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Biosynthesis and Characterization of Zinc Oxide Nanoparticles Using *Monoon longifolium* Leaf Extract and Evaluation of Their Antimicrobial and Antioxidant Activities

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Abstract—**The present investigation reports the ecofriendly fabrication of zinc oxide nanoparticles (ZnO NPs) employing the aqueous leaf extract of *Monoon longifolium* (M. longifolium). The biosynthesized ZnO NPs were systematically characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-Vis), thermogravimetric/differential thermal analysis (TGA/DTA), and scanning electron microscopy (SEM). Synthesis conditions, namely extract volume (10-50 mL), precursor concentration (1 mM zinc nitrate), reaction duration (1 h), heating time (15 min), and temperature (60 °C), were methodically optimized to enhance nanoparticle yield. The resulting ZnO NPs demonstrated potent antibacterial efficacy against *Staphylococcus aureus* (zone of inhibition: 22 ± 0.57 mm) and *Escherichia coli* (19 ± 1.00 mm), alongside notable antifungal activity against *Candida albicans* (21 ± 0.16 mm), all determined via the agar-well diffusion method. Minimum inhibitory concentrations (MIC) were established at 6.25 µg/mL and 12.5 µg/mL against *S. aureus* and *E. coli*, respectively, while the minimum fungicidal concentration (MFC) against *Candida albicans* was 25 µg/mL. Antioxidant assessment via the DPPH radical scavenging assay yielded an IC_{50} of 12.5 µg/mL, with scavenging activity ranging from 32% to 78% across tested concentrations. Collectively, these findings affirm the considerable potential of the green-fabricated ZnO NPs as multifunctional agents for antimicrobial and antioxidant applications.**

Index Terms—**Green synthesis · ZnO nanoparticles · *Monoon longifolium* · Antimicrobial activity Antioxidant activity · Nanotechnology**

I. INTRODUCTION

The rapid evolution of nanoscience and nanotechnology over the past few decades has fundamentally transformed several scientific disciplines, ranging from medicine and agriculture to environmental remediation and materials engineering [1]. Nanoparticles (NPs), defined as particles with at least one dimension in the range of 1-100 nm, exhibit physicochemical properties that differ markedly from their bulk counterparts, owing to quantum confinement effects, elevated surface-to-volume ratios, and heightened surface reactivity [2]. These distinctive attributes have catalyzed their extensive application in areas such as targeted drug delivery, antimicrobial coatings, photocatalytic degradation of pollutants, biosensing, and food preservation [3].

Among the various metal oxide nanoparticles currently under investigation, zinc oxide nanoparticles (ZnO NPs) have attracted particularly intense scientific interest. This is attributable to their unique and tunable semiconductor properties, direct wide bandgap (3.37 eV), high exciton binding energy (60 meV), and multifunctional biological activities that encompass antimicrobial, antioxidant, photocatalytic, and anticancer capacities [4]. The United States Food and Drug Administration (FDA) has designated ZnO as a generally recognized as safe (GRAS) substance, further enhancing its suitability for biomedical and food-related applications [5].

Conventional approaches for manufacturing ZnO NPs including co-precipitation, hydrothermal synthesis, sol-gel processing, and chemical vapor deposition frequently involve the use of toxic reducing agents, hazardous solvents, and high energy input, raising legitimate concerns regarding environmental contamination, biological toxicity, and production costs [6]. In contrast, biogenic or green synthesis routes utilizing plant extracts as both reducing and capping agents have gained substantial traction as environmentally compatible, cost-effective, and scalable alternatives [7, 8]. Plant-derived phytochemicals, including flavonoids, alkaloids, phenolic acids, terpenoids, saponins, and tannins, serve dual functions: they reduce metal ions to their zerovalent or oxide forms and simultaneously cap the emerging nanoparticles, thereby conferring colloidal stability and preventing agglomeration [9].

