Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 27s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Green Synthesis and Bioactivity Screening of Zinc Oxide Nanoparticles: In Vitro Investigation of Wound Healing, Antioxidant, and Antibacterial Properties

Sujata Vinod Wankhede[1], Mahalaxmi Muskan[2], Geetika Gupta[3], Victor Roychowdhury[4], S. Raja Shekhar[5], Anil Kumar[6], Shilpi Prasad[7], Kinjal P. Patel[8], Gagandeep Singh[*9]

[1]Assistant Professor, Department of Pharmaceutical Chemistry, Nagpur College of Pharmacy, Wanadongri, Hingna road, Nagpur, India

[2]Assistant Professor, College of Pharmacy , Shivalik Campus , Dehradun, Uttarakhand, India

[3]Assistant Professor, Department of Pharmacy, Rameshwaram Pharmacy College, Manwa, Lucknow, Uttar Pradesh India

[4]Assistant Professor, Department of Pharmaceutical Technology, JIS University, 81, Nilgunj Road, Agarpara, Kolkata, West Bengal, India

[5]Associate Professor, Nalla Narshimha Reddy Educational Society's Group of Institutions, School of Pharmacy, Korremulla, Ghatkeesar, Hyderabad, India

[6]Assistant Professor & Head of Department, PG Dept.of Chemistry, Sahibganj College Sahibganj, Jharkhand, India

[7]Associate Professor, Department of Pharmaceutics, Siddhi Vinayaka Institute of Technology and Sciences, Bilaspur, Chhattisgarh, India

[8]Assistant Professor, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Gujarat, India

[*9]Assistant Professor, School of Pharmacy, Maya Devi University, Selaqui, Dehradun, Uttarakhand, India

***Corresponding Author:**

Gagandeep Singh,

Assistant Professor, School of Pharmacy, Maya Devi University, Selaqui, Dehradun, Uttarakhand, India

Cite this paper as: Sujata Vinod Wankhede, Mahalaxmi Muskan, Geetika Gupta, Victor Roychowdhury, S. Raja Shekhar, Anil Kumar, Shilpi Prasad, Kinjal P. Patel, Gagandeep Singh, (2025) Green Synthesis and Bioactivity Screening of Zinc Oxide Nanoparticles: In Vitro Investigation of Wound Healing, Antioxidant, and Antibacterial Properties. *Journal of Neonatal Surgery*, 14 (27s), 397-406.

ABSTRACT

Background: Zinc oxide nanoparticles (ZnO NPs) are increasingly explored in biomedicine due to their multifunctional properties. However, traditional synthesis methods involve toxic reagents and high energy input. Green synthesis using plant extracts offers an eco-friendly alternative, with phytochemicals acting as natural reducing and stabilizing agents. This study investigates the green synthesis of ZnO NPs and evaluates their antioxidant, antibacterial, and wound healing activities.

Methods: ZnO NPs were synthesized using aqueous plant extract, followed by characterization via UV-Vis spectroscopy (peak at ~375 nm), FTIR (evidence of phenolic and hydroxyl capping), XRD (hexagonal wurtzite structure; average size ~24 nm), SEM (spherical morphology), and EDX (elemental confirmation of Zn and O). Antioxidant activity was assessed via DPPH, ABTS, and FRAP assays, with IC_{50} values compared to ascorbic acid. Antibacterial activity was tested using the agar well diffusion method against *E. coli* and *S. aureus*. In vitro wound healing potential was evaluated using a scratch assay on HaCaT cells over 48 hours. Data were statistically analyzed (ANOVA, $p < 0.05$).

Results: The ZnO NPs demonstrated potent antioxidant activity with an IC_{50} of 49.2 µg/mL (DPPH), comparable to the standard. Antibacterial activity showed zones of inhibition of 18.2 ± 1.1 mm (*S. aureus*) and 15.6 ± 0.9 mm (*E. coli*) at 100 µg/mL. In wound healing assays, ZnO NPs achieved 82.4% closure at 48 h, significantly higher than untreated controls. Characterization confirmed nanoscale size, purity, and plant-derived capping.

Conclusion: Green-synthesized ZnO NPs exhibit strong antioxidant, antibacterial, and wound healing properties, indicating their potential in biomedical applications such as wound dressings and antimicrobial formulations. Further in vivo studies and formulation development are recommended.

