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Green Synthesis of ZnO Nanoparticles Using Five Medicinal Leaf Extracts: A Comparative Review on Physicochemical and Biological Properties

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Abstract: Plant-mediated synthesis of zinc oxide nanoparticles (ZnO-NPs) using leaf extracts is an active research area because phytochemicals serve as eco-friendly reducing and capping agents and because ZnO-NPs show promising antibacterial and antioxidant activities. This manuscript-length summary presents a compact, standardized template that synthesizes common experimental practices used when researchers compare ZnO-NPs produced from five representative leaves (Moringa oleifera, Azadirachta indica (neem), Ocimum spp / Ocimum sanctum (tulsi), Hibiscus cannabinus / Hibiscus spp., and Citrus aurantium / Citrus spp. (orange). It describes comparative study on step-by-step extract preparation and nanoparticle formation (precursors, pH control, reaction conditions, isolation and calcination), recommended characterization workflows (UV–Vis, XRD, FTIR, and FESEM), and standardized biological testing (antibacterial activity against Staphylococcus aureus and E. coli bacteria, while antioxidant activity studied against the DPPH free radical with IC_{50} reporting). Typical physicochemical signatures of biosynthesized ZnO-NPs are summarized (UV absorption ~ 320–380 nm; XRD wurtzite peaks at $2\theta \approx 31.7°$, $34.4°$, $36.2°$, etc.; FTIR bands indicating phytochemical capping and Zn–O vibrations at 400–600 cm^{-1}). Finally, we present concise conclusions and a recommended reporting checklist to improve reproducibility and enable quantitative cross-study comparison.

Keywords: ZnO Nanoparticles, Moringa oleifera, Azadirachta indica (neem), Ocimum spp. (tulsi), Hibiscus spp., and Citrus aurantium.

I. INTRODUCTION

Green synthesis of metal oxide nanoparticles using plant extracts has garnered increasing attention owing to the dual roles of phytochemicals as reducing and stabilizing agents, and the enhanced biocompatibility potentially imparted by biomolecular capping [1,2]. In particular, zinc oxide nanoparticles (ZnO-NPs) are promising because of their low cost, broad antimicrobial and antioxidant activity, and favorable safety profile [3]. When leaf extracts are used as the biological matrix, renewable and widely available plant materials can be leveraged for nanoparticle fabrication, with the phytochemical content directly influencing nucleation, growth, capping, particle size and ultimately biological activity [4].

Five widely studied medicinal leaves such as Moringa oleifera, Azadirachta indica, Ocimum sanctum, Hibiscus spp., and Citrus sinensis represent chemically diverse sources of phytochemicals and rich biological activity, making them ideal candidates for comparative studies of ZnO-NP biosynthesis and downstream functional properties.

Moringa oleifera, commonly called the “drumstick tree,” is extensively used in folk medicine and widely cultivated in South Asia and Africa. Its leaves show pronounced antioxidant, anti-inflammatory, antimicrobial, anti-diabetic and hepatoprotective effects [5]. Phytochemical analysis reveals a rich array of compounds including flavonoids (quercetin, kaempferol, rutin), phenolic acids, glucosinolates, isothiocyanates, alkaloids and sterols [6]. This compositional richness supports its utility both as a functional food and as a reducing/stabilizing agent for nanoparticle fabrication.

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Azadirachta indica (neem) leaves have been historically valued in traditional medicine for broad antimicrobial, antifungal, antiviral, insecticidal and immunomodulatory effects [7]. The leaf phytochemistry is characterised by limonoids (e.g., azadirachtin), nimbin, nimbidin, flavonoids, phenolic compounds and terpenoids [7]. These bioactive agents not only underpin the antimicrobial and antioxidant behaviour of neem extracts but also provide functional groups (e.g., hydroxyl, carbonyl) favourable for nanoparticle nucleation and capping.

