VOLUME 12 ISSUE 1 2026

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 12, No. 1, 13-20 (2026)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

In Vitro Antimicrobial and Antioxidant Abilities of the *Phyllanthus emblica* Seed Mediated Zincoxide Nanoparticles

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Received: 8th September, 2025; **Accepted:** 22nd December, 2025; **Published online:** 4th January, 2026

https://doi.org/10.33745/ijzi.2026.v12i01.002

Abstract: The current work is focussed on green synthesized ZnO NPs using Indian gooseberry *Phyllanthus emblica* seeds. Green synthesis, characterization, *in vitro* antioxidants and antimicrobial parameters were focussed. The band gap energies, active component, shape, and surface charge of the green synthesized ZnO NPs were determined by UV, FTIR, DLS, and SEM analyses. Significant growth inhibition actions on gram positive, gram negative, and fungal species were found in the antimicrobial data. The evaluated ZnO NPs' reducing and scavenging properties were also demonstrated by antioxidant investigations. Our research revealed that green synthesized ZnO NPs based on *P. emblica* seeds produced more suitable outcomes and can be utilized as a traditional substitute source for nanoparticles than other hazardous chemicals.

Keywords: *Phyllanthus emblica*, ZnO NPs, SEM, Antioxidant, Antimicrobial

Citation: Manoranjitham M., Ravikumar R., Velmurugan S. and Suganthi P.: *In vitro* antimicrobial and antioxidant abilities of the *Phyllanthus emblica* seed mediated zincoxide nanoparticles. Intern. J. Zool. Invest. 12(1): 13-20, 2026.

https://doi.org/10.33745/ijzi.2026.v12i01.002

Introduction

Nanoparticles have gained great importance because of their small size (1 to 100 nm) and strong surface reactivity. Nanomaterials have special properties like high surface and volume ratios (Geonmonond *et al.*, 2018). According to Sobha *et al.* (2010), nanobiotechnology is a recently developed field that focuses on enhanced biotechnological methods for the synthesis of nanoparticles from a variety of substrates, including microbes and plants. These days, green synthesized nanoparticles made from plant products are replacing bulk materials and chemically synthesized nanoparticles (Zhong *et al.*, 2018). Green nanoparticles (NPs) are the safe materials with elite features and also considered as an eco-friendly, biocompatible, bio-safe, cost effective and less hazardous chemicals (Rosi and Mirkin, 2005; Raghupathi *et al.*, 2011; Sabir *et al.*, 2014). For the purpose of creating nanoparticles and using them in various application studies, the majority of researchers concentrated on a variety of plants and plant parts.

Zinc oxide (ZnO) nanoparticles are a type of metallic oxide compound that exhibits a variety of remarkable properties, including photocatalysis, optical, electrical, magnetic, and piezoelectric sensors (Dagdeviren *et al.*, 2003; Teodore, 2006; Wang *et al.*, 2008), as well as antimicrobial and anticorrosive properties (Okitsu *et al.*, 2007; Siyanbola *et al.*, 2013). These days, ZnO NPs are a promising material with a wide range of uses, including medication delivery systems, gene guns, anti-inflammatory, antioxidant, anti-cancerous, and wound healing. These are employed in the rubber, ceramic, pharmaceutical, and environmental remediation processes (Iravani, 2011; Duan *et al.*, 2015; Bala *et al.*, 2015). Thus, green synthesized ZnO NPs are ultimately thought to be a quick, efficient, and environmentally benign method (Gunalana *et al.*, 2012).

Phyllanthus emblica, or Indian gooseberry, is one of the main fruits used in medicine. In Asian locations, the *P. emblica* plant species is widely distributed. The fields of Unani and Ayurveda medicine are the main uses for the *P. emblica* fruit. Fruit has the ability to chill the body when it becomes hot, and it contains a lot of vitamin C (Dinesh *et al.*, 2017). The fruits' therapeutic and nutritional value led to their enormous significance in indigenous traditional medical systems such as Ayurveda, Siddha, etc. It is also used for Hepatopathy, anemia, diarrhea, jaundice, hemorrhages, fever, menorrhagia, leucorrhea, common cold, cardiac disorders, cough, bronchitis, asthma, cephalalgia, diabetes, hyperacidity, dyspepsia, erysipelas, dermal diseases and peptic ulcers treatments (Saini *et al.*, 2022).