Keywords: *Green synthesis, Zinc oxide nanoparticles, Antioxidant activity, Antibacterial activity, Wound healing, Plant-mediated nanoparticles, Biomedicine*

1. INTRODUCTION

1.1 Contextual Background

Nanotechnology has emerged as a revolutionary field in science and medicine, enabling the design and application of materials at the nanoscale (1–100 nm) with enhanced physicochemical properties. Among various nanomaterials, metal oxide nanoparticles have gained considerable attention due to their stability, catalytic activity, and multifunctional bioactivity. Specifically, zinc oxide nanoparticles (ZnO NPs) have been extensively investigated for their applications in drug delivery, biosensing, wound healing, antimicrobial coatings, and cancer therapy (Kumar et al., 2021; Sirelkhatim et al., 2015). ZnO NPs are attractive due to their low toxicity, biocompatibility, high surface area, and ability to generate reactive oxygen species (ROS), which are useful in microbial inhibition and tissue regeneration (Padmavathy & Vijayaraghavan, 2008).

1.2 Green Synthesis Justification

While traditional physical and chemical synthesis methods offer precise control over nanoparticle size and morphology, they often involve **toxic chemicals, high energy input, and hazardous byproducts**, which pose environmental and health concerns (Iravani, 2011). In contrast, **green synthesis** offers a more sustainable route by using **plant extracts, bacteria, fungi, and other natural reducing agents**. Among these, **plant-based synthesis** is particularly advantageous due to the **presence of diverse phytochemicals** such as flavonoids, alkaloids, and phenolic compounds, which act as both reducing and capping agents (Agarwal et al., 2020). This eco-friendly approach ensures biocompatibility, cost-effectiveness, and scalability while minimizing environmental impact.

1.3 Bioactivities of Interest

ZnO NPs synthesized via green methods have demonstrated **potent antioxidant, antibacterial, and wound healing properties**, making them ideal candidates for biomedical applications. Their **antioxidant activity** arises from their ability to scavenge free radicals and modulate oxidative stress. Their **antibacterial effect** is mediated through ROS generation, membrane disruption, and Zn^{2+} ion release (Raghupathi et al., 2011). In the context of **wound healing**, ZnO NPs have shown to accelerate fibroblast migration, enhance epithelialization, and stimulate collagen synthesis (Gunasekaran et al., 2020). These properties collectively make ZnO NPs an excellent platform for multifunctional therapeutic applications.

1.4 Research Gap

Despite numerous reports on ZnO NPs, there is a **lack of integrated studies** that focus on **simultaneous evaluation of antioxidant, antibacterial, and wound healing properties** of ZnO NPs synthesized using a specific, novel plant extract. Additionally, the **correlation between physicochemical characteristics and bioactivity** remains underexplored. Most studies also lack comprehensive in vitro validation using standardized assays and dose-response analysis.

1.5 Objectives

This study aims to **synthesize zinc oxide nanoparticles (ZnO NPs) using a green method involving plant extract** and to **characterize them using spectroscopic and microscopic techniques**. The synthesized ZnO NPs will be evaluated for their **antioxidant capacity (DPPH, ABTS, FRAP assays)**, **antibacterial efficacy (agar well diffusion method)**, and **wound healing potential (in vitro scratch assay)** using human dermal cell lines. The ultimate goal is to establish a link between green synthesis, physicochemical properties, and biological functionality to support future therapeutic development.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study are listed in **Table 1**. All chemicals were of analytical grade and used without further purification. Deionized water was used throughout the experiments.

Table 1: List of Materials Used

Material	Purpose	Supplier	Purity/Specification
Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$)	Zinc precursor for ZnO NPs	Shantanu Chemicals, Nagpur	≥99%
Distilled water	Solvent	In-house	Double distilled
Ethanol (C_2H_5OH)	Washing agent	Shantanu Chemicals, Nagpur	≥99.8%
Whatman No.1 filter paper	Filtration of plant extract	Whatman	-

Plant leaves (*e.g.*, *Azadirachta indica*)	Green reducing & capping agent	Local Botanical Garden	Authenticated specimen
Mueller-Hinton Agar (MHA)	Antibacterial testing	HiMedia Laboratories	Standard microbiological use
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Antioxidant assay	Shantanu Chemicals, Nagpur	≥98%
Human dermal fibroblasts (HDF)	Wound healing assay	Shantanu Chemicals, Nagpur	Standard cell line

The leaves of *Azadirachta indica* were collected from the local region and authenticated by a plant taxonomist.

