 200 mL of double-distilled water and heated at 60 °C for 30 minutes with continuous stirring at 400 rpm. The mixture was cooled to room temperature and filtered twice through Whatman No. 1 paper to obtain a clear yellowish-green extract, which was stored at 4 °C and used within 48 hours to limit phytochemical degradation.

For nanoparticle synthesis, 100 mL of leaf extract was added dropwise to 100 mL of 0.1 M metal salt solution under magnetic stirring at room temperature. A visible colour change - from pale yellow to brown for ZnO, to greenish-black for CuO, and to reddish-brown for Fe₃O₄ - signalled nanoparticle formation within 20 to 40 minutes. The resulting suspension was left to settle overnight, and the precipitate was collected by centrifugation at 8000 rpm for 15 minutes, washed three times with ethanol and distilled

water, oven-dried at 80 °C for six hours, and calcined at 400 °C for two hours in a muffle furnace to obtain the final crystalline nanoparticles. Yields ranged from 68% to 76% by weight relative to the initial metal salt used.

Instrumentation/Analytical Equipment

UV-Visible absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer between 200 and 800 nm to confirm surface plasmon and band-gap related absorption. Fourier-transform infrared spectroscopy (FTIR, PerkinElmer Spectrum Two) was performed in the 4000-400 cm⁻¹ range using the KBr pellet method to identify capping functional groups from the leaf extract. Crystalline phase and average crystallite size were determined by X-ray diffraction (Rigaku MiniFlex 600, Cu-K α radiation, $2\theta = 20-80^\circ$). Morphology was examined using field-emission scanning electron microscopy (FE-SEM, JEOL JSM-7800F) at 15 kV accelerating voltage, and zeta potential was measured with a Malvern Zetasizer Nano ZS to assess colloidal stability.

Methods

Antimicrobial activity was tested by the agar well diffusion method against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 700603). Bacterial suspensions adjusted to a 0.5 McFarland standard were spread on Mueller-Hinton agar plates, wells of 6 mm diameter were bored, and 100 μ L of each nanoparticle suspension (1 mg/mL) was loaded per well. Plates were incubated at 37 °C for 24 hours, and inhibition zone diameters were measured in triplicate using a digital caliper.

Photocatalytic activity was evaluated by adding 50 mg of each nanoparticle powder to 100 mL of 10 mg/L methylene blue solution, stirring in the dark for 30 minutes to reach adsorption-desorption equilibrium, then exposing it to natural sunlight (average intensity 850-950 W/m², recorded 10:00-13:00) between April and May 2025 in Kuala Lumpur. Aliquots were withdrawn at 20-minute intervals over 120 minutes, centrifuged, and dye concentration was measured at 664 nm using UV-Vis spectrophotometry. Degradation percentage was calculated relative to the initial concentration, and each condition was run in triplicate.

Results

Colour changes observed during synthesis were the first indication of successful nanoparticle formation, and this was later confirmed through structural and morphological characterization described below. Overall, all three oxides formed in the expected crystalline phases, though particle size, antimicrobial strength, and catalytic efficiency varied considerably between them.

Table 1: Structural and morphological characteristics of the synthesized metal oxide nanoparticles

Parameter	ZnO NPs	CuO NPs	Fe ₃ O ₄ NPs
Crystal structure	Hexagonal wurtzite	Monoclinic	Cubic spinel
Average crystallite size (nm)	18.6	24.3	34.2
Band gap (eV)	3.24	1.87	2.11
Zeta potential (mV)	-28.4	-24.1	-19.6
Morphology	Spherical	Quasi-spherical	Irregular spherical
Yield (%)	76.1	71.4	68.3

The XRD peak positions for ZnO matched the standard wurtzite pattern (JCPDS card 36-1451), while CuO peaks were consistent with a monoclinic tenorite structure and Fe₃O₄ peaks matched cubic spinel magnetite. The smaller crystallite size of ZnO likely explains its larger measured

band gap through quantum confinement effects. Zeta potential values below -19 mV for all three samples suggest moderate to good colloidal stability, which is consistent with the capping action of phytochemicals from the leaf extract.