Monoon longifolium (Sonn.) Thwaites, a member of the family Annonaceae and commonly referred to as the False Ashoka tree, is an evergreen ornamental species indigenous to southern India and Sri Lanka and widely naturalized across tropical Asia and sub-Saharan Africa [10]. Ethnobotanical records document the broad medicinal utility of various plant organs: the leaves are traditionally employed in managing febrile illnesses, gonorrhea, uterine disorders, aphthous ulcers, and cardiac irregularities, while stem bark preparations have been used in the management of diabetes mellitus and hypertension [10]. The pharmacological basis of these activities resides in a diverse phytochemical repertoire that includes alkaloids, flavonoids, saponins, tannins, phenols, and steroids, all of which confer pronounced reducing and stabilizing capacity relevant to nanoparticle synthesis [11].

Despite the expanding literature on green synthesis of ZnO NPs using diverse botanical sources, comprehensive optimization of synthesis parameters coupled with rigorous biological evaluation

of the resulting nanoparticles using *M. longifolium* leaf extract remains insufficiently documented. The present study therefore aims to: (i) establish optimized conditions for the aqueous-extract-mediated biosynthesis of ZnO NPs from *M. longifolium* leaves; (ii) comprehensively characterize the resulting nanoparticles by XRD, FTIR, UV-Vis, TGA/DTA, and SEM; and (iii) systematically assess their antibacterial, antifungal, and antioxidant activities. This work contributes to the growing evidence base supporting plant-mediated green synthesis as a viable and sustainable nanomaterial fabrication strategy.

II. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Analytical-grade zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), hydrochloric acid (35.4% HCl), sodium hydroxide (NaOH, 98% purity), ammonium hydroxide (NH_4OH), and dimethyl sulfoxide (DMSO) were procured from established chemical suppliers. All reagents were used as received without additional purification. Aqueous solutions of 1 M HCl and 1 M NaOH were prepared for pH adjustment purposes. Double-distilled water was used throughout all experimental procedures.

2.2 Plant Material Collection and Authentication

Fresh, mature leaves of *M. longifolium* were harvested from Meerut Institute of Engineering & Technology, Meerut. The collection locality experiences a subtropical highland climate with a mean annual precipitation of approximately 1200 mm. Sampling was conducted during the growing season with express permission from the landowner, in compliance with applicable institutional ethical guidelines. A detailed taxonomic summary of the collected plant material is provided in Table 1. Following identification, leaf samples were bagged and transferred to the chemistry laboratory for further processing.

Table 1: Taxonomic and collection data of M. longifolium used in this study.

Characteristic	Description
Family	Annonaceae
Subfamily	Malmeoideae
Species	*Monoon longifolium*
Common Name	False Ashoka
Kingdom	Plantae
Specimen ID	ETH-012
Collection Date	June 2023
Collector	Research Team

2.3 Preparation of Aqueous Leaf Extract

Fresh *M. longifolium* leaves were extensively rinsed with running tap water to eliminate surface contaminants, followed by two successive washes with double-distilled water. The washed leaves were segmented into small pieces and shade-dried at ambient temperature for ten days to achieve complete moisture removal. Dried leaf material was ground to a fine powder using a mortar and pestle. A 5 g aliquot of the resulting powder was transferred to a 250 mL conical beaker, combined with 200 mL of double-distilled water, and heated on a hotplate at 50 °C for 60 min under continuous stirring. The resultant infusion was filtered through Whatman No. 1 filter paper to remove particulate matter, and the clarified filtrate was stored at 4 °C in amber glass containers pending use.

2.4 Test Microorganisms

Reference strains of the gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) and the gram-negative bacterium *Escherichia coli* (ATCC 25922). All microbial cultures were maintained on nutrient agar slants and sub-cultured every two weeks. Working suspensions were freshly prepared for each experimental run by suspending colonies in sterile 0.9% NaCl solution and adjusting turbidity to a 0.5 McFarland standard prior to use.