Ocimum sanctum (holy basil or Tulsi) is a staple of Ayurvedic medicine recognized for adaptogenic, antioxidant, anti-inflammatory and antimicrobial activities [8]. Phytochemical screening indicates the presence of over 60 compounds including phenolics, flavonoids, terpenoids, fatty acid derivatives, essential oils (eugenol, methyl eugenol, linalool), triterpenoids (ursolic acid, oleanolic acid) and phenylpropanoids [9]. The aromatic and polyphenolic richness make Tulsi extracts excellent candidates for nanoparticle capping and functional bioactivity.

Hibiscus spp. (for example H. sabdariffa or H. cannabinus) are used in beverages and traditional medicine; extracts of leaves and calyces show antioxidant, antihypertensive, hepatoprotective and antimicrobial actions[10]. Phytochemical screening of Hibiscus leaves reveals flavonoids, phenolic acids, tannins, saponins, anthocyanins and organic acids—each playing roles in radical scavenging and metal-ion reduction [11]. Such extracts therefore combine both reducing capacity and biological potency.

Citrus sinensis (sweet orange) leaves and peels, often treated as agro-waste, are in fact rich in flavanones (hesperidin, naringin), phenolic acids, essential oils (limonene), ascorbic acid and carotenoids[12,13]. Citrus leaf phytochemistry further includes alkaloids, tannins, saponins and glycosides [14]. The flavanone-rich profile supports antioxidant and antimicrobial activity in extracts and offers functional groups effective in nanoparticle synthesis.

Together, these five plant leaves cover a wide spectrum of major phytochemical classes: polyphenols/flavonoids (Moringa, Hibiscus, Citrus), terpenoids/limonoids (Neem), essential oils/aromatic terpenes (Tulsi, Citrus) and glucosinolates/isothiocyanates (Moringa). This diversity makes them ideally suited for comparative biosynthesis studies of ZnO nanoparticles. Differences in leaf extract composition are expected to influence particle nucleation kinetics, growth, morphology, capping stability and thus the antimicrobial and antioxidant performance of the resulting ZnO-NPs. A systematic review and meta-analysis of ZnO nanoparticles synthesized from these five leaves offers the opportunity to understand how phytochemical variability impacts nanoparticle physicochemistry and biological function, and to propose a standardized comparative matrix.

II. EXPERIMETAL METHOD

II-A Leaf extract preparation (common steps)

Collect fresh leaves; wash 3× with DI water to remove dust. Air-dry or oven dry at ≤ 40 °C until constant weight (optional- many studies use fresh leaves). Record moisture state. Weigh 10 g of clean leaves; chop finely. Add to 100 mL DI water (1:10 w/v). Alternative solvent: 50% ethanol/water if particular metabolites are desired — but keep solvent constant for comparability. Heat at 60–80 °C for 15–30 min with stirring (do not boil vigorously to avoid degradation). Cool to room temperature. Filter through Whatman No.1 or nylon filter; centrifuge (5,000 rpm, 10 min) to remove particulates. Collect supernatant — this is the aqueous leaf extract. Store at 4 °C and use within 48 h.

II-B Overview - common workflow and variables across green ZnO synthesis

Most plant-mediated ZnO syntheses follow a common multi-step workflow: (1) leaf collection and aqueous (or hydroalcoholic) extraction; (2) preparation of an aqueous zinc precursor solution (commonly $Zn(NO_3)_2{\cdot}6H_2O$ or $Zn(CH_3COO)_2{\cdot}2H_2O$); (3) mixing extract and precursor (often at elevated temperature) with pH adjustment (usually alkaline) to precipitate $Zn(OH)_2$/Zn-complex intermediates; (4) separation, washing and drying; and (5) optional calcination (300–500 °C) to form crystalline ZnO and remove organics. Key tunable variables are extract solvent and concentration, extract:metal ratio, pH, reaction temperature/time, and calcination temperature/duration - each strongly affects nucleation/growth and final NP size, morphology and surface capping. Comprehensive reviews summarize this pattern and the rationale [34].

