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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *Catharanthus roseus* PLANT EXTRACT AND ITS ANTIMICROBIAL ACTIVITY AGAINST FOOD-BORNE PATHOGENS

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ABSTRACT

Green synthesis of nanoparticles using plant extracts has emerged as an eco-friendly alternative to conventional chemical methods. In the present study, zinc oxide nanoparticles (ZnO-NPs) were synthesized using aqueous leaf and flower extracts of Catharanthus roseus. The synthesized nanoparticles were characterized using UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and SDS-PAGE analysis. UV-Visible analysis showed characteristic absorption peaks at 345 nm and 356 nm for leaf- and flower-mediated ZnO-NPs, respectively, confirming nanoparticle formation. FTIR analysis revealed the presence of hydroxyl, carbonyl, and alkyl functional groups involved in the reduction and stabilization of nanoparticles. SEM analysis demonstrated the formation of rod- and cuboid-shaped ZnO-NPs. SDS-PAGE analysis indicated the presence of proteinaceous biomolecules that may contribute to nanoparticle synthesis and stabilization. The antimicrobial activity of the synthesized nanoparticles was evaluated against Escherichia coli, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, and Fusarium spp. ZnO-NPs exhibited enhanced antimicrobial activity compared with crude plant extracts. Flower-derived ZnO-NPs demonstrated the highest antibacterial activity against Escherichia coli, producing 62.14% bacterial cell death at 30 µL concentration during MIC analysis, which may be attributed to reactive oxygen species generation and membrane disruption. The results indicate that Catharanthus roseus-mediated ZnO-NPs possess promising antimicrobial potential and may serve as eco-friendly nanomaterials for biomedical and food-related applications.

INTRODUCTION

Nanotechnology represents a pivotal interdisciplinary field spanning biology, chemistry, physics, and materials science. Due to their unique structural, optical, and electronic properties—which differ significantly from both individual molecules and bulk solids—nanoparticles (NPs) find extensive application in medicine, electronics, therapeutics, and diagnostics (Thangadurai et al., 2020) . Recent advancements in the precise fabrication of ordered NPs have catalysed the development of novel biocidal agents, leading some to characterize nanomaterials as a cornerstone of modern biomedical innovation (Gunalan et al., 2012). The interaction between NPs and biological systems establishes complex interfaces governed by colloidal forces and dynamic biophysiochemical interactions, which dictate the resulting material's surface chemistry, roughness, and coating (Nel et al., 2009).

Recently, metal oxide NPs have gained prominence as bactericidal agents, drug delivery vehicles, and tools for the targeted destruction of carcinogenic cells (Stoimenov et al., 2002). While various physicochemical synthesis methods exist, they often entail environmental drawbacks. Consequently, research has shifted toward eco-friendly, "green" synthesis protocols to ensure environmental sustainability(Khan, 2020). Among these, zinc oxide nanoparticles (ZnO-NPs) are particularly noteworthy due to their applications in optics, magnetism, gas sensing, and wastewater treatment(Kołodziejczak-Radzimska & Jesionowski, 2014).

Numerous studies have reported the green synthesis of ZnO-NPs using plant extracts, including the leaves of *Coriandrum sativum*, *Calotropis gigantea*, and *Acalypha indica*, as well as the latex of *Calotropis procera* (Akhter et al., 2018). Furthermore, quasi-spherical ZnO-NPs have been synthesized using *Agathosma betulina* (12–26 nm) and *Aloe vera* (Ahmed et al., 2016).

Catharanthus roseus (family Apocynaceae), commonly known as Madagascar periwinkle, is a renowned medicinal plant(Singh et al., 2001). Traditionally used to manage diabetes and hypertension, its modern clinical utility stems from chemotherapeutic alkaloids used in cancer treatment and pain management (Adeyemi et al., 2002).While the isolation of secondary metabolites from *C. roseus* is well-documented, its application in nanometal synthesis remains sparse(Jaleel et al., 2007). Existing reports on *C. roseus*-mediated ZnO-NP synthesis are limited and the resulting

knowledge base remains fragmented(Bhumi & Savithramma, 2014).