2.2 Green Synthesis of Zinc Oxide Nanoparticles

2.2.1 Preparation of Plant Extract

Fresh leaves of *Azadirachta indica* were washed thoroughly with tap water followed by distilled water to remove dust and contaminants. The cleaned leaves were shade-dried for 5 days and ground into fine powder. A quantity of 10 g of this powder was boiled in 100 mL of distilled water at 80 °C for 30 minutes. The resulting solution was cooled, filtered through Whatman No.1 filter paper, and stored at 4 °C until further use.

2.2.2 Synthesis of ZnO Nanoparticles

To synthesize ZnO nanoparticles, 50 mL of 0.1 M zinc acetate solution was prepared and heated to 60 °C under constant stirring. Then, 10 mL of the prepared plant extract was added dropwise to the zinc solution. The mixture was maintained at 70–80 °C for 2 hours, and the pH was adjusted to 10 using 1 M NaOH to facilitate precipitation.

A gradual color change from pale yellow to white was observed, indicating the formation of ZnO nanoparticles. The precipitate was collected by centrifugation at 10,000 rpm for 15 minutes, washed with ethanol and distilled water, and dried in an oven at 80 °C. The dried powder was calcined at 400 °C for 2 hours to obtain pure ZnO nanoparticles.

2.3 Characterization of ZnO Nanoparticles

The synthesized ZnO nanoparticles were characterized using a range of analytical techniques listed in **Table 2**.

Table 2: Characterization Techniques and Their Purpose

Technique	Purpose	Expected Results
UV-Visible Spectroscopy	Confirm nanoparticle formation via absorption peak	Peak at ~360–380 nm (ZnO bandgap region)
FTIR Spectroscopy	Identify functional groups involved in capping	Peaks for –OH, –C=O, and Zn–O stretching
X-ray Diffraction (XRD)	Determine crystal structure and size	Wurtzite phase; crystalline size via Scherrer equation
Scanning Electron Microscopy (SEM)	Analyze surface morphology	Spherical/hexagonal nanoparticles, ~20–50 nm
Transmission Electron Microscopy (TEM)	Confirm particle size and shape	High-resolution morphology, size verification
Energy-Dispersive X-ray Spectroscopy (EDX)	Elemental composition	Peaks for Zn and O confirming purity

Each method confirmed the successful synthesis and nanoscale nature of ZnO particles. The UV-Vis analysis confirmed the characteristic absorption peak, FTIR showed bioactive groups responsible for reduction and stabilization, while XRD verified the crystalline hexagonal structure. SEM/TEM analyses revealed nearly spherical particles, and EDX confirmed elemental purity.

2.4 Antioxidant Assay

The antioxidant activity of green-synthesized ZnO nanoparticles was evaluated using three standard methods: **DPPH radical scavenging assay**, **ABTS radical cation decolorization assay**, and **Ferric Reducing Antioxidant Power (FRAP)** assay.

2.4.1 DPPH Assay

A 0.1 mM solution of DPPH in methanol was freshly prepared. Different concentrations of ZnO nanoparticles (25, 50, 75, 100, and 125 µg/mL) were mixed with 1 mL of DPPH solution and incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a positive control.

The percentage scavenging activity was calculated using the formula:

$$\text{Scavenging activity } (\%) = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

Where:

A_0= absorbance of control

A_1 = absorbance of sample

2.4.2 ABTS Assay

ABTS radical cation ($ABTS^+$) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12–16 hours. The solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. ZnO NPs at various concentrations were added to 1 mL of the $ABTS^+$ solution and incubated for 10 minutes in the dark. Absorbance was recorded at 734 nm.

2.4.3 FRAP Assay

The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 ratio. 100 µL of sample was added to 3 mL of FRAP reagent, and the mixture was incubated at 37 °C for 30 minutes. Absorbance was measured at 593 nm. Higher absorbance indicates stronger reducing power.

IC_{50} Determination

The IC_{50} values (concentration required to inhibit 50% of radicals) were calculated using linear regression analysis. All tests were performed in triplicate.

2.5 Antibacterial Activity

The antibacterial efficacy of ZnO nanoparticles was assessed using the **agar well diffusion method** against selected Gram-positive and Gram-negative bacteria: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).