Green synthesis of zinc oxide nanoparticles based on *Phyllanthus emblica* seeds are the study's main goal. The size and shape of the ZnO NPs are characterized by FTIR, UV, DLS, and SEM. The ZnONP *in vitro* antimicrobial and antioxidant properties were also assessed.

Materials and Methods

Amla or *Phyllanthus emblica* plant berries were collected from Raja Herbal Garden (Indira Nagar, Tiruchirappalli, India) and authenticated. In a sample bag, *P. emblica* green berries transported to the research laboratory (KIRND, Tiruchirappalli) and washed with tap water and then with distilled water. Berries were spread in the tray and the fleshy (mesocarp) parts were removed with the help of new scalpel. Endocarp part which encases the seeds were collected. Finally the seeds were collected from stony endocarp and air dried in the laboratory for the next 48 h. Then, the dried seeds were grounded and sieved. The seed powder was used for further analysis. Based on the phytochemical screening and bioactive compound analysis of *P. emblica* seeds (results not included here), the seed extracts were used for ZnO nanoparticle synthesis.

Synthesis of green synthesized ZnO NPs

Zinc hydroxide (Sigma Aldrich Chemicals Ltd., Mumbai, India) and Ethanol (Spectrochem Research Laboratories, Mumbai, India) were purchased. By downstream process, the zinc oxide nanoparticles were synthesised.

Characterization of green synthesized ZnO NPs

The functional or active groups and bandgap of the investigated substances can be identified using UV spectroscopy (Shimadzu UV-2450) and FTIR (FTIR SHIMADZU 8400) based on potassium bromide pellets, which is crucial for molecular analysis. Synthesized chemical structure are anticipated by Gemini 500 (Zeiss) field emission scanning electron microscopy (SEM) combined with DLS.

Antimicrobial activity

Klebsiella pneumonia, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus*

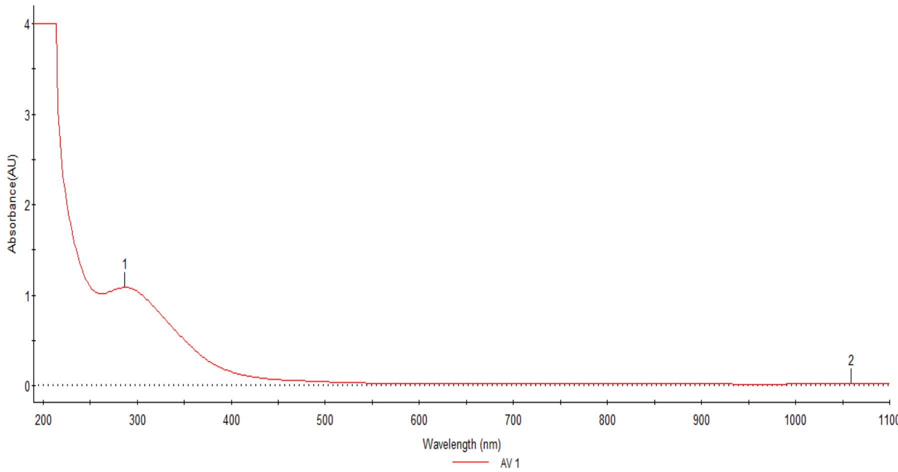

Fig. 1: Green synthesized ZnO nanoparticles - UV spectra.

niger were used for the antimicrobial assay. *Phyllanthus emblica*-based green synthesized ZnO NPs were evaluated for antimicrobial activity utilizing the agar well diffusion method (Rajasekar *et al.*, 2013). By using the spreading plate method, nutrient agar medium was made, placed onto the petriplates, and then infected with the appropriate microorganisms (concentration 10^4 cells/ml). Four wells were prepared in each plate. Three wells were filled with three distinct concentrations of green synthesized ZnO NPs (50, 75, and 100µg/ml); and one well with a positive control (fungi: clotrimazole; bacteria: tetracycline). The inhibitory zone was measured (in mm) after the plates were incubated for 24 h at 37°C.

