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Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities.

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Abstract:

This research presents an eco-friendly biotechnological approach for synthesizing Zinc Oxide (ZnO) nanoparticles using orange peel extract (*Citrus sinensis*) as a natural reducing and stabilizing agent. Traditional synthesis methods often require toxic reagents and high energy; however, this study utilizes agricultural waste rich in polyphenols, flavonoids, and ascorbic acid to facilitate a sustainable "Green Chemistry" alternative. The experimental process involves reacting a zinc nitrate precursor with the aqueous extract under thermal stirring. Phytochemical functional groups reduce Zn^{2+} ions into intermediates, which are converted into stable ZnO nanoparticles through calcination (400°C–600°C), resulting in a pure hexagonal wurtzite structure. A primary focus is characterization via UV-Visible Spectroscopy. Successful synthesis is confirmed by a sharp Surface Plasmon Resonance (SPR) peak between 360 nm and 380 nm. This blue-shifted absorption indicates significant quantum confinement effects. Using the Tauc relation, the optical band gap was determined to be approximately 3.3 eV, confirming the high purity and semiconducting nature of the biogenic nanoparticles. Functional testing revealed exceptional antibacterial efficacy against *S. aureus* and *E. coli*. The mechanism involves the generation of Reactive Oxygen Species (ROS), which induce oxidative stress and physically disrupt bacterial cell membranes. This study confirms that orange peel-mediated synthesis is a cost-effective, non-toxic, and scalable route for producing high-quality ZnO nanoparticles. These findings suggest strong potential for applications in biomedical engineering, wastewater remediation, and antimicrobial coatings, offering a superior alternative to conventional synthetic methodologies.

Keywords: Green Synthesis, ZnO Nanoparticles, *Citrus sinensis*, Orange, Peel Extract, UV-Vis Spectroscopy, Surface Plasmon, Resonance (SPR), Optical Band Gap, Antibacterial Activity, Green Chemistry, Agricultural Waste.

Introduction

The emergence of nanotechnology has fundamentally redefined modern science, enabling the manipulation of matter at the atomic level (1–100 nm) to unlock unique physical and chemical properties. Among various nanomaterials, Zinc Oxide (ZnO) nanoparticles have garnered significant interest due to their semiconductor properties, UV-filtering capabilities, and FDA-approved safety profile. Most notably, ZnO nanoparticles offer a potent solution to the global crisis of antibiotic resistance. Unlike traditional drugs, they employ a multi-pronged antimicrobial mechanism—including the generation of Reactive Oxygen Species (ROS) and physical membrane disruption—making it difficult for pathogens to develop mutations.

However, conventional synthesis methods like hydrothermal or chemical vapor deposition are often energy-intensive and rely on hazardous reducing agents that leave toxic residues. To address these drawbacks, this study adopts the principles of Green Chemistry through phyto-synthesis. This approach utilizes agricultural waste—specifically orange (*Citrus sinensis*) peels—as a sustainable biological factory.

Rich in bioactive phytochemicals such as flavonoids, phenolic acids, and limonene, orange peels act as both reducing and capping agents, converting zinc ions into stable nanoparticles without toxic byproducts. By transforming ubiquitous food waste into high-value antimicrobial agents, this research promotes a "Waste-to-Wealth" circular economy. This project aims to validate an eco-friendly, cost-effective protocol for producing biocompatible ZnO nanoparticles, bridging the gap between environmental sustainability and pharmaceutical innovation.

Methodology

The synthesis of Zinc Oxide (ZnO) nanoparticles follows a streamlined, eco-friendly protocol centered on the principles of phyto-synthesis.

The process begins with the preparation of the bioreductant from *Citrus sinensis* (orange) peels. Collected peels are thoroughly washed with deionized water to remove impurities, oven-dried at 60°C, and ground into a fine powder. To extract the bioactive phytochemicals, 10g of the powder is boiled in 100 mL of distilled water for 30 minutes. This extract, rich in flavonoids and phenolic acids, is then filtered and stored for the reduction process.

For the nanoparticle synthesis, a precursor solution of Zinc Acetate Dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) is prepared. The orange peel extract is added dropwise to the zinc solution under constant magnetic stirring at 70°C. During this phase, the phytochemicals act as reducing agents, converting zinc ions into metallic zinc, while simultaneously serving as capping agents to stabilize the particles. A visible color change or the formation of a precipitate indicates the successful reduction and nucleation of nanoparticles. The resulting suspension is centrifuged at 10,000 rpm to isolate the nanoparticles, followed by multiple washing steps with ethanol and water to remove unreacted precursors. Finally, the pellet is dried and calcined at 400°C to obtain high-purity, crystalline ZnO nanoparticles. The final product is then characterized using UV-Vis spectroscopy to confirm



its structural