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Plant-Based Synthesis of ZnO Nanoparticles using Neem Leaves

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Abstract: This study presents an eco-friendly approach for synthesizing zinc ox- ide nanoparticles (ZnO NPs) using Azadirachta indica (neem) leaf extract as a natural reducing and stabilizing agent. The green synthesis method offers a sustainable alternative to conventional chemical synthesis routes that often involve hazardous substances and high energy consumption. Fresh neem leaves were processed to prepare an aqueous extract, which was then reacted with zinc nitrate hexahydrate, followed by calcination at 400°C for 2 hours. The synthesized nanoparticles were comprehen- sively characterized using UV-visible spectrophotometry, scanning elec- tron microscopy with energy dispersive X-ray analysis (SEM-EDX), and Fourier transform infrared spectroscopy (FTIR). The results confirmed the formation of spherical ZnO nanoparticles with sizes ranging from 20- 40 nm. UV-visible analysis revealed characteristic absorption peaks at 273.0 nm and 360.5 nm, confirming the successful synthesis of ZnO NPs. The antibacterial evaluation demonstrated significant inhibitory effects against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria, with inhibition zones of 15 mm and 12 mm, respectively. Additionally, the nanoparticles exhibited excellent photo- catalytic activity, achieving approximately 75% degradation of methylene blue dye under UV irradiation within 120 minutes. This green synthesis approach eliminates the use of toxic chemicals, operates under mild condi- tions, and produces multifunctional nanoparticles suitable for applications in antimicrobial treatments, environmental remediation, and sustainable agriculture. Green synthesis Zinc oxide nanoparticles Neem extract antimicrobial activity Photocatalytic degradation Sustainable nanotechnology.

Keywords: *Azadirachta Indica, Nanoparticles, Green Synthesis¸ Zinc Oxide, Antimicrobial Activity.*

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I. INTRODUCTION

Nanotechnology has emerged as a revolutionary field with profound implications across diverse disciplines, including medicine, electronics, environmental science, and agriculture. Among the various nanomaterials, metal oxide nanoparticles, particularly zinc oxide nanoparticles (ZnO NPs), have garnered significant at- tention due to their unique physicochemical properties and versatile applications [1].

Traditional synthesis methods for nanoparticles predominantly rely on phys- ical and chemical approaches that, while efficient, often involve hazardous chem- icals, high energy consumption, and generate toxic byproducts, raising serious environmental and health concerns. In response to these challenges, green syn- thesis has emerged as a promising alternative—an eco-friendly approach that harnesses the reducing and stabilizing capabilities of biological entities such as plants, algae, fungi, and bacteria [2].

Plant-mediated synthesis stands out among various green synthesis methods for its simplicity, cost-effectiveness, and scalability. Plants possess a rich reposi- tory of phytochemicals including flavonoids, alkaloids, terpenoids, and polyphe- nols, which serve as natural reducing and capping agents, eliminating the need for toxic chemicals typically employed in conventional synthesis methods [4].

Azadirachta indica (neem), belonging to the Meliaceae family, has been revered in traditional medicine for centuries due to its diverse therapeutic appli- cations. The plant's leaves contain numerous bioactive compounds with proven antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant proper- ties. The rich phytochemical profile of neem makes it an ideal candidate for the biosynthesis of nanoparticles, as these compounds can effectively reduce metal ions while simultaneously stabilizing them against agglomeration.

Zinc oxide nanoparticles have gained considerable interest due to their re- markable properties, including strong catalytic activity, large surface-to-volume ratio, enhanced optical behavior, and notable antimicrobial effects. These fea- tures make ZnO NPs highly versatile, with applications spanning from electron- ics to healthcare. Additionally, zinc oxide is classified as ”Generally Recognized As Safe” (GRAS) by the U.S. Food and Drug Administration, highlighting its biocompatibility and suitability for biological and medical applications.

The agricultural sector stands to benefit significantly from ZnO NP technol- ogy. Zinc deficiency affects approximately 50% of cereal-growing soils globally, leading to significant yield reductions. ZnO NPs, with their enhanced bioavail- ability and surface reactivity, offer a promising solution to zinc deficiency in crops, potentially improving nutrient use efficiency and crop productivity [6].

This study focuses on developing a simple, eco-friendly method for syn- thesizing ZnO nanoparticles using neem leaf extract and evaluating their mul- tifunctional properties for potential applications in antimicrobial treatments, environmental remediation, and sustainable agriculture.