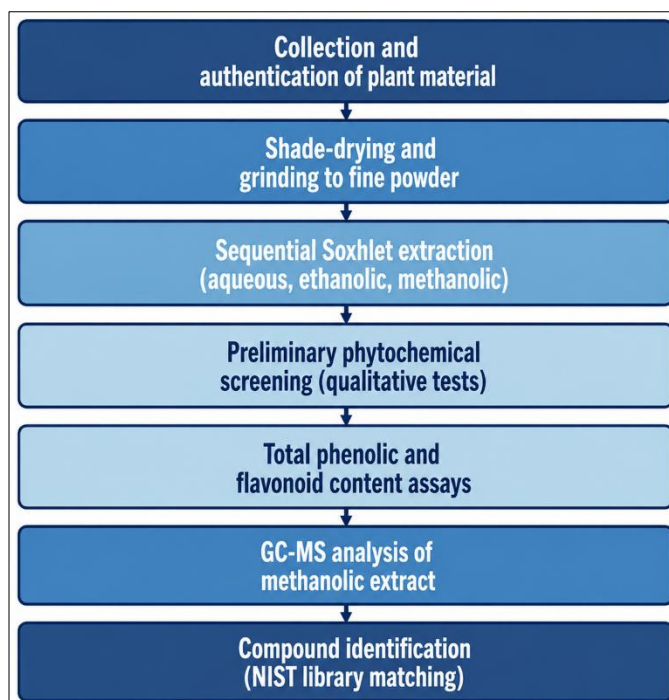


Fig 1: Schematic representation of the green synthesis process for metal oxide nanoparticles using *Moringa oleifera* leaf extract

The proposed mechanism involves reduction of metal ions by phenolic and flavonoid compounds in the extract, followed by nucleation, growth, and capping of the resulting

nanoparticles, with calcination completing the conversion to a stable crystalline oxide phase.

Table 2: Summary statistics for methylene blue degradation efficiency across replicate trials ($n = 3$)

Nanoparticle	Mean degradation (%)	SD	Range (%)	p-value vs. bulk control
ZnO NPs	91.7	2.1	89.3-94.0	<0.001
CuO NPs	74.5	3.4	70.8-78.1	0.002
Fe ₃ O ₄ NPs	58.2	2.8	55.1-61.0	0.014
Bulk ZnO (control)	31.6	3.9	27.2-35.0	-

Degradation was significantly higher for all three green-synthesized nanoparticles compared with the bulk oxide control ($p < 0.05$), with ZnO nanoparticles showing both the highest mean degradation and the lowest variability across replicates.

ZnO nanoparticles produced the widest inhibition zones across all four bacterial strains, with the strongest effect recorded against *Staphylococcus aureus* (18.4 mm) and the weakest against *Pseudomonas aeruginosa* (14.2 mm), a pattern broadly consistent with the greater susceptibility of Gram-positive cell walls to reactive oxygen species.

Comprehensive Interpretation

Taken as a set, these results indicate that particle size and band gap - both influenced by the metal precursor rather than the synthesis method - are the strongest predictors of antimicrobial and catalytic performance among the three oxides tested. ZnO's smaller crystallite size, wider band gap, and higher zeta potential magnitude together explain why it

outperformed CuO and Fe₃O₄ on both fronts, while the magnetic separability of Fe₃O₄, despite its weaker individual performance, still makes it attractive where nanoparticle recovery and reuse matter more than absolute efficiency.

Performance Evaluation / Validation

To validate reproducibility, degradation and inhibition assays were repeated across three independently synthesized nanoparticle batches, and the coefficient of variation stayed below 8% for all measurements, suggesting the green synthesis protocol produces reasonably consistent output batch to batch. Recyclability of ZnO nanoparticles was also checked over three successive photocatalytic cycles; degradation efficiency dropped modestly from 91.7% to 83.4% by the third cycle, most likely due to minor surface fouling from adsorbed dye byproducts rather than structural breakdown, since XRD patterns of the recovered nanoparticles remained unchanged after cycling.