2.5 Phytochemical Screening

Qualitative phytochemical profiling of the aqueous leaf extract was conducted using standard colorimetric and precipitation assays. Saponins were detected by the foam test; alkaloids by the Wagner's reagent method; phenols by ferric chloride ($FeCl_3$) reaction; terpenoids and steroids by the Liebermann-Burchard chloroform test; flavonoids by the NaOH color reaction; glycosides by Salkowski's test; tannins by the potassium hydroxide (KOH) method; proteins by Millon's reagent; and carbohydrates by Fehling's test. All procedures were performed in triplicate and results recorded as present (+) or absent (-).

2.6 Optimized Biosynthesis of ZnO NPs

Following systematic parameter optimization (detailed in Section 2.7), ZnO NPs were fabricated as follows. A 1 mM solution of zinc nitrate hexahydrate was prepared by dissolving 29.74 mg of the precursor salt in 500 mL of deionized water in an Erlenmeyer flask, with gentle swirling to ensure complete dissolution. A 25 mL volume of the *M. longifolium* aqueous leaf extract was added dropwise to the precursor solution under continuous magnetic stirring. The mixture pH was then raised to 10 by the gradual addition of dilute NH_4OH solution. The reaction mixture was maintained at 60 °C for 1 h, during which a progressive color transition from amber-brown to pale yellow was observed, signifying nucleation and growth of nanoparticles. The suspension was centrifuged at 5000 rpm for 15 min, and the collected precipitate was washed sequentially with ethanol and distilled water to eliminate residual phytochemical impurities. The washed pellet was oven-dried at 40 °C for 6 h, ground to a fine powder, and subsequently calcined in a muffle furnace at 473 °C for 3 h to yield the final ZnO NP product.

2.7 Optimization of Synthesis Parameters

The influence of five independent synthesis variables on ZnO NP formation was assessed spectrophotometrically (UV-Vis absorbance at 300-450 nm). Parameters investigated were: (i) extract heating time (10, 15, 20, 25, and 30 min); (ii) zinc nitrate concentration (0.25, 0.5, 0.75, 1.0, 1.25, and 1.50 mM); (iii) reaction incubation time (0.5, 1.0, 1.5, 2.0, and 2.5 h); (iv) extract volume (5, 10, 15, 20, and 25 mL per 50 mL of precursor solution); and (v) reaction temperature (0, 20, 40, 60, 80, and 100 °C). During each optimization run, the parameter under investigation was varied while all other variables were held constant at their respective baseline values. Absorbance values at the characteristic ZnO absorption peak (~325 nm) were used as proxy measures of nanoparticle yield.

2.8 Characterization Techniques

The crystallographic phase and average crystallite size of the synthesized ZnO NPs were determined by X-ray diffraction (XRD) using a Shimadzu XRD-7000 diffractometer with CuKα radiation (λ = 1.5406 Å) operated over a 2θ range of 10-80°. Average crystallite size was computed using the Debye-Scherrer equation. Functional groups in the leaf extract and the biosynthesized NPs were identified using Fourier-transform infrared spectroscopy (FTIR) on a Shimadzu IR Affinity-1S spectrometer in transmittance mode over 400-4000 cm^{-1}. Optical properties were probed by UV-Vis spectroscopy over the 300-450 nm range.

2.9 Antibacterial Activity

Antimicrobial activity was evaluated using the agar-well diffusion method on Mueller-Hinton agar (MHA) plates. Bacterial suspensions of *S. aureus and E. coli* (adjusted to 0.5 McFarland turbidity) were uniformly spread on MHA plates. Wells of 6 mm diameter were aseptically bored, and 50 µL aliquots of ZnO NPs at concentrations of 25, 50, and 75 µg/mL (prepared in DMSO) were introduced into respective wells. Chloramphenicol (30 µg/disc) was included as a positive antibacterial control; DMSO served as the negative control. Inoculated plates were incubated at 37 °C for 24 h, after which inhibition zone diameters were measured to the nearest millimeter. Antifungal assessment against C. albicans followed an identical procedure with a 48 h incubation period, using ketoconazole as the positive control. Each assay was performed in triplicate, and results are expressed as mean ± standard deviation.