Antioxidant activity

By using the *in vitro* DPPH, hydroxyl reducing power, and hydrogen peroxide assay protocols described by Anzabi (2018), Jacob and Rajiv (2019), and Gocer *et al.* (2012), respectively, the antioxidant capabilities of the green synthesized ZnO nanoparticles were investigated. The solution's absorbance was measured by a spectrophotometer (Shimadzu UV-2450) at 517 nm.

Statistical analysis

SPSS (7.0 version) and Duncan post hoc testing was used to compare the groups' mean±SD values and find the least significant differences. Regression analysis was used for determining the IC_{50} values.

Results and Discussion

During green synthesis of ZnO NPs, the chemical colour changed into yellowish white precipitate which represented the reduction of reactants (Zinc nitrate) into the product ZnO nanoparticles. Various herbal plants mediated ZnO NPs synthesis were reported by Rathika *et al.* (2023) and Jagajothi *et al.* (2024).

Characterization of P. emblica seed mediated ZnONPs

Green synthesized ZnO NPs UV spectrum is ranged between 200 to 1100 nm. Two peaks were observed at 287.30 and 1059.20 nm (Fig. 1) and their respective absorbance were 1.087 and 0.021. The direct band gap energies are measured as 4.32 and 1.17eV, respectively. UV spectral analysis (Fig. 2) revealed the presence of alkyne (C-H), (C=C), alkane (CH_3) bending and C-I for their respective peaks at 3397, 1628, 1384 and 569cm^{-1}, respectively. SEM analysis revealed the aggregated hexagonal particles and DLS also proved the size of the ZnO NPs as ~30nm (Figs. 3, 4). Similar green synthesized ZnO NPs characterization results are evidenced by Vahidi *et al.* (2009), Chikkanna *et al.* (2019) and Mahvash *et al.* (2020).

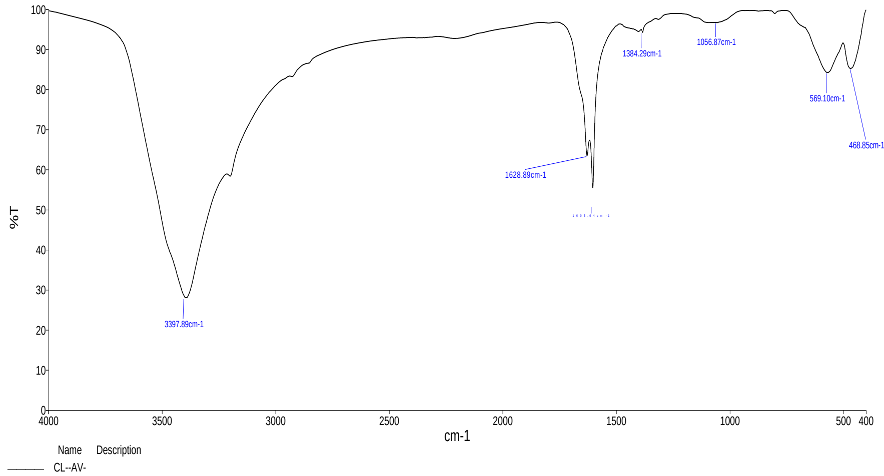

Fig. 2: FTIR spectrum of green synthesized ZnO NPs.

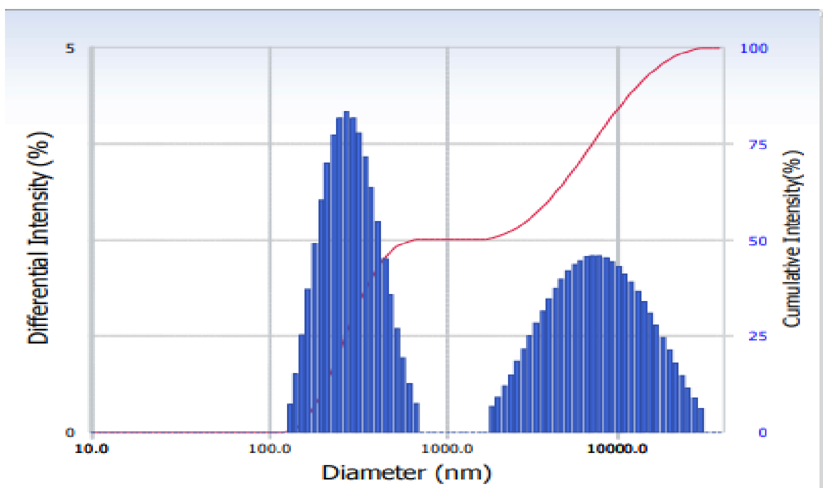

Fig. 3: Green synthesized ZnONPs DLS analysis for intensity distribution.