Biosynthesized ZnO-NPs exhibit multifunctional behaviours and potent antibacterial activity even at low concentrations (Dizaj et al., 2014). Their inherent biocompatibility makes them ideal candidates for biomedical and food-safety applications, particularly as cost-effective alternatives to conventional antibiotics in controlling resistant pathogens (Jin et al., 2009). Although Catharanthus roseus is well known for its medicinal properties, comparative studies evaluating leaf- and flower-mediated ZnO nanoparticle synthesis together with comprehensive antimicrobial profiling against food-borne pathogens remain limited. Therefore, the present study aimed to synthesize ZnO nanoparticles using both leaf and flower extracts of C. roseus, characterize the synthesized nanoparticles, and compare their antimicrobial activity against selected food-borne pathogens.

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh leaves and flowers of *Catharanthus roseus* were collected from Coimbatore, Tamil Nadu, India. The botanical samples were thoroughly washed, sectioned, and shade-dried. The dried material was pulverized into a fine powder. To prepare the aqueous extract, 2 g of the powder was suspended in 250 mL beakers containing 20 mL of double-distilled water. The mixture was incubated at 40°C and agitated at 70–80 rpm for 24 hours. The resulting solution was filtered through Whatman No. 1 filter paper and stored at 4°C for subsequent experimental use. (Gupta et al., 2018).

Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs)

For the synthesis, 11.84 g of zinc nitrate $Zn(NO_3)_2$ was dissolved in 40 mL of distilled water. A 5 mL aliquot of the *C. roseus* extract was added to 20 mL of the zinc nitrate solution. The mixture was subjected to continuous magnetic stirring for 3 hours. During this process, 1 M sodium hydroxide (NaOH) was added dropwise until a pale yellow precipitate formed. The solution was then incubated in the dark for 24 hours to allow complete crystallization. The resulting ZnO-NPs were collected and stored for characterization (Anvekar et al., 2017).

Characterization Techniques

The bio reduction of zinc ions was monitored using UV-Visible spectroscopy (Labotronics LT 291) within a scanning range of 200–400 nm. Functional group analysis was performed via Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu). The samples were prepared using the KBr pellet method (10 mg KBr) and recorded in the region of 500–4000 cm^{-1}. Morphological analysis was conducted using Scanning Electron Microscopy (SEM, ZEISS), where a thin film of the sample was deposited onto a carbon-coated copper grid and air-dried prior to imaging (Gupta et al., 2018)

Analysis of Bioreductase (SDS-PAGE)

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to identify potential bioreductase enzymes in the *C. roseus* extract responsible for NP stabilization. Purified samples (20 µL) were mixed with 10 µL of 2X loading dye and denatured at 90°C for 10 minutes. Electrophoresis was performed at 50V for 45 minutes. Following staining and destaining, protein bands were visualized to estimate molecular weights against a standard protein marker (Gupta et al., 2018).

Antibacterial Assay

The antibacterial efficacy of the synthesized ZnO-NPs was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method on Nutrient Agar (NA). The media was sterilized at 121°C (15 lbs) for 20 minutes. Bacterial inoculum was swabbed onto the plates, and wells were loaded with the ZnO-NPs. Gold nanoparticles were utilized as a comparative control. After incubation at 37°C for 24 hours, the zones of inhibition (ZOI) were measured in millimetres (Zarrindokht Emami-Karvani, 2012).

Antifungal Assay

Antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium* spp. was assessed using Malt Extract Agar (MEA). Ampicillin was added to the medium to suppress bacterial contaminants. The plates were inoculated via the swabbing method, and wells were treated with the extracts and ZnO-NPs. The plates were sealed and incubated for 72 hours, after which the ZOI was recorded (Jamdagni et al., 2018).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the 96-well microtiter plate method. *E. coli* culture (100 µL) served as the control. ZnO-NPs synthesized from both leaf and flower extracts were added in varying volumes (10, 20, and 30 µL). Following a 24-hour incubation, the optical density (OD) was measured using an ELISA plate reader to quantify growth inhibition (Liu et al., 2009). Statistical analysis was performed using Microsoft Excel. All experiments were carried out in triplicate (n = 3), and the results are presented as mean values.

Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean values. Data were tabulated and analysed using Microsoft Excel.