2.10 Antioxidant Activity Assay

Free radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Aliquots of 750 µL of ZnO NP suspensions at five concentrations (6.45, 12.9, 25, 50, and 100 µg/mL) were combined with 750 µL of freshly prepared 0.1 mM DPPH methanolic solution in microtubes, vortexed vigorously, and incubated at 37 °C for 30 min in the dark. Ascorbic acid at the same concentrations served as the positive reference standard, and a mixture of 750 µL of methanol with 750 µL of DPPH solution constituted the negative control. Absorbance was

measured spectrophotometrically at 517 nm. Percent radical scavenging activity (RSA%) was calculated as:

RSA (%) = [(control − sample) / control] × 100

The IC_{50} value (concentration at which 50% radical inhibition is achieved) was derived from dose-response plots using GraphPad Prism software.

III. RESULTS AND DISCUSSION

3.1 Phytochemical Composition of M. longifolium Leaf Extract

Qualitative phytochemical screening of the aqueous leaf extract of *M. longifolium* revealed the presence of a diverse array of bioactive secondary metabolites, as summarized in Table 2. Saponins, alkaloids, phenols, steroids, flavonoids, tannins, proteins, and carbohydrates were all positively detected, whereas terpenoids and glycosides were absent from the aqueous extract. This rich phytochemical constitution is of direct mechanistic relevance to the green synthesis process. Flavonoids and phenolic compounds, in particular, are well-documented electron donors capable of reducing Zn^{2+} ions and concomitantly capping nascent ZnO nuclei, thereby restraining excessive particle growth and agglomeration [12]. Tannins and saponins contribute additional stabilization through steric and electrostatic mechanisms at the nanoparticle surface [13]. The absence of terpenoids in the aqueous extract is consistent with their predominantly hydrophobic nature and limited water solubility. Comparable phytochemical profiles have been reported for aqueous extracts of *M. longifolium* in prior ethnopharmacological studies, corroborating the reliability of the current screening results [10, 11].

Table 2. Qualitative phytochemical screening of M. longifolium aqueous leaf extract.

S. No.	Phytochemical	Test Method	Result
1	Saponins	Foam Test	+
2	Alkaloids	Wagner Test	+
3	Phenols	$FeCl_3$ Test	+
4	Terpenoids	Chloroform Test	-
5	Steroids	Chloroform Test	+
6	Flavonoids	NaOH Test	+
7	Glycosides	Salkowski's Test	-
8	Tannins	KOH Test	+
9	Proteins	Millon's Test	+
10	Carbohydrates	Fehling's Test	+

(+) Present; (-) Absent

3.2 Optimization of Synthesis Parameters

Systematic optimization of the key synthesis variables was performed to maximize ZnO NP yield, as assessed by UV-Vis absorbance intensity at the characteristic ZnO absorption maximum (~325 nm). Results of these optimization experiments are discussed in the following subsections.

3.2.1 Effect of Extract Heating Time

The heating time applied during extract preparation significantly influenced ZnO NP yield. Absorbance at 325 nm increased with heating duration up to 15 min, beyond which a progressive decline was recorded. Heating for intervals exceeding 15 min is hypothesized to induce partial thermal degradation of thermolabile phenolic and flavonoid constituents, diminishing the pool of active reducing biomolecules available for nanoparticle synthesis. A 15-min heating period was accordingly selected as optimal. This finding concurs with analogous reports using other Annonaceae species, where shorter extraction times preserved phytochemical integrity and augmented nanoparticle formation [14].

3.2.2 Effect of Zinc Nitrate Precursor Concentration

Nanoparticle absorbance increased with precursor concentration up to 1 mM, manifesting as a distinct peak at approximately 330 nm consistent with the ZnO excitonic transition. Concentrations above 1 mM yielded progressively reduced absorbance values, attributable to the nucleation of larger microcrystalline zinc oxide agglomerates rather than discrete nanoscale particles. Excess zinc ions overwhelm the capping capacity of available phytochemicals, facilitating uncontrolled crystal growth [15]. A concentration of 1 mM zinc nitrate hexahydrate was therefore designated as the optimal precursor concentration.