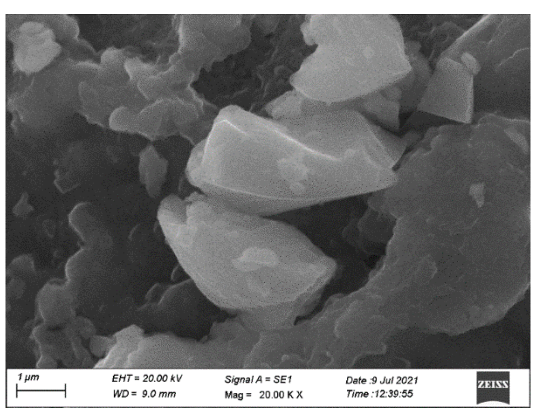

Fig. 4: Scanning electron microscopic image of green synthesized ZnO NPs.

Table 1: Antimicrobial activity of green synthesized ZnO nanoparticles

Microbes	Green synthesized ZnO NPs			Control
	50 µg/ml	**75 µg/ml**	**100 µg/ml**	**200 µg/ml**
Aspergillus niger	17.06±0.03	16.03±0.01	15.05±0.01	16.09±0.01
Escherichia coli	15.02±0.02	17.09±0.03	17.09±0.07	16.08±0.04
Klebsiella pneumonia	15.05±0.03	16.10±0.02	16.03±0.04	16.02±0.06
Bacillus subtilis	18.05±0.04	15.03±0.01	17.06±0.05	17.03±0.03
Staphylococcus aureus	17.01±0.06	16.02±0.01	16.05±0.04	15.05±0.02

Control: Tetracycline (Bacteria); Clotrimazole (Fungi)

Table 2: Antioxidant studies of *P. emblica* seed mediated ZnO nanoparticles

ZnO NPs Concentrations (µg/ml)	Percentage of inhibition			
	DPPH	**Hydroxyl scavenging**	**FRAP**	**H_2O_2**
0.2	61.57±2.46	34.68±1.78	0.13±0.09	5.68±0.25
0.4	70.89±1.68	45.78±1.21	0.19±0.05	14.67±1.35
0.6	75.80±1.97	52.36±1.46	0.25±0.07	26.78±2.01
0.8	79.90±1.58	56.78±1.85	0.32±0.08	47.89±3.58
1.0	82.57±1.78	67.85±1.64	0.56±0.07	50.91±2.11

Antimicrobial activities of Phyllanthus emblica based green synthesized ZnO NPs

Treated groups showed significant results against the microbes at all the concentrations (Table 1). The maximum inhibition zone was observed against gram positive *Bacillus subtilis* (18.05±0.04 mm) treated with 50 µg/ml green synthesized ZnO NPs. ZnO NPs treated group growth inhibition results are similar to their respective positive control growth inhibition. Plant compound mediated nanoparticles contain numerous bioactive compounds which acts as capping and stabilizing agents for nanoparticles and also acts as capping and reducing agents against various gram positive and negative bacteria (Talbot *et al.*, 2006; Mohamed and Akladious 2017; Rehman *et al.*, 2017; Rajeshkumar *et al.*, 2019; Sergiev *et al.*, 2019). Green synthesized ZnO NPs showed significant antimicrobial activities due to the activation of reactive oxygen species by hydrogen peroxide, hydroxyl radial and superoxide anion development in the microbial system (Rasmussen *et al.*, 2010; Xiong *et al.*, 2013).