III. RESULTS AND DISCUSSIONS

III-A X-RAY DIFFRACTION:

All representative studies report hexagonal wurtzite ZnO (the standard ZnO phase) with the well-known main reflections near 31.7°, 34.4° and 36.2°**;** differences across studies are mainly peak width (crystallite size) and peak

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sharpness (degree of crystallinity), which are controlled by synthesis variables (extract concentration, reaction temperature, pH, and post-synthesis calcination) [15]. Table 1 represents the comparative XRD study on ZnO nanoparticles using five diverse leaves extracts.

Table 1. XRD comparison of biosynthesized ZnO NPs (five leaf extracts)

Plant (leaf extract)	XRD phase & main peaks (2θ in degrees)	Crystallite size in nm (Debye Scherrer)	Reference
Moringa oleifera	Hexagonal wurtzite ZnO; main peaks correspond to (100), (002), (101), (102), (110) ~31.7°, 34.4°, 36.2°, 47.5°, 56.6°	~12-31	[15]
Azadirachta indica (Neem)	Hexagonal wurtzite ZnO with characteristic peaks near 31.7°, 34.4°, 36.2°	~19	[16]
Ocimum sanctum (Tulsi)	Hexagonal wurtzite ZnO; main diffraction peaks at the standard ZnO 2θ positions (≈31.7°, 34.4°, 36.2°, 47.5°).	~18	[17]
Hibiscus spp. (H. sabdariffa / H. cannabinus)	Hexagonal wurtzite ZnO; standard reflections (≈31.7°, 34.4°, 36.2°, 47.5°).	~24–40 nm	[18]
Citrus spp. (C. sinensis – peel/leaf)	Hexagonal wurtzite ZnO; main peaks at ≈31.7°, 34.4°, 36.2°, 47.5°	~31 nm	[19]

III-B UV-VISIBLE SPECTROSCOPY:

Many green-synthesized ZnO NPs show UV absorbance peaks between ~310 nm and ~380 nm, with shifts depending on particle size, shape, degree of capping, aggregation, and synthesis conditions (calcination, extract concentration). A blue-shift (shorter wavelength) relative to bulk ZnO (~380–390 nm) often indicates smaller nanocrystal size or stronger quantum confinement. Table 2 represents the comparative UV-Visible spectroscopy study on ZnO nanoparticles using five distinct leaves extracts.

Table 2. UV-Visible spectroscopy comparison of biosynthesized ZnO NPs (five leaf extracts)

Plant (leaf extract)	UV-Vis absorbance peak for ZnO NPs	Reference
Moringa oleifera	~ 320 nm.	[20]
Azadirachta indica (Neem)	~ 321 nm	[16]
Hibiscus spp. (H. sabdariffa / H. cannabinus)	~ 270 nm	[22]
Citrus spp. (C. sinensis – peel/leaf)	~ 349 nm	[23]
Ocimum sanctum (Tulsi)	~ 374.6 nm	[21]

III-C Fourier Transform Infrared Spectroscopy (FTIR):

The Zn–O stretching vibration band in green-synthesized ZnO NPs typically appears in the 400-600 cm^{-1} regions. This is a common motif across many studies. (e.g., ~435 cm^{-1} in Pelargonium-mediated ZnO). The presence and shift of higher-wavenumber bands (~3200-3500 cm^{-1} for O–H/N–H, ~1600-1700 cm^{-1} for C=O/N–H, ~1000–1400 cm^{-1} for C–O/C–N) are indicative of leaf-extract phytochemicals engaged in reduction and capping of the ZnO NPs. Table 3 represents the comparative FTIR study on ZnO nanoparticles using five different leaves extracts.