II. MATERIALS AND METHODS

➢ *Materials*

- *Chemicals and Reagents*

Zinc nitrate hexahydrate [Zn (NO3)2·6H2O] was used as the primary zinc source. Fresh neem leaves (Azadirachta indica) were obtained from the medicinal plant garden at KLE College of Pharmacy, Hubballi, Karnataka, with proper botan- ical verification. Double-distilled water was prepared using glass distillation equipment. Methylene blue (MB, C16H18ClN3S, 95% purity, Merck) was used for photocatalytic studies. Microbiological testing required nutrient agar and Mueller-Hinton agar from Hi Media Laboratories. Standard bacterial strains Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) were obtained from the Department of Microbiology.

➢ *Equipment*

Various glassware including beakers (100 mL, 250 mL, 500 mL), silica crucibles, and graduated cylinders were used. A laboratory oven (Labtech, Model LDO- 080N), muffle furnace (Thermo Scientific, Model FB1410M), UV-visible spec- trophotometer (Shimadzu, UV-1800), scanning electron microscope with EDX (JEOL, JSM-7600F), and FTIR spectrometer (Bruker, Alpha II) were employed for synthesis and characterization.

➢ *Methods*

- *Preparation of Neem Leaf Extract*

Fresh, fully-developed neem leaves were collected during early morning hours and thoroughly washed under flowing tap water, followed by three sequential rinses with distilled water. The leaves were air-dried at ambient temperature (25 ± 2°C) for 48 hours, then oven-dried at 30°C for 6-8 days until completely dehydrated.

The dried leaves were ground into fine powder using a commercial mixer grinder and passed through a 40-mesh sieve. For extract preparation, 10 g of neem leaf powder was mixed with 100 mL of double-distilled water (1:10 w/v ratio) and heated at 80 ± 2°C for 30-45 minutes with gentle stirring. The mix- ture was cooled to room temperature and filtered through muslin cloth followed by Whatman No. 1 filter paper to obtain a clear extract.

- *Green Synthesis of ZnO Nanoparticles*

Ten milliliters of fresh neem extract was heated to 80°C in a 100 mL beaker. Zinc nitrate hexahydrate (1.0 g) was added dropwise over 5 minutes under con- tinuous stirring. The reaction mixture was maintained at boiling temperature for 2 hours, during which the color changed from light amber to dark brown, indicating the reduction of zinc ions.

The resulting dark paste was transferred to a pre-weighed silica crucible and calcined in a muffle furnace at 400°C for 2 hours with a heating rate of 10°C/min. After cooling, the white ZnO powder was ground using a mortar and pestle and stored in airtight glass vials.

- *Characterization Techniques*

UV-Visible Spectrophotometry: ZnO powder (5 mg) was dispersed in 10 mL of double-distilled water and sonicated for 30 minutes. The absorbance was measured from 300 to 800 nm using a UV-visible spectrophotometer.

SEM-EDX Analysis: The powder was sputter-coated with gold and ex- amined under SEM at accelerating voltages up to 15 kV with magnifications ranging from 5,000× to 50,000×. EDX spectra were collected simultaneously for elemental analysis.

FTIR Spectroscopy: KBr pellets were prepared for pure neem extract, Zn-neem precursor mixture, and calcined ZnO. Spectra were recorded from 4000 to 400 cm−1 at 4 cm−1 resolution.

- *Antibacterial Activity Assessment*

Disk diffusion assays were performed using S. aureus and E. coli cultured to 0.5 McFarland density. Mueller-Hinton agar plates were inoculated, and 6 mm filter disks loaded with 25-100 µg/mL ZnO suspensions were placed alongside controls. Inhibition zones were measured after 24 hours of incubation at 37°C. Minimum inhibitory concentration (MIC) was determined using serial two- fold dilutions of ZnO (3.9-500 µg/mL) in Mueller-Hinton broth inoculated to 5×104 CFU/mL. After 24 hours, resazurin (0.02% w/v) was added, and the lowest concentration preventing color change was recorded as MIC.

- *Photocatalytic Activity Evaluation*

A 10 mg/L methylene blue solution was equilibrated with 50 mg ZnO for 30 minutes in darkness. Samples were exposed to UV light (365 nm, 15 W) under magnetic stirring. Aliquots were collected at various time intervals, centrifuged,

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and their absorbance measured at 664 nm. Degradation efficiency was calculated as:

Degradation (%) = $C0 - Ct \times 100$ (1)

C0

where C0 is the initial concentration and Ct is the concentration at time t.