Antioxidant activities of green synthesized ZnO NPs

Based on the absorbance of the control and tested samples, the inhibitory concentration and its significant antioxidant potential of *Phyllanthus emblica* seed based green synthesized ZnONPs was evaluated by DPPH radical scavenging assay having IC_{50} 2.018 µg/ml (Table 2). Similarly, IC_{50} values for hydroxyl, FRAP and hydrogen peroxide assay are evaluated as 0.259, 2.812 and 0.624 µg/ml, respectively.The antioxidant activities of the tested compound are based on their reducing properties (Taylor *et al.*, 1997). In general, increased ROS species by various plant metabolic pathways as well as chemically synthesized NPs leads to mutations and lethality to cells (Yen *et al.*, 1993; Halliwel and Gutteridge, 1999) whereas *Phyllanthus emblica* based green ZnO NPs showed significantly increased antioxidant levels than the control.

In microbial cytoplasm, ROS production resulted the gradient variation which leads to the increased membrane permeability, genomic instability and cytotoxicity (Dutta *et al.*, 2013; Shi *et al.*, 2014; Jiang *et al.*, 2016). Due to the increased microbial resistance towards the drugs, the identification of enhanced and improved antimicrobial agents is essential for the present situation (Romero *et al.*, 2005; Boucher *et al.*, 2009).

P. emblica ability to scavenge free radicals was tested using the DPPH radical scavenging assay (Suttijit, 2020). The results showed that the extracts from the fruit and seeds had strong antioxidant properties towards oxygen radicals and the maximum antioxidant activity was obtained at the highest dose of 10 mg/ml. The results of the bioactivities indicate that the *Phyllanthus emblica* seed has the ability to take the position of the fruit in terms of medical benefits, therefore increasing the plant's value (Suttijit, 2020).

Green synthesis of ZnO NPs utilizing *Phyllanthus emblica* extract was reported by Vala *et al.* (2023). The XRD data validate the pure hexagonal wurtzite structure. The production of quasi-spherical NPs is confirmed by FESEM. The purity of ZnO NPs is revealed by the EDAX analysis. FTIR results proved that the NPs contain Zn-O stretching vibration. ZnONPs optical response is displayed by the UV-vis curve, and their optical bandgap is 3.29 eV.

Anbukkarasi *et al.* (2015) investigated the antibacterial properties of ZnO nanoparticles from *Emblica officinalis*. Six bacterial pathogens—*Bacillus subtilis*, *Streptococcus pneumonia*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Salmonella typhi*, and *Escherichia coli* as well as two fungal pathogens *Aspergillus niger* and *Candida albicans* were tested for antimicrobial activity using the agar well diffusion method. Both the minimum bactericidal concentration (MBC) and the minimal inhibitory concentration (MIC) against certain pathogens were assessed. ZnONPS had the highest antibacterial activity against *S. epidermidis* (35 mm) with the lowest MIC and MBC values (7.81 µg/ml), followed by *E. coli*.

Palladium nanoparticles (PdNPs) were synthesized in an environmentally friendly manner by employing *P. emblica* seeds as a reducing agent (Murugesan *et al.*, 2017). The metallic PdNPs were tested using a range of analytical methods, including FT-IR, UV-Vis, XRD, Zeta potential, SEM, and TEM. According to the findings, the green PdNPs that were synthesized had a spherical shape and an average particle size of 28 ± 2 nm, with a moderate stability (Murugesan *et al.*, 2017).. Additionally, using the agar well diffusion method, the produced PdNPs and extract were tested for their antibacterial properties against a range of disease-causing microorganisms. The results against *B. subtilis* and *S. aureus* were 8.9 ± 1.46 mm and 9.6 ± 1.10 mm for the seed extract and PdNPs, respectively (Murugesan *et al.*, 2017).

Conclusion

This study is focused about the green synthesis of ZnO NPs from Indian gooseberry *Phyllanthus emblica* seed extract which is an eco-friendly compound and used as a conventional and promising compound for the toxic chemical based nanoparticles. Synthesized ZnO NPs characterization results revealed the nanoparticle size, morphology, band gap energies and surface charges. *In vitro* antimicrobial and antioxidant studies also revealed the compatibility and efficiency of *P. emblica* seed based green synthesized ZnO NPs.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the KIRND Institute of Research and Development Pvt. Ltd., Tiruchirappalli 620020, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors are thankful to the authorities at PG and Research Department of Botany, Alagappa

Government Arts College, Kariakudi, India for their support during this study.

Conflict of Interest

The authors declare no conflicts of interest.

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