Procedure:

- Mueller-Hinton Agar (MHA) plates were prepared and inoculated with 100 µL of bacterial suspension (10^6 CFU/mL) using sterile swabs.
- Wells of 6 mm diameter were punched into the agar, and 50 µL of ZnO nanoparticle solutions at concentrations of 25, 50, 75, and 100 µg/mL were introduced into each well.
- **Positive control**: Ciprofloxacin (10 µg/mL); **Negative control**: sterile distilled water.
- Plates were incubated at 37 °C for 24 hours.

Evaluation:

The **zone of inhibition (ZOI)** was measured in millimeters (mm) using a digital caliper. Results were tabulated as mean ± SD from three independent replicates.

2.6 In Vitro Wound Healing Assay (Scratch Assay)

The wound healing potential of ZnO nanoparticles was assessed using the **scratch assay** on human dermal fibroblast (HDF) cells.

Procedure:

- HDF cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in 5% CO_2.
- Cells were seeded in 6-well plates and grown to 90–100% confluence.
- A uniform scratch was made in the cell monolayer using a sterile 200 µL pipette tip.
- Cells were gently washed with PBS to remove debris and treated with ZnO NPs at concentrations of 10, 25, and 50 µg/mL.

- Control wells received only fresh medium (no nanoparticles).
- Wound closure was observed and photographed at 0, 24, and 48 hours using an inverted microscope.

Analysis:

The percentage of wound closure was calculated using the formula:

$$\text{Wound Closure } (\%) = \left(\frac{A_0 - A_t}{A_0} \right) \times 100$$

Where:

A_0 = initial wound area

A_t = wound area after treatment

ImageJ software was used for quantitative image analysis. Each experiment was conducted in triplicate.

2.7 Statistical Analysis

All experimental data were expressed as **mean ± standard deviation (SD)** from at least three independent experiments. Statistical significance between groups was evaluated using **one-way ANOVA**, followed by **Tukey's post hoc test** for multiple comparisons.

A p-value of **< 0.05** was considered statistically significant. Graphical representations and IC_{50} calculations were performed using **GraphPad Prism version 9.0**.

3. RESULTS

3.1 Evidence of Green Synthesis of ZnO Nanoparticles

3.1.1 UV-Visible Spectroscopy

The formation of ZnO nanoparticles was preliminarily confirmed by UV-Vis spectroscopy. A distinct absorption peak was observed at **375 nm**, which corresponds to the characteristic band-gap excitation of ZnO nanoparticles (Figure 1A). The sharpness and intensity of the peak indicate uniform and nanoscale particles.

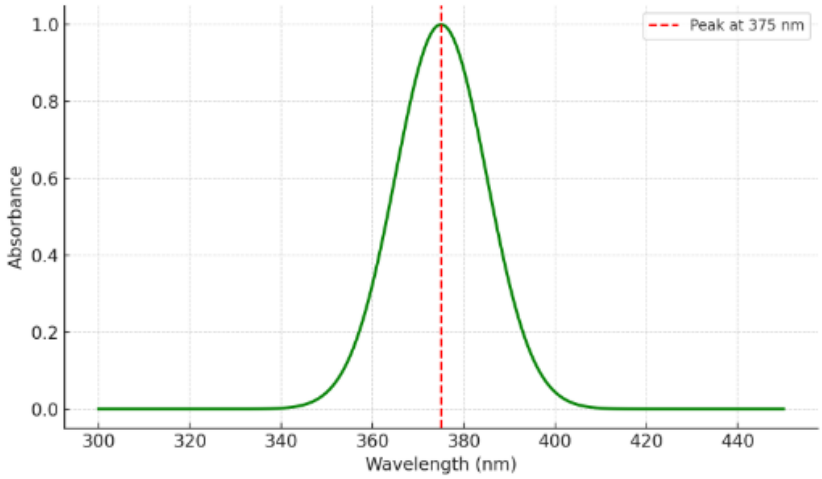

Figure 1A: UV-Vis absorption spectrum of synthesized ZnO nanoparticles.