RESULTS

UV-Visible Spectroscopy

In this study, C. roseus served as an efficient medium for the green synthesis of ZnO-NPs. The absorption peaks were observed within the 200–400 nm range. During biosynthesis, zinc ions were reduced by the leaf and flower extracts, which are rich in secondary metabolites such as vincristine, vinblastine, and serpentine. These metabolites likely act as reducing, stabilizing, and capping agents. The extracts provided distinct surface plasmon resonance (SPR) bands at 345 nm (leaf) and 356 nm (flower), as shown in Figure 1. The reduction process is governed by the excitation of surface plasmon vibrations of the metallic NPs (Gupta et al., 2018)

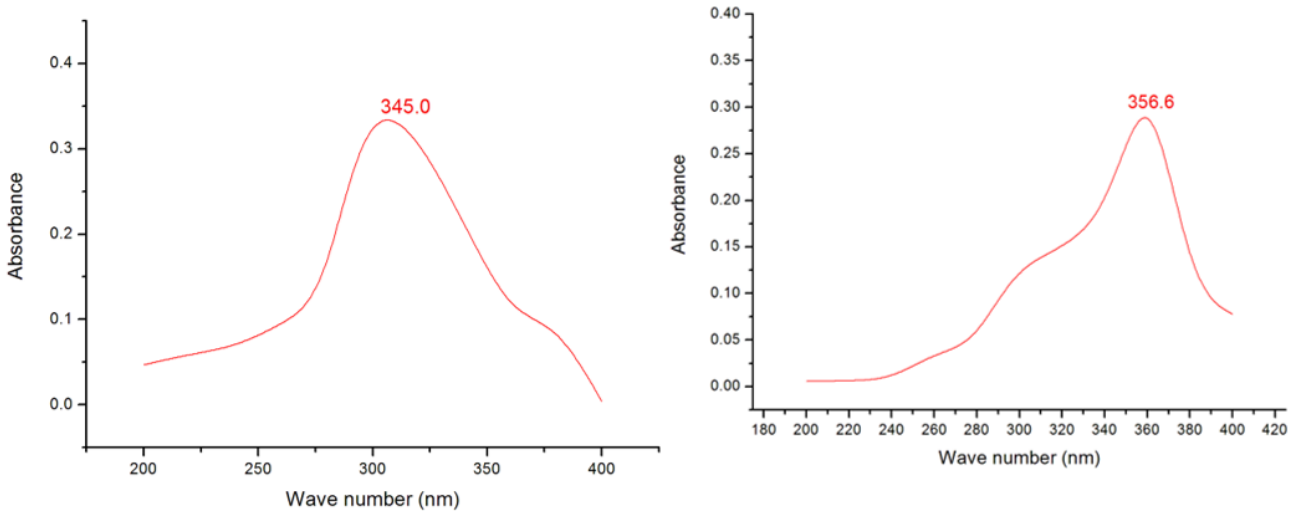

Fig. 1: The surface plasmon resonance bands of ZnO-NPs synthesized from leaf and flower of *Catharanthus roseus* plant.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum (Figure 2) identified the functional groups involved in the reduction and stabilization of ZnO-NPs. Broad peaks at 3278.99 cm^{-1} are assigned to O-H stretching or alkenyl C-H stretches. Peaks at 2943.37 and 2908.65 cm^{-1} correspond to C-H stretching vibrations of alkyl groups (Jayachandran et al., 2021a). The absorption at 1724.36 cm^{-1} indicates C=O stretching, while the range near 1427.32 cm^{-1} and 1323.17 cm^{-1} suggests the presence of nitro (NO_2) groups or aromatic ring vibrations (Rastogi & Arunachalam, 2011). The fingerprint region (921–640 cm^{-1}) and the intense peaks between 432–597 cm^{-1} confirm the formation of the Zn-O bond, consistent with the reduction of zinc nitrate salts into ZnO-NPs by *C. roseus* metabolites.