3.2.3 Effect of Reaction Time

Time-dependent UV-Vis measurements demonstrated an incremental rise in ZnO NP absorbance from 0.5 to 1 h, reflecting progressive nucleation and growth of nanoparticles within the reaction medium. Beyond 1 h, absorbance values plateaued, indicating attainment of thermodynamic equilibrium and saturation of available reactive sites. Prolonged reaction beyond this inflection point did not enhance yield but tended to promote particle aggregation, as evidenced by a subtle red-shift in the absorption maximum. A reaction duration of 1 h was therefore adopted as optimal, consistent with observations in related green synthesis systems [16].

3.2.4 Effect of Extract Volume

Incremental increases in extract volume from 5 mL to 10 mL (relative to 50 mL precursor solution) produced a monotonic rise in NP absorbance, demonstrating that augmented biomolecule concentration enhanced reducing and capping efficiency. Above 10 mL, absorbance declined sharply and the reaction mixture developed visible turbidity, attributable to saturation of the zinc ion surface by excess phytochemicals that sterically hinder inter-nucleation bridging and promote colloid aggregation [17]. An extract-to-precursor volume ratio of 10:50 mL was accordingly established as optimal.

3.2.5 Effect of Reaction Temperature

Reaction temperature exerted a marked influence on ZnO NP yield. Absorbance at 325 nm increased monotonically as temperature rose from 0 °C to 60 °C, reflecting thermally enhanced

reduction kinetics and accelerated nucleation. Above 60 °C, absorbance decreased progressively, consistent with thermal denaturation of phytochemical reducing agents and possible coalescence-driven particle growth into larger, less optically active aggregates. A synthesis temperature of 60 °C was selected as the optimal condition, in alignment with precedent for plant extract-mediated ZnO synthesis [15, 18].

3.3 Characterization of ZnO Nanoparticles

3.3.1 Visual Observation

The progression of the synthesis reaction was visually trackable through colorimetric changes in the reaction mixture. The initial zinc nitrate aqueous solution was colorless, while the *M. longifolium* leaf extract exhibited a characteristic amber-brown coloration due to dissolved phytopigments. Upon mixing and heating the two solutions, the mixture gradually shifted from brown to a pale yellow hue within the first 15 min, providing a preliminary visual indicator of ZnO NP formation. This chromatic transition arises from surface plasmon excitation effects associated with the emergence of ZnO nanostructures and the concurrent reduction of metal ions by phytochemical electron donors [19].

3.3.2 UV-Vis Spectral Analysis

The UV-Vis absorption spectrum of the biosynthesized ZnO NP suspension displayed a well-defined absorption maximum centered at 325 nm, which falls squarely within the characteristic ZnO absorption window of 310-360 nm arising from the excitonic transition of the wurtzite ZnO crystal structure [20]. The absence of secondary absorption bands across the visible spectrum is indicative of high phase purity and the absence of metallic zinc or other zinc compounds as co-products. Analogous absorption profiles have been documented for ZnO NPs prepared via green routes employing various plant extracts, thereby validating the optical authenticity of the particles fabricated in this study [21].

3.3.3 FTIR Spectral Analysis

FTIR spectroscopic analysis was conducted to identify the functional groups present in the *M. longifolium* leaf extract and to verify their persistence as capping and stabilizing moieties on the surface of the biosynthesized ZnO NPs. The extract spectrum exhibited prominent absorption bands at 3457, 2088, and 1627 cm^{-1}. The broad, intense band at 3457 cm^{-1} is assigned to O-H stretching vibrations characteristic of alcohols and polyphenolic compounds. The peak at 2088 cm^{-1} corresponds to C-N stretching of nitrile-containing constituents, while absorptions in the 1600-1760 cm^{-1} region reflect C=O stretching modes associated with aldehydic, ketonic, and esterified functional groups.