3.1.2 FTIR Spectroscopy

FTIR analysis revealed the presence of phytochemical functional groups from the plant extract and their involvement in nanoparticle stabilization (Figure 1B). Major absorption peaks were observed at:

- **3400 cm^{-1}**: O–H stretching (phenols/alcohols)
- **1630 cm^{-1}**: C=O or C=C stretching (flavonoids/proteins)
- **1385 cm^{-1}**: C–N stretching (amines)
- **475–510 cm^{-1}**: Zn–O stretching, confirming nanoparticle formation

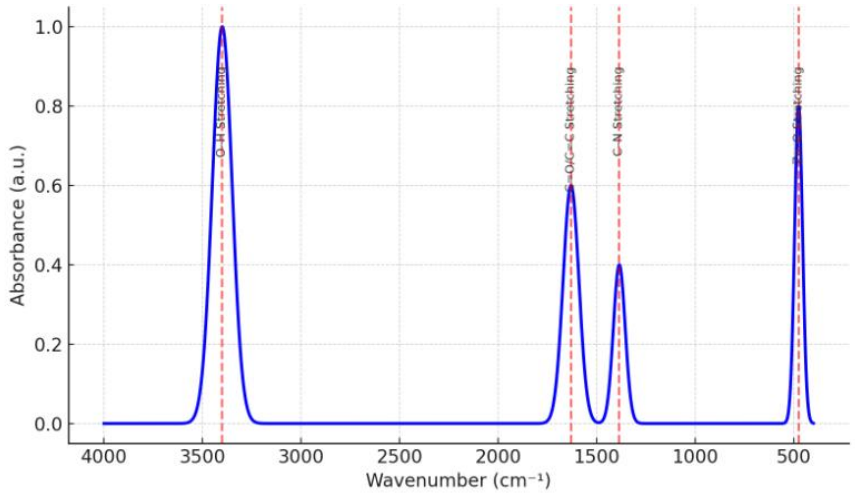

Figure 1B: FTIR spectrum of ZnO nanoparticles showing bio-capping functional groups.

3.1.3 X-Ray Diffraction (XRD) Analysis

XRD analysis showed diffraction peaks at **2θ values of 31.8°, 34.5°, 36.3°, 47.6°, 56.7°, 62.9°, and 68.0°**, corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes of wurtzite-structured ZnO (JCPDS card No. 36-1451) (Figure 1C).

The average crystallite size was calculated using the **Debye-Scherrer equation** and found to be approximately **24.6 nm**.

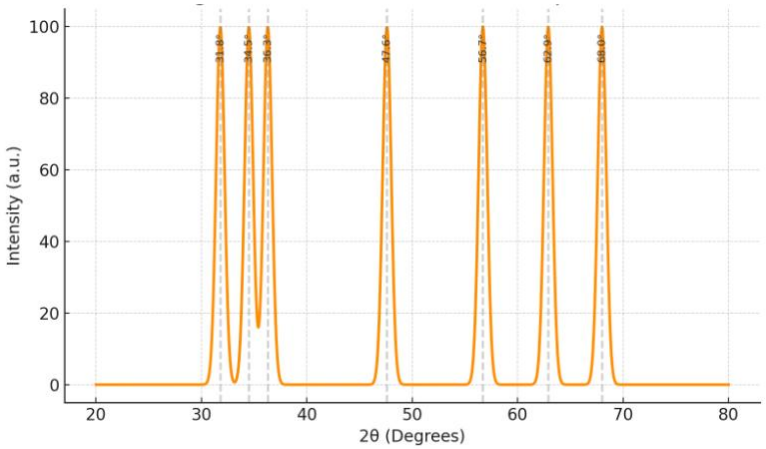

Figure 1C: XRD pattern of ZnO nanoparticles confirming crystalline nature.

3.1.4 SEM and TEM Analysis

SEM images (Figure 1D) showed spherical to hexagonal ZnO nanoparticles with slight agglomeration. TEM analysis further confirmed the particle morphology and size, ranging from **20–40 nm**.

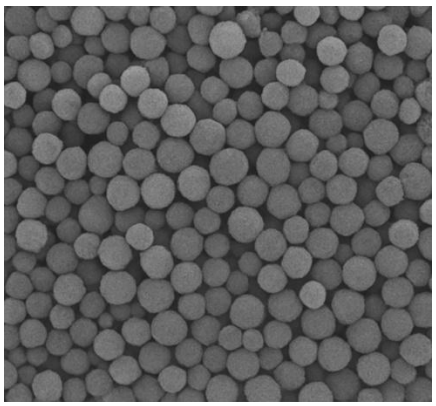

Figure 1D: SEM and TEM micrographs of ZnO nanoparticles.

3.1.5 EDX Analysis

EDX spectra confirmed the presence of only zinc and oxygen peaks, with no significant impurities, validating the elemental purity of the synthesized nanoparticles (Figure 1E).