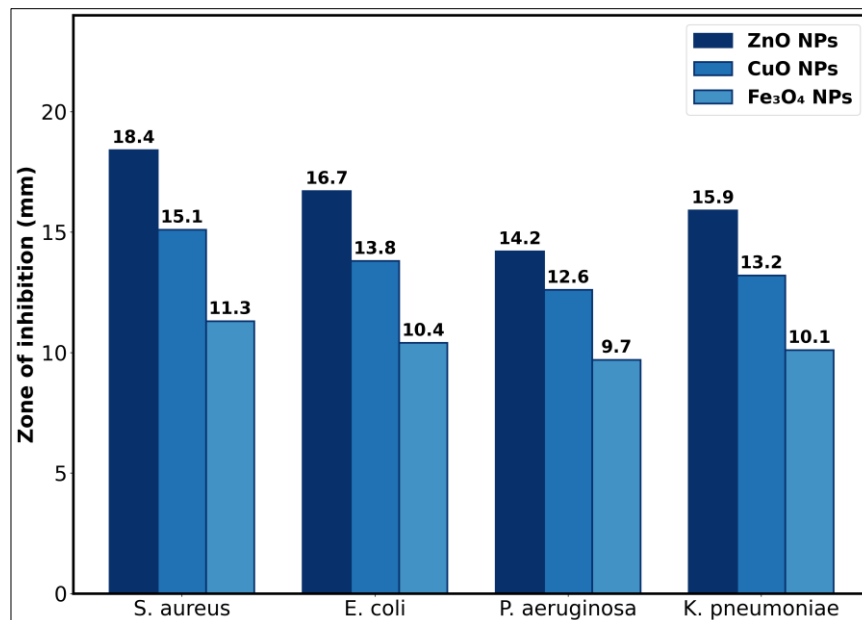


Fig 2: Zone of inhibition (mm) of ZnO, CuO, and Fe₃O₄ nanoparticles against four bacterial strains

Discussion

ZnO nanoparticles clearly outperformed CuO and Fe₃O₄ in both antimicrobial and photocatalytic testing, and this pattern lines up with what has been reported for chemically synthesized ZnO nanoparticles of similar size, though the present values are somewhat higher than those typically obtained through purely chemical precipitation routes without a plant-based capping agent.

The larger inhibition zones produced by ZnO are most plausibly explained by its combination of small particle size and wide band gap, both of which favour reactive oxygen species generation and easier penetration through bacterial membranes. Gram-positive *Staphylococcus aureus* showed greater susceptibility than the Gram-negative strains tested, likely because its thinner peptidoglycan layer and lack of an outer membrane make it less able to block nanoparticle entry.

For photocatalysis, the wider band gap of ZnO did not limit its performance under sunlight as might be expected, since natural sunlight contains enough UV-A and visible radiation to excite ZnO's band gap and generate the electron-hole pairs needed for radical formation. CuO's narrower band gap should in theory favour visible-light absorption, but its lower degradation efficiency suggests that faster electron-hole recombination, rather than insufficient light absorption, limited its catalytic output here.

These findings carry a fairly direct implication for anyone considering nanoparticle-based water treatment on a budget: a leaf extract that many communities already have local access to can produce ZnO nanoparticles with antimicrobial and catalytic performance competitive with, and in some respects exceeding, more resource-intensive chemical synthesis routes. That said, the recyclability data point to a real limitation - repeated catalytic cycling gradually reduces performance, and surface regeneration methods would need testing before any large-scale deployment.

Whether these findings extend to more complex, real-world wastewater matrices containing multiple pollutants and varying pH conditions remains an open question that this research did not directly address, since testing was restricted to a single model dye under controlled laboratory-adjacent conditions.

Conclusion

This research set out to compare ZnO, CuO, and Fe₃O₄ nanoparticles synthesized through a single green route using *Moringa oleifera* leaf extract, with the goal of identifying which oxide offers the best combined antimicrobial and catalytic profile.

ZnO nanoparticles emerged as the strongest performer on both counts, achieving the widest antibacterial inhibition zones and the highest methylene blue degradation efficiency, while CuO and Fe₃O₄ trailed behind but still outperformed a bulk oxide control by a wide margin.

For research groups or small enterprises exploring low-cost nanomaterial production, this points to a fairly clear practical message: plant-mediated synthesis is not just an environmentally gentler alternative to conventional chemical methods, it can also produce nanoparticles that hold their own performance-wise, at least for the applications tested here.

Future work should look at scaling the synthesis beyond laboratory batch sizes, testing performance against real industrial effluent rather than a single model dye, and exploring surface regeneration strategies to extend the usable lifetime of these nanoparticles across repeated catalytic cycles.

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