III. RESULTS AND DISCUSSION

➢ *Green Synthesis and Physical Characteristics*

The eco-friendly synthesis of ZnO nanoparticles using neem leaf extract was successfully accomplished. The synthesis procedure produced a fine, white crystalline powder after thermal treatment, exhibiting characteristics consis- tent with ZnO nanoparticles reported in the literature. The color change from light amber to dark brown during the reaction indicates the reduction of zinc ions by neem phytochemicals.

Fig 1 Extract of Zinc oxide nano particle

➢ *UV-Visible Spectroscopy Analysis*

UV-visible spectroscopic analysis confirmed the successful formation of ZnO nanoparticles through optical characterization. The spectrum displayed distinc- tive absorption characteristics with a prominent peak at 273.0 nm (absorbance = 0.081) and an additional peak at 360.5 nm (absorbance = 0.045). These peaks correspond to the characteristic UV absorption of ZnO nanoparticles e- sulting from electronic transitions within the material, specifically O2p → Zn3d transitions. The absorption characteristics demonstrate excellent agreement with previ- ously published UV-visible spectra of ZnO nanoparticles, confirming successful synthesis. The slight blue shift compared to bulk ZnO suggests quantum con- finement effects due to the nanoscale dimensions.

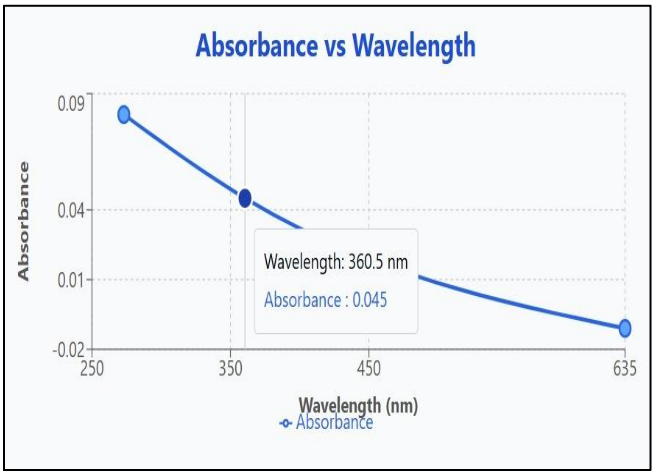

Fig 2 Result of UV Spectroscopy

➢ *Morphological Analysis*

SEM analysis revealed that the synthesized ZnO nanoparticles predominantly exhibited spherical and near-spherical morphologies with relatively consistent size distribution. The average particle size was determined to be in the range of 20-40 nm, which is optimal for various applications including antimicrobial activity and photocatalytic processes. Some degree of particle clustering was ob- served, which is commonly reported for nanoparticles synthesized using botan- ical extracts.

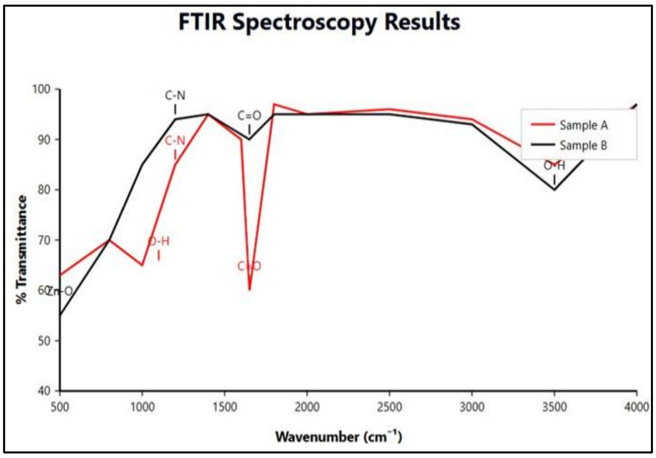

Fig 3 FTIR Result

➢ *FTIR Analysis*

FTIR spectroscopy was utilized to identify functional groups and elucidate the involvement of neem phytochemicals in the synthesis process. The spectrum revealed several characteristic absorption bands:

- A broad band at 3400-3500 cm^{-1} attributed to O-H stretching vibrations of adsorbed water molecules
- Bands around 1600-1650 cm^{-1} corresponding to C=O stretching vibra- tions from flavonoids and terpenoids
- A strong band at 400-500 cm^{-1} representing characteristic Zn-O stretching vibration
- Additional bands in the 1000-1400 cm^{-1} region assigned to C-O-C and C-O stretching vibrations from polyphenolic compounds

The FTIR analysis confirms the presence of various functional groups derived from neem extract on the nanoparticle surface, indicating their active role in zinc ion reduction and stabilization.