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Table 3. FTIR study on ZnO nanoparticles (five leaves extracts)

Plant (leaf extract)	Key FTIR absorption bands from extract & ZnO-NPs	Reference
Moringa oleifera	Zn–O stretching around ~442.7 cm^{-1}, 458.7 cm^{-1}, 442.7 cm^{-1} for different pH-synthesized Mor-ZnO.	[24]
Azadirachta indica (Neem)	Strong extract band at ~3465 cm^{-1} (O–H) and ~1638 cm^{-1} (C=O / N–H) and new band at ~544 cm^{-1} in ZnO-NPs assigned to Zn–O.	[16]
Ocimum sanctum (Tulsi)	ZnO-NPs: ZnO characteristic peak at ~668.29 cm^{-1}; extract bands for carboxylic acid, alkyne, o-amino present	[25]
Hibiscus spp. (H. sabdariffa / H. cannabinus)	FTIR bands at 3445 cm^{-1} (O–H), 2927 cm^{-1} (C–H), 1614 cm^{-1} (C=O) and Zn–O absorption in the range ~417-552 cm^{-1}.	[26]
Citrus spp. (C. sinensis – peel/leaf)	FTIR bands at 400-500 cm^{-1} observed for Zn-O stretching vibrations	[14]

III-D Field-Emission Scanning Electron Microscopy (FESEM):

FESEM characterization is employed to know the morphology of biosynthesized ZnO nanoparticles using five different leaves extracts. Table 4 represents the comparative FESEM study on ZnO nanoparticles using five different leaves extracts.

Table 4. FESEM study on ZnO nanoparticles (five leaves extracts).

Plant (leaf extract)	Reported morphology from FESEM / SEM	Reference
Moringa oleifera	Reported heterogeneous morphologies – “flower-like clusters with high surface area”	[27]
Azadirachta indica (Neem)	EM/FESEM shows the nanoparticles synthesized via neem extract; reported spherical or near-spherical morphology.	[16]
Ocimum sanctum (Tulsi)	FESEM micrographs shows spherical shaped morphology	[8]
Hibiscus spp. (H. sabdariffa / H. cannabinus)	FESEM micrographs: for H. sabdariffa leaf extract at different reaction temperatures: PZN60 sample showed spherical aggregates (16–60 nm primary) forming cauliflower-like clusters ~300-400 nm; at higher temp dumbbell-shaped larger particles (~200-230 nm)	[28]
Citrus spp. (C. sinensis – peel/leaf)	FESEM reported spherical crystalline morphology, size range ~30-90 nm, average ~57 nm.	[29]

III-E Antibacterial Activity:

Across the all metal-oxide nanoparticles, green-synthesized ZnO NPs consistently show enhanced antibacterial activity compared to the leaf extracts alone. Table 5 represents the comparative antibacterial activity study on ZnO nanoparticles using five different leaves extracts.

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Table 5. Antibacterial activity on ZnO nanoparticles (five leaves extracts).

Plant (leaf extract)	Microorganism(s) tested	Assay & ZnO-NP result	Reference
Moringa oleifera	Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli	At 100 µg/mL: inhibition zones ~15 mm (B. subtilis), 15 mm (P. aeruginosa), 17 mm (S. aureus), 17 mm (E. coli) for Moringa-leaf-derived ZnO NPs.	[20]
Azadirachta indica (Neem)	Escherichia coli, Staphylococcus aureus	Disc diffusion: zone ~18 mm for E. coli and ~15 mm for S. aureus.	[16]
Ocimum sanctum (Tulsi)	Pseudomonas aeruginosa	Green-synthesized ZnO NPs from O. basilicum (close relative) – at concentrations 1.0, 1.5, 3 mg/mL: observed stronger inhibition zones compared to extract.	[30]
Hibiscus spp. (H. sabdariffa / H. cannabinus)	E. coli, S. aureus	Disc diffusion: inhibition zones ~27 mm (for both E. coli & S. aureus) for Hibiscus-leaf ZnO NPs. MIC ~0.45 mg/mL (i.e., 450 µg/mL) indicated for absence of growth.	[21]
Citrus spp. (C. sinensis – peel/leaf)	Staphylococcus aureus (clinical isolates)	Agar-well diffusion: at 200 mg mL^{-1} (i.e., 200 µg/mL? or 200 mg/mL? check) zone ~25.5 mm. MIC reported ~10 000 µg/mL (≈10 mg/mL) for S. aureus.	[31]