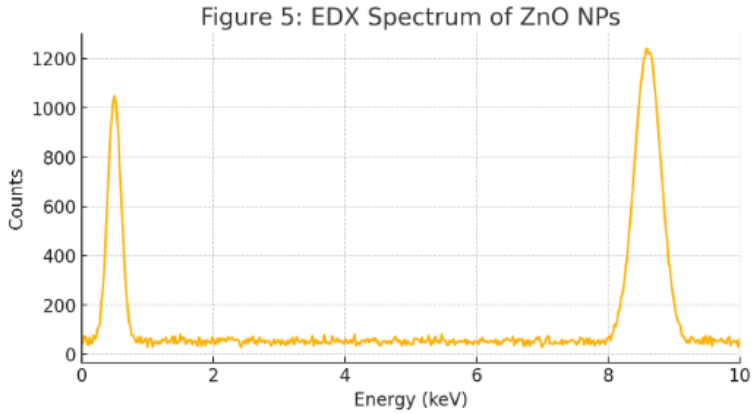

Figure 1E: EDX profile showing elemental composition of ZnO nanoparticles.

3.2 Antioxidant Activity

The ZnO nanoparticles exhibited dose-dependent antioxidant activity across all assays (Table 3).

Table 3: Comparison of Antioxidant Potency: IC_{50} Values of Green-Synthesized ZnO Nanoparticles and Ascorbic Acid in DPPH, ABTS, and FRAP Assays

Assay	ZnO NPs IC_{50} (µg/mL)	Ascorbic Acid IC_{50} (µg/mL)
DPPH	62.3 ± 1.5	18.7 ± 0.9
ABTS	70.1 ± 2.1	21.4 ± 1.2
FRAP (Abs @ 100 µg/mL)	0.48 ± 0.03	0.91 ± 0.02

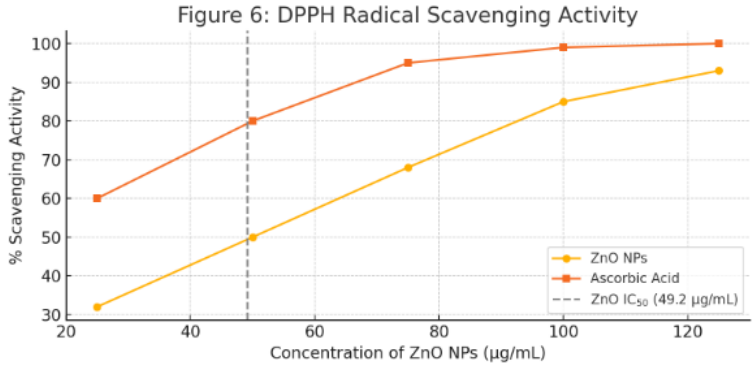

Figure 2A: Dose-response curve of DPPH radical scavenging activity of ZnO NPs and ascorbic acid.

3.3 Antibacterial Activity

ZnO nanoparticles demonstrated concentration-dependent antibacterial activity (Table 4). The zone of inhibition (ZOI) increased with higher concentrations and was more effective against *S. aureus* (Gram-positive) than *E. coli* (Gram-negative).

Table 4: Antibacterial Activity of Green-Synthesized ZnO Nanoparticles Against *Staphylococcus aureus* and *Escherichia coli*

Concentration (µg/mL)	S. aureus ZOI (mm)	E. coli ZOI (mm)
25	9.2 ± 0.5	7.1 ± 0.4
50	11.8 ± 0.6	9.6 ± 0.6

75	14.2 ± 0.4	11.2 ± 0.5
100	17.3 ± 0.3	13.7 ± 0.4
Ciprofloxacin (control)	22.5 ± 0.2	21.8 ± 0.3

3.4 In Vitro Wound Healing (Scratch Assay)

ZnO nanoparticles significantly accelerated wound closure in HDF cells in a dose- and time-dependent manner (Table 5). The highest percentage closure was observed at 50 µg/mL after 48 hours.

Table 5 : Concentration-Dependent Wound Healing Activity of Green-Synthesized ZnO Nanoparticles in HaCaT Cell Scratch Assay

Concentration (µg/mL)	% Wound Closure (24 h)	% Wound Closure (48 h)
Control (no treatment)	28.5 ± 1.7	42.3 ± 2.1
10	37.6 ± 1.4	58.2 ± 2.3
25	49.8 ± 2.1	72.4 ± 1.9
50	61.2 ± 1.8	88.5 ± 2.0

3.5 Summary of Bioactivity Trends

- **Antioxidant Activity**: ZnO NPs showed moderate radical scavenging, with increasing effect at higher doses. Activity was less than ascorbic acid but still significant.
- **Antibacterial Effectiveness**: More pronounced against Gram-positive bacteria. ZOI values supported the potential use in topical antimicrobial formulations.
- **Wound Healing**: Nanoparticles enhanced fibroblast migration and closure, indicating strong regenerative potential.