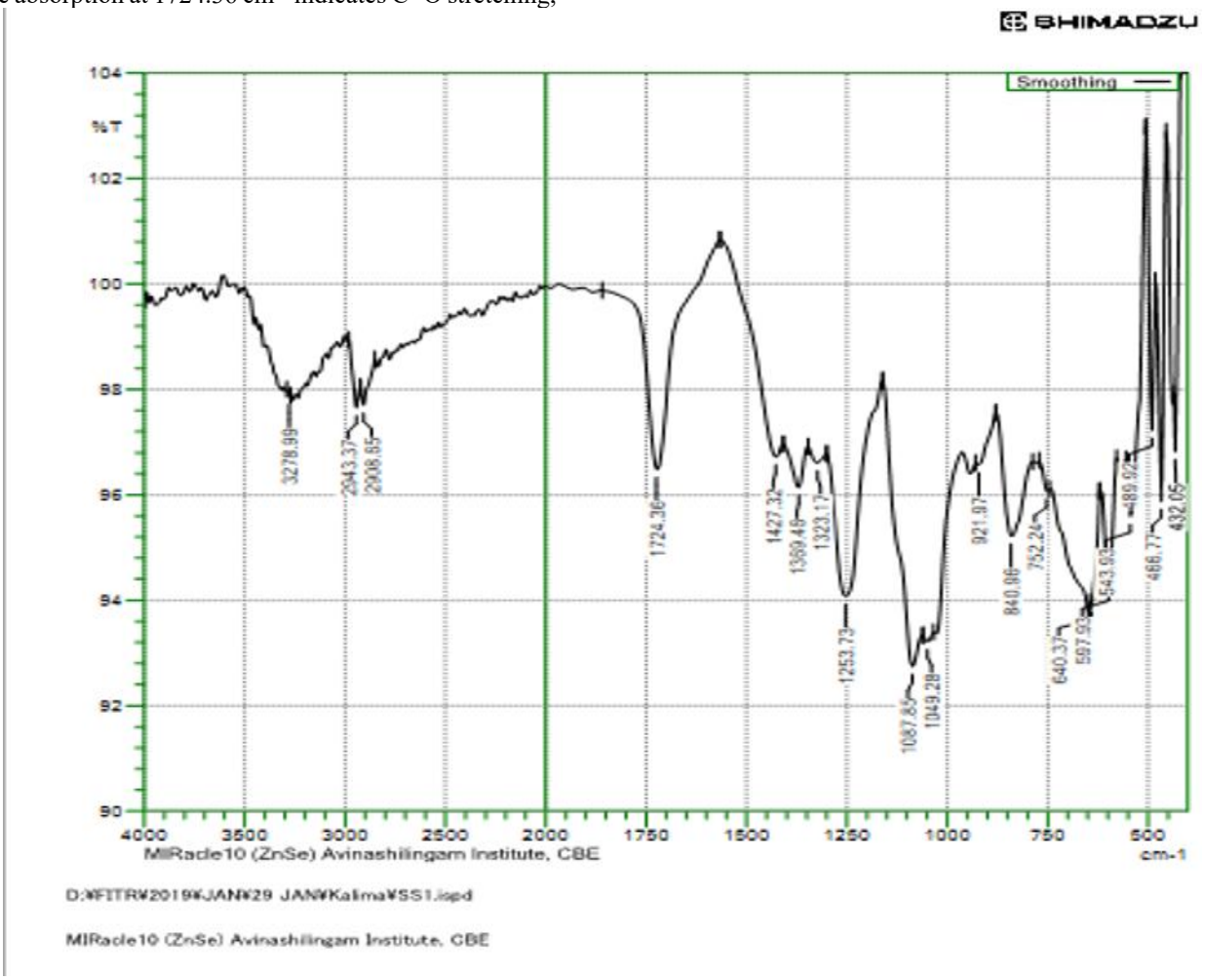

Fig. 2: The FT-IR spectrum peak at the range of 3280-433cm^{-1}.

SEM Analysis of ZnO-NPs

Scann Scanning Electron Microscopy (SEM) was used to examine the morphology of the synthesized ZnO-NPs (Molla et al., 2025). The SEM micrographs (Figure 3) showed a mixture of irregular and cuboid-shaped ZnO-NPs. The observed morphology indicates that the

phytochemicals present in Catharanthus roseus influenced the growth and shape of the ZnO crystals during the green synthesis process.

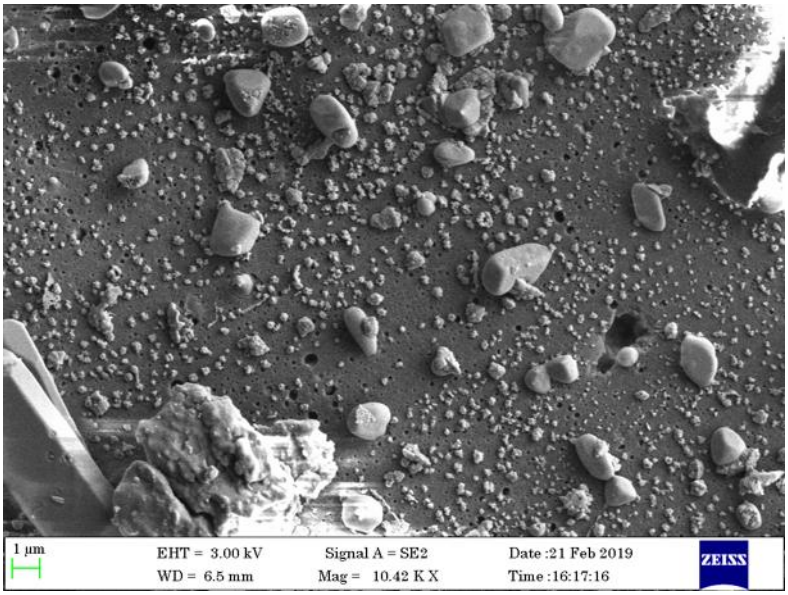

Fig. 3: The irregular and cuboid shaped zinc oxide nanoparticle synthesized from *Catharanthus roseus*

Bioreductase Analysis (SDS-PAGE)

The presence of bioreductase enzymes in the *C. roseus* extract was analysed via SDS-PAGE. As shown in Figure 4, the distinct bands in the sample well confirm the presence of proteinaceous biomolecules that may participate in synthesis and stabilization of zinc precursors. These biomolecules serve as the primary agents for transforming zinc nitrate into stable nanoparticles.

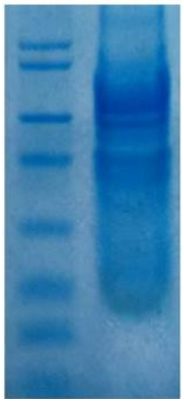

Fig. 4: SDS-PAGE profile of *Catharanthus roseus* extract showing protein bands involved in the biosynthesis and stabilization of ZnO nanoparticles. Lane 1: Plant extract sample; Lane 2: Protein marker.

Antibacterial Activity

The synthesized ZnO-NPs demonstrated significant antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria (Table 1). ZnO-NPs showed superior inhibition compared to the crude plant extracts. Notably, *S. aureus* exhibited higher sensitivity to the treatment than *E. coli*, evidenced by larger zones of inhibition (ZOI). Both leaf- and flower-derived ZnO-NPs exhibited comparable antibacterial activity, with flower-derived nanoparticles showing slightly greater inhibition against E. coli. No antibacterial activity was observed for the crude flower extract under the experimental conditions, whereas its corresponding ZnO nanoparticles exhibited clear antibacterial activity, indicating that nanoparticle synthesis significantly enhanced the antimicrobial potential as shown in figure 5.

Fig. 5:Antibacterial activity of plant extract of *C. roseus* and synthesized ZnO-Nps against *Escherichia coli* and *Staphylococcus aureus*

Table 1. Zones of Inhibition (mm) for *C. roseus* extract and ZnO-NPs

Culture	Leaf Extract	Flower Extract	Leaf ZnO-NPs	Flower ZnO-NPs
E. coli	5	Nil	9	10
S. aureus	4	Nil	10	10

Antifungal Activity

The antifungal efficacy was most pronounced against *Fusarium* spp. compared to *Aspergillus* species (Table Flower-derived ZnO-NPs showed slightly higher antifungal activity against *A. niger* and *A. flavus* than leaf-derived particles (Figure 6).

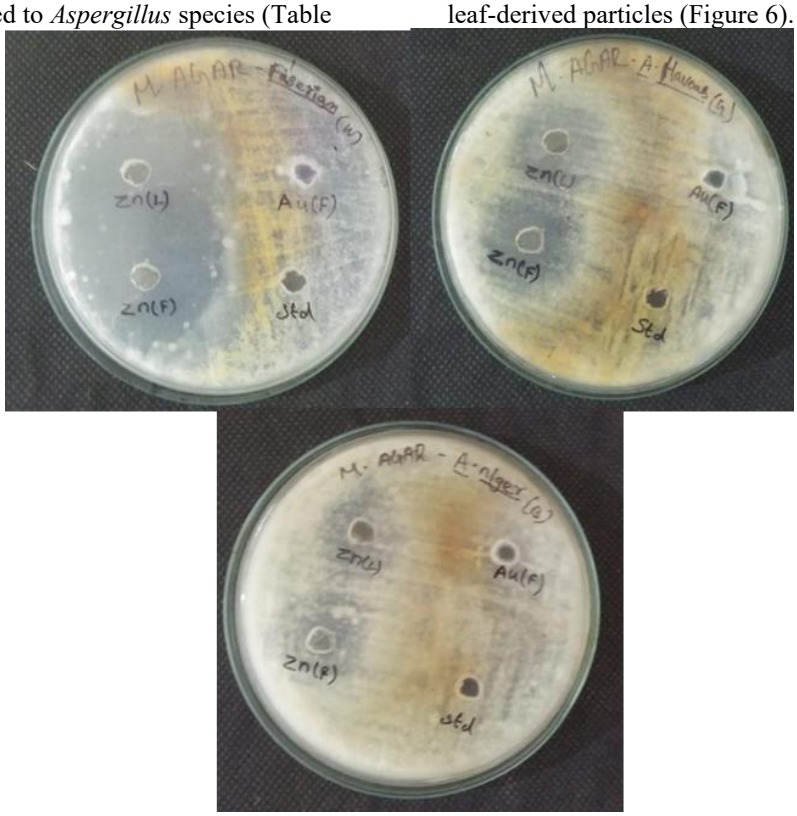

Fig. 6: Antifungal activity of synthesized ZnO-NPs from plant extract of *C.roseus* against *Fusarium, A.flavus* and *A.niger.*

Table 2. Antifungal activity of synthesized ZnO-NPs

Culture	Leaf ZnO-NPs (mm)	Flower ZnO-NPs (mm)	Standard (mm)
Fusarium	20	20	2
A. flavus	11	11	2
A. niger	10	11	3

Minimum Inhibitory Concentration (MIC)

The MIC was determined by measuring the Optical Density (OD) at 600 nm. A clear dose-dependent relationship was observed: higher concentrations of ZnO-NPs resulted in increased cell death percentages. Flower-derived ZnO-NPs at 30 µl concentration achieved the highest mortality rate at 62.14% (Table 3).

The MIC assay was performed in triplicate (n = 3), and the results are presented as mean values. Optical density measurements were analysed using Microsoft Excel. The percentage of cell death was calculated using the following formula:

$$\text{Cell Death \%} = \frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}} \times 100$$

Table 3. Cell Death Percentage based on OD Values

Sample	Replicate 1	Replicate 2	Replicate 3	Average OD value	Average cell death (%)
Control *E.coli*	0.554	0.654	0.584	0.597	-
ZnO from leaf at 10µl concentration	0.318	0.317	0.319	0.318	46.73
ZnO from leaf at 20µl concentration	0.298	0.290	0.295	0.294	50.75
ZnO from leaf at30µl concentration	0.260	0.252	0.250	0.254	57.45
ZnO from flower at 10µl concentration	0.314	0.322	0.315	0.317	46.90
ZnO from flower at 20µl concentration	0.286	0.285	0.288	0.286	52.09
ZnO from flower at 30µl concentration	0.224	0.225	0.231	0.226	62.14

DISCUSSION

The current investigation highlights the successful green synthesis of ZnO-NPs over an aqueous plant extract of *Catharanthus roseus* leaves and flowers, based on the fact that this technique is simple, cheap and environmentally benign compared to conventional chemical or physical approaches utilizing toxic reagents and excess energy(Ahmed et al., 2016). The confirmation of synthesized particles through UV-Visible spectral analysis showed the presence of a band of surface plasmon resonance within 300-400 nm in UV region for all aqueous leaf and flower extract-mediated ZnO-NPs (modes of experiment), common in reports of Phyto reduction of metal ions to nanoparticles (Gupta et al., 2018). The redshift of the absorption peak from 345nm leaf extract to 356nm flower extract likely results from distinct differences in nanoparticle size, shape, and the density of biomolecular capping. (Bhumi & Savithramma, 2014,).

The FTIR spectra elucidated the mechanistic function of the C. Roseus biomolecules considerably, by illustrating a dominant O-H broad/stretch (~ 3278cm) of phenols, alcohols, C-H vibrations (~ 2943 & 2908cm) of alkyl groups and a significant C=O peak (~1724cm) of carbonyls-hallmarks of the polyphenols, terpenoids and alkaloids such as vincristine & vinblastine (also electron donators to Zinc reduction peaking at the FTIR levels – all taking part in the reduction of Zn (Jayachandran et al., 2021). Moreover, the presence of lattice vibrations of Zinc-oxide (432-597 cm) signified the fact that extraction had resulted in complete conversion of zinc nitrate- in addition to pointing towards the dual role played by the biomolecules as the stabilizing and capping agents (in addition to reduction)(Kołodziejczak-Radzimska & Jesionowski, 2014). These results reinforced the efficiency of the green synthesis process by preventing aggregation and minimizing energy input respectively- much like the protocols for plant *Acalypha indica* or *Calotropis gigantea*

In addition to that, SDS-PAGE analysis revealed distinct protein bands in the aqueous extract of *Catharanthus roseus*, indicating the presence of soluble proteins that may participate in nanoparticle synthesis. These biomolecules are believed to facilitate electron transfer during the reduction of zinc ions and may contribute to nanoparticle stabilization by adsorbing onto the particle surface. The proteinaceous compounds present in the extract can function as natural capping agents, preventing aggregation and promoting the formation of stable and well-dispersed ZnO nanoparticles. Therefore, the SDS-PAGE results support the potential role of proteins in the green synthesis and stabilization of ZnO-NPs (Gupta et al., 2018).

SEM images showed the presence of crystalline rod- and cuboid-shaped ZnO nanoparticles. The observed morphological anisotropy may be attributed to differences in phytochemical adsorption on ZnO crystal facets during growth, which are influenced by pH, temperature, and extract composition (Molla et al., 2025). Such anisotropy can be further exploited in efficiency of reactivity via augmenting surface area to volume ratio (by casting anisotropic crystal shapes as opposed to isotropic spheres), which accurately accounts for the improved microbial inhibition effects of other phytocompound generated ZnO(Gunalan et al., 2012). This particular mechanism ensures the direct transference onto an effective concentration of microbial species active on the microbial cells to elicit increased antimicrobial effects, with observed zones of inhibition exceeding that of the ethanol extract provisioned controls towards Escherichia coli and Staphylococcus aureus with the latter more susceptible (Zarrindokht Emami-Karvani, 2012).

Further antifungal testing demonstrated nanoparticles' broad spectrum of activity, as Fusarium spp. was most susceptible (up to 20mm zone of inhibition), with flower-derived NPs being marginally more effective than *Aspergillus flavus* and *A.niger*-probably in response to matching floral chemistry/exotic compounds that boost their affinity for a target cell (Jamdagni et al., 2018). The antifungal and antibacterial activities of ZnO-NPs are attributed to multiple mechanisms, including reactive oxygen species (ROS) generation, membrane disruption, ion release, and damage to intracellular proteins and DNA, all of which are enhanced by their nanoscale dimensions and surface properties (Nel et al., 2009). MIC analysis further proved this dose-related phenomenon, with flower-made ZnO-NPs at 30 µl resulting in 62.1% bacterial kill rate, again comparable to commercial alternatives and justifies scale-up for clinical efficacy studies (Liu et al., 2009).

The synthesis integrates the ethnomedicinal properties of *C. roseus* to produce a green nanomaterial with significant bioactivity, manufactured economically and safely for application such as antimicrobial food-packaging film, wound-dressing, and anti-bacterial surface-coating (Khan, 2020). A limitation of the present study is that advanced characterization techniques such as XRD, TEM, DLS, and zeta potential analysis were not performed. Therefore, particle size distribution, crystallinity, and surface charge characteristics could not be determined. Future investigations should incorporate these analyses, along with cytotoxicity studies and scale-up experiments, to facilitate biomedical and industrial applications of the synthesized ZnO nanoparticles.

CONCLUSION

The present study successfully synthesized zinc oxide nanoparticles (ZnO-NPs) using aqueous leaf and flower extracts of Catharanthus roseus, with plant phytochemicals serving as reducing, capping, and stabilizing agents. Among the synthesized nanoparticles, flower-derived ZnO-NPs exhibited the highest antimicrobial activity, particularly against Escherichia coli, producing 62.14% bacterial cell death during the MIC assay, which may be attributed to reactive oxygen species (ROS) generation, Zn^{2+} ion release, and membrane disruption. A limitation of the present study is the absence of advanced characterization techniques such as XRD and TEM; therefore, particle crystallinity and precise size distribution could not be confirmed. Future studies should include detailed physicochemical characterization, cytotoxicity evaluation, and in vivo investigations to support the biomedical, food packaging, and industrial applications of the synthesized ZnO nanoparticles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

AUTHORS CONTRIBUTION

Sushmitha M conceived and designed the study, performed the experiments, analysed the data, and prepared the

manuscript. Sangeetha A contributed to manuscript editing and review. All authors read and approved the final manuscript.

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