➢ *Antibacterial Activity*

The antimicrobial properties were assessed using the disk diffusion method against representative bacterial strains. The results demonstrated significant inhibitory effects with inhibition zones of approximately 15 mm against S. au- reus and 12 mm against E. coli. The stronger activity against Gram-positive bacteria can be attributed to differences in cell wall structure, where the thicker peptidoglycan layer facilitates greater nanoparticle adsorption.

➢ *The Antibacterial Mechanism Likely Involves Multiple Pathways:*

- Generation of reactive oxygen species (ROS)
- Direct interaction with bacterial cell walls
- Release of Zn^{2+} ions interfering with enzyme systems
- Synergistic effects with retained neem bioactive compounds

➢ *Photocatalytic Activity*

The photocatalytic performance was evaluated by monitoring methylene blue degradation under UV irradiation. The results demonstrated substantial degra- dation with approximately 75% of the dye being degraded after 120 minutes. The degradation process followed pseudo-first-order kinetics with a rate constant of 0.012 min^{-1}.

The photocatalytic mechanism involves the generation of electron-hole pairs under UV irradiation, which react with water and oxygen to produce ROS that subsequently oxidize and degrade organic pollutants. The high surface area- to-volume ratio of the nanoparticles provides numerous active sites, enhancing efficiency compared to bulk materials.

➢ *Mechanism of ZnO Nanoparticle Formation*

The formation mechanism can be attributed to the phytochemical constituents in neem extract. Polyphenols, flavonoids, and terpenoids act as reducing agents, facilitating the conversion of Zn^{2+} ions to ZnO. Simultaneously, these com- pounds, along with proteins and polysaccharides, serve as natural capping agents, preventing agglomeration and contributing to size uniformity.

The calcination step at 400°C was crucial for obtaining crystalline ZnO by removing organic components while allowing stable crystal structure formation. The complete conversion to zinc oxide with minimal impurities is evidenced by the white color of the final product.

➢ *Comparative Analysis and Significance*

The characteristics of the synthesized ZnO NPs compare favorably with previous studies. The observed particle size range (20-40 nm) is consistent with reports by Elumalai and Velmurugan [1] and Bhuyan et al. [2], validating the repro- ducibility of the green synthesis approach. The antibacterial and photocatalytic activities are also comparable to previous reports, confirming the potential for practical applications.

➢ *The Green Synthesis Approach Offers Several Advantages:*

- Environmental sustainability through elimination of toxic chemicals
- Cost-effectiveness using readily available plant materials
- Enhanced biocompatibility due to natural capping agents
- Mild reaction conditions reducing energy consumption
- Potential Applications and Future Prospects

- Based on the demonstrated properties, several applications can be envisioned:

➢ *Agricultural Applications*

The synthesized ZnO NPs could address zinc deficiency in crops while providing antimicrobial protection against phytopathogens. The enhanced bioavailability compared to conventional zinc supplements could improve nutrient use efficiency and crop productivity.

➢ *Environmental Remediation*

The excellent photocatalytic activity suggests potential applications in wastew- ater treatment, particularly for removing organic dyes and pollutants from in- dustrial effluents. The sustainable synthesis approach aligns with environmental conservation goals.

➢ *Biomedical Applications*

The demonstrated antibacterial properties and biocompatibility make these nanoparticles suitable for wound healing applications, antibacterial coatings for medical devices, and potential drug delivery systems.

IV. CONCLUSION

This study successfully demonstrates an eco-friendly approach for synthesizing ZnO nanoparticles using Azadirachta indica leaf extract. The green synthesis method produces spherical nanoparticles (20-40 nm) with excellent antibacterial activity against both Gram-positive and Gram-negative bacteria and significant photocatalytic activity for organic dye degradation.

The comprehensive characterization confirms the successful formation of crystalline ZnO nanoparticles with properties suitable for multiple applications. The method's environmental sustainability, cost-effectiveness, and mild reaction conditions make it attractive for large-scale production.

The multifunctional nature of these nanoparticles, combining antimicrobial, photocatalytic, and potential agricultural benefits, positions them as valuable materials for addressing contemporary challenges in healthcare, environmen- tal remediation, and sustainable agriculture. Future research should focus on optimization for specific applications, scale-up studies, and comprehensive tox- icological evaluations.

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This work contributes to the expanding field of green nanotechnology by demonstrating how traditional botanical knowledge can be integrated with mod- ern nanotechnology to develop sustainable solutions for global challenges.

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