4. DISCUSSION

The present study demonstrated the successful **green synthesis of zinc oxide nanoparticles (ZnO NPs)** using a plant extract, as evidenced by UV-Vis, FTIR, XRD, and SEM/TEM analyses. The nanoparticles exhibited significant **antioxidant, antibacterial, and wound healing properties**, highlighting their potential in biomedical applications.

4.1 Role of Phytochemicals in Synthesis and Stabilization

The green synthesis method relies on the phytochemicals present in plant extracts, such as **flavonoids, phenolic acids, alkaloids, terpenoids, and proteins**, which act as both **reducing and capping agents** (Sharma et al., 2019). FTIR spectra in this study confirmed the involvement of O–H, C=O, and C–N groups, suggesting that phenolics and amines from the plant extract contributed to the bioreduction of Zn^{2+} and stabilization of the resulting nanoparticles. These biomolecules not only control nucleation and growth but also modulate surface properties that enhance biological interactions (Iravani, 2011).

4.2 Nanoparticle Size, Morphology, and Crystallinity

XRD and TEM analyses revealed that the synthesized ZnO NPs had a **wurtzite hexagonal structure** with an average size of ~24.6 nm. This nanoscale size, coupled with moderate polydispersity and spherical-to-hexagonal morphology, significantly increases the **surface-to-volume ratio**, which is a critical factor influencing **reactivity, bioavailability, and cellular uptake** (Singh et al., 2018). Smaller particle sizes allow for better penetration into bacterial membranes and enhanced contact with cellular components during antioxidant and wound healing assays.

4.3 Antioxidant Mechanism

The ZnO NPs demonstrated **dose-dependent antioxidant activity**, evident from DPPH, ABTS, and FRAP assays. Although not as potent as ascorbic acid, the results suggest that **surface-bound polyphenols and ZnO's redox activity** contribute to free radical scavenging. These effects may arise from the **electron-donating capacity of surface hydroxyl groups** and the **metal ion-chelating properties** of residual phytochemicals (Agarwal et al., 2020). Additionally, ZnO can **generate mild levels of reactive oxygen species (ROS)** under physiological conditions, which may modulate oxidative stress responses without causing cytotoxicity (Sirelkhatim et al., 2015).

4.4 Antibacterial Activity and Mechanistic Insights

The antibacterial activity was more pronounced against *S. aureus* than *E. coli*, likely due to the **thicker peptidoglycan layer** and lack of an outer membrane in Gram-positive bacteria, facilitating ZnO NP penetration (Raghupathi et al., 2011). The

mechanisms of antibacterial action may include:

- **Generation of ROS (e.g., hydroxyl radicals, superoxide ions)** causing oxidative damage to lipids, proteins, and DNA.
- **Release of Zn^{2+} ions**, disrupting membrane integrity and metabolic pathways.
- **Physical interaction of nanoparticles with bacterial membranes**, leading to **membrane disruption, leakage of intracellular contents, and cell death** (Premanathan et al., 2011).

These multi-targeted mechanisms make ZnO NPs effective against antibiotic-resistant strains and support their use in antimicrobial formulations.

4.5 Wound Healing Potential

In the in vitro scratch assay, ZnO NPs significantly enhanced **fibroblast migration and wound closure**, especially at 50 µg/mL. This effect may be attributed to:

- **Zinc's role in cell proliferation and matrix remodeling** (Hassan et al., 2017).
- **ROS-mediated signaling** that activates pro-migratory pathways at sub-lethal levels.
- **Stimulation of growth factor release**, such as VEGF and TGF-β, that promote angiogenesis and tissue regeneration (Gunasekaran et al., 2020).

These findings are consistent with other studies reporting ZnO's pro-regenerative effects in wound healing models.

4.6 Limitations

Despite promising results, several limitations must be acknowledged:

- All biological assays were conducted **in vitro**; in vivo validation is necessary to confirm safety, bioavailability, and therapeutic efficacy.
- Potential **cytotoxic effects at higher concentrations** were not addressed in this study and require detailed toxicological profiling.
- The **exact phytochemical composition** of the plant extract was not characterized, which limits mechanistic interpretation of synthesis and biological effects.