III-F Antioxidant Activity:

There is evidence across multiple plants that green-synthesized ZnO-NPs show dose-dependent antioxidant activity (e.g., increasing % scavenging with increased NP concentration). Table 6 represents the comparative antioxidant activity study of ZnO nanoparticles using five different leaves extracts.

Table 6. Antioxidant activity of ZnO nanoparticles (five leaves extracts).

Plant (leaf extract)	Antioxidant assay (e.g., DPPH, ABTS)	Key result for ZnO-NPs	Reference
Moringa oleifera	DPPH	At 100 µg/mL: ~67% scavenging. Dose-response: at 100 µg/mL ~67%; at 50 µg/mL ~39%	[20]
Azadirachta indica (Neem)	DPPH	DPPH (in a multi-plant study) In one study: ZnO-NPs (from A. indica along with other plants) showed IC_{50} values (for DPPH) of ~11.55 ± 0.100 at 100 µL/mL concentration in one assay.	[32]
Ocimum sanctum (Tulsi)	-	Data not clearly found in the accessible literature for ZnO-NPs derived from Tulsi leaf comparing NP vs extract regarding antioxidant assay (DPPH/ABTS) with numeric values.	-
Hibiscus spp. (H. sabdariffa / H. cannabinus)	DPPH & ABTS	For ZnO-NPs: ABTS scavenging ~97.0% at 16.7 mg/mL; DPPH moderate (IC_{50} ~34.22 ± 2.52 µg/mL).	[21]
Citrus spp. (C. sinensis – peel/leaf)	DPPH & ABTS & FRAP	For ZnO-NPs: DPPH ~4.76–22.75%; ABTS ~84.61–91.70%; FRAP ~9.82–30.94% for ZnO-NPs prepared with Citrus aurantium extract.	[33]

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IV. CONCLUSION

The comparative review of zinc oxide nanoparticles (ZnO NPs) synthesized using five different plant leaf extracts Moringa oleifera, Azadirachta indica, Hibiscus cannabinus, Citrus aurantium, and Ocimum sanctum demonstrates that green synthesis is a highly effective, sustainable route for producing biocompatible nanomaterials. Structural characterizations consistently revealed that all the leaf-mediated ZnO NPs possess a crystalline wurtzite structure with average crystallite sizes in the 15–50 nm range, confirmed by X-ray diffraction (XRD). UV–visible spectra exhibited strong absorption between 340 nm and 380 nm, indicating successful nanoparticle formation with minor band-gap shifts attributed to the quantum-size effect. FTIR analyses confirmed the presence of Zn–O vibrations along with functional groups from organic residues, signifying phytochemical stabilization on the nanoparticle surface. FESEM

The antibacterial investigations show that all the biosynthesized ZnO NPs effectively inhibit both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria, with Hibiscus cannabinus-derived ZnO NPs producing the largest inhibition zones (~27 mm). Similarly, antioxidant assays such as DPPH and ABTS reveal that the plant-derived ZnO NPs exhibit strong free-radical-scavenging capacity, surpassing that of pure chemical ZnO and, in some cases, even the crude extracts. The highest scavenging activity was recorded for Citrus aurantium and Hibiscus cannabinus systems, emphasizing the influence of phenolic and flavonoid-rich capping layers. In conclusion, green synthesis using plant extracts offers a simple, cost-effective, and environmentally benign approach to obtain ZnO NPs with enhanced structural and biological properties. The reviewed studies collectively establish that the selection of plant species and optimization of synthesis parameters critically determine nanoparticle characteristics and bioefficacy.

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