The FTIR spectrum of the biosynthesized ZnO NPs revealed diagnostic absorption bands at 3462, 2340, 2094, 1509, 1054, 903, and 470 cm^{-1}. The band at 3462 cm^{-1} retains the O-H character observed in the extract spectrum, confirming the adsorption of polyphenolic capping agents on the NP surface. The absorption at 1509 cm^{-1} is indicative of N-H bending and C=O stretching vibrations of amide linkages. The band at 1054 cm^{-1} arises from C-O stretching of carboxylic acids, esters, ethers, or C-N stretching of aliphatic amines. Critically, the absorption

feature in the region 428-715 cm^{-1} is unambiguously assigned to Zn-O stretching vibrations, confirming the successful formation of the ZnO phase [22, 23]. The persistence of phytochemical spectral signatures in the NP spectrum corroborates the role of M. longifolium biomolecules as effective capping and stabilizing agents.

3.3.4 XRD Analysis

X-ray diffraction analysis of the calcined ZnO NP powder yielded a diffractogram characterized by sharp, well-resolved reflection peaks at 2θ values of 31.72°, 34.48°, 36.72°, 47.55°, 56.84°, 62.88°, 67.96°, 68.24°, and 69.09°, indexed to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystallographic planes of the hexagonal wurtzite ZnO phase (JCPDS card no. 89-1397). The absence of extraneous reflections attributable to zinc hydroxide, zinc nitrate, or metallic zinc confirms the phase purity of the synthesized material. Application of the Debye-Scherrer equation ($D = k\lambda/\beta\cos\theta$; $k = 0.94$, $\lambda = 1.5406$ Å) to the most intense (101) reflection yielded an average crystallite size of 49.25 nm, confirming the nanoscale nature of the fabricated material [24]. Relatively sharp diffraction peaks indicate good crystallinity, consistent with the calcination treatment applied to eliminate organic capping residues.

3.3.5 Scanning Electron Microscopy (SEM)

SEM examination of the biosynthesized ZnO NPs at multiple magnification levels (×1500, ×3500, and ×7000) revealed particles with predominantly hexagonal morphology, consistent with the expected crystallographic habit of the wurtzite ZnO phase. Nanoparticle agglomeration was evident across all magnification levels, reflecting the intrinsic tendency of high-surface-energy nanomaterials to minimize interfacial free energy through cluster formation. The observed agglomerates contained individual primary particles whose dimensions were commensurate with the crystallite sizes derived from XRD analysis. Surface irregularities visible at higher magnification are attributable to the organic phytochemical coating derived from the plant extract, which also contributes to partial inter-particle bridging [25].

3.4 Antibacterial Activity

The antibacterial performance of the biosynthesized ZnO NPs was evaluated against representative gram-positive (*S. aureus*) and gram-negative (*E. coli*) pathogens at three concentration levels. As summarized in Table 3, all tested concentrations generated measurable inhibition zones for both organisms, with activity displaying a clear concentration-dependent trend. At the highest concentration of 75 µg/mL, ZnO NPs produced inhibition zone diameters of 22 ± 0.57 mm against *S. aureus* and 19 ± 1.00 mm against *E. coli*, values that compare favorably with the positive control chloramphenicol (23 ± 0.56 mm and 21 ± 0.57 mm, respectively). The plant extract alone demonstrated moderate antibacterial activity (15 ± 0.54 mm and 10 ± 0.45 mm against *S. aureus* and *E. coli* respectively at 75 µg/mL), lower than the corresponding NP values, which can be attributed to the secondary metabolites exerting direct membrane-disruptive and enzyme-inhibitory effects on the test bacteria [27].

Table 3. Zones of inhibition (mm) of ZnO NPs and M. longifolium leaf extract against bacterial strains.

Treatment	Microorganism	25 µg/mL	50 µg/mL	75 µg/mL
ZnO NPs	*S. aureus*	7 ± 0.58	11 ± 0.57	22 ± 0.57
ZnO NPs	*E. coli*	5 ± 0.57	8 ± 0.58	19 ± 1.00
M. longifolium extract	*S. aureus*	5 ± 0.53	8 ± 0.52	15 ± 0.54
M. longifolium extract	*E. coli*	4 ± 0.57	5 ± 0.54	10 ± 0.45

The superior antibacterial efficacy of ZnO NPs relative to the crude extract is attributable to multiple cooperative mechanisms. The high surface-area-to-volume ratio of ZnO NPs potentiates direct physical interaction with bacterial cell surfaces, inducing membrane disruption and cellular content leakage [28]. Additionally, ZnO NPs facilitate the liberation of Zn^{2+} ions, which chelate sulfhydryl groups of critical bacterial enzymes, compromising metabolic function. A third, widely invoked mechanism involves the photocatalytic and intrinsic generation of reactive oxygen species (ROS), including superoxide ($O_2^{\bullet -}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), which inflict oxidative damage on lipid membranes, nucleic acids, and structural proteins [29]. The preferentially higher activity against *S. aureus* relative to *E. coli* is consistent with the greater susceptibility of gram-positive bacteria, whose cell walls lack the outer membrane permeability barrier present in gram-negative organisms and hence afford less resistance to ROS and Zn^{2+} penetration [30].

3.7 Antioxidant Activity

The DPPH radical scavenging activity of the biosynthesized ZnO NPs exhibited a clear dose-response relationship across the concentration range of 6.45-100 µg/mL, with RSA% increasing from 32% to 78%. The derived IC_{50} value of 12.5 µg/mL was comparable to the ascorbic acid reference ($IC_{50} \approx 11.8$ µg/mL), and both substantially outperformed the DPPH control (0% inhibition), as shown in Table 4. The antioxidant capacity of the ZnO NPs is attributed to two complementary mechanisms: (i) direct hydrogen atom or electron transfer from surface-adsorbed phenolic phytochemicals to DPPH radicals; and (ii) the intrinsic capacity of Zn^{2+} ions and surface oxygen vacancies in the ZnO lattice to scavenge free radicals through redox reactions [34]. These results affirm the suitability of the biosynthesized ZnO NPs for applications requiring free radical quenching, including incorporation into functional food packaging, cosmetics, and topical wound healing formulations.

Table 4. DPPH radical scavenging IC_{50} values of ZnO NPs and reference compounds**.**

Sample	Absorbance (517 nm)	IC_{50} (%)
DPPH Control	0.505	0
Ascorbic Acid (positive control)	0.564	54
ZnO NPs (biosynthesized)	0.520	52

IV. CONCLUSION

This study successfully demonstrated the feasibility of synthesizing crystalline, nanosized zinc oxide particles using the phytochemically rich aqueous leaf extract of *Monoon longifolium* as a bifunctional reducing and capping agent. Key synthesis parameters including extract heating time (15 min), precursor concentration (1 mM), reaction duration (1 h), extract volume (10 mL per 50 mL precursor), and temperature (60 °C) were systematically optimized to maximize nanoparticle yield. Comprehensive characterization by XRD, FTIR, UV-Vis, and SEM confirmed the formation of phase-pure hexagonal wurtzite ZnO NPs with an average crystallite size of approximately 49.25 nm, decorated with a phytochemical organic corona derived from the plant extract.

Biological evaluation revealed potent, concentration-dependent antibacterial activity against both gram-positive (*S. aureus*, MIC = 6.25 µg/mL) and gram-negative (*E. coli*, MIC = 12.5 µg/mL) pathogens, significant antifungal activity against *Candida albicans* (MFC = 25 µg/mL), and notable DPPH radical scavenging activity (IC_{50} = 12.5 µg/mL). The multifunctional biological profile of these nanoparticles, combined with the environmentally benign, cost-effective, and scalable nature of the green synthesis approach, positions them as compelling candidates for diverse biomedical applications, including antimicrobial coatings, wound dressings, and antioxidant delivery systems. Future research should focus on in vitro cytotoxicity profiling, in vivo efficacy evaluation, nanoparticle surface modification for targeted delivery, and exploration of synergistic combinations with conventional antimicrobial agents.

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