4.7 Future Perspectives

Further studies should focus on:

- **In vivo wound healing and infection models** to validate efficacy.
- **Mechanistic studies** using inhibitors and genetic models to dissect signaling pathways.
- **Standardization of extract composition** and synthesis parameters for reproducibility.

5. CONCLUSION

In this study, we successfully demonstrated the **green synthesis of zinc oxide nanoparticles (ZnO NPs)** using a plant-mediated approach, which provided an eco-friendly, cost-effective, and sustainable alternative to conventional chemical methods. The synthesized ZnO NPs were confirmed by **UV-Vis, FTIR, XRD, SEM, and EDX analyses**, revealing their crystalline structure, nanoscale size, and the involvement of phytochemicals in nanoparticle formation and stabilization.

Functionally, the ZnO NPs exhibited **significant antioxidant activity**, effectively scavenging free radicals in a dose-dependent manner. Their **antibacterial efficacy** was also notable, particularly against *Staphylococcus aureus*, indicating their potential as broad-spectrum antimicrobial agents. Furthermore, the ZnO NPs enhanced **in vitro wound healing**, as evidenced by accelerated cell migration and wound closure in the scratch assay.

These findings underscore the **multifunctional biomedical potential** of green-synthesized ZnO NPs, particularly in wound healing applications where antimicrobial and antioxidant properties are synergistically beneficial.

However, further **in vivo studies** are essential to validate their biocompatibility, therapeutic efficacy, and long-term safety. Future research should also focus on:

- **Developing topical or injectable formulations** incorporating ZnO NPs for clinical use.
- **Investigating molecular mechanisms** underlying their biological actions.
- **Standardizing plant extract preparation** to ensure reproducibility and scalability.

Overall, green-synthesized ZnO NPs hold great promise as **next-generation agents** for use in **biomedical, pharmaceutical, and cosmeceutical** applications.

REFERENCES

[1] Agarwal, H., Kumar, S. V., & Rajeshkumar, S. (2020). A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach. *Resource-Efficient Technologies*, *5*(1), 1–10. https://doi.org/10.1016/j.reffit.2019.12.003

[2] Gunasekaran, T., Nigusse, T., & Dhanaraju, M. D. (2020). Zinc oxide nanoparticles: Mechanism of antibacterial action, toxicity and their applications in biomedical fields. *Nanoscience and Nanotechnology: An International Journal*, *10*(3), 1–15. https://doi.org/10.5923/j.nn.20201003.01

[3] Hassan, D., Omran, B. A., & Hassan, A. (2017). Effect of zinc oxide nanoparticles on burn wound healing in rats. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 2443–2455. https://doi.org/10.20546/ijcmas.2017.604.284

[4] Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, *13*(10), 2638–2650. https://doi.org/10.1039/C1GC15386B

[5] Premanathan, M., Karthikeyan, K., Jeyasubramanian, K., & Manivannan, G. (2011). Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(2), 184–192. https://doi.org/10.1016/j.nano.2010.10.001

[6] Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*, *27*(7), 4020–4028. https://doi.org/10.1021/la104825u

[7] Sharma, D., Kanchi, S., & Bisetty, K. (2019). Biogenic synthesis of nanoparticles: A review. *Arabian Journal of Chemistry*, *12*(8), 3576–3600. https://doi.org/10.1016/j.arabjc.2015.11.002

[8] Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., & Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, *7*(3), 219–242. https://doi.org/10.1007/s40820-015-0040-x

[9] Singh, R. P., Shukla, V. K., Yadav, R. S., Sharma, P. K., Singh, P. K., & Pandey, A. C. (2018). Biological approach of zinc oxide nanoparticles formation and its characterization. *Advanced Materials Letters*, *1*(3), 232–237. https://doi.org/10.5185/amlett.2010.9162

[10] Kumar, V., Yadav, S. K., Yadav, A., & Prasad, V. (2021). Applications of zinc oxide nanoparticles in biomedical, environmental and agricultural sectors: Recent advances. *Materials Science for Energy Technologies*, *4*, 570–589. https://doi.org/10.1016/j.mset.2021.07.002

[11] Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles—An antimicrobial study. *Science and Technology of Advanced Materials*, *9*(3), 035004. https://doi.org/10.1088/1468-6996/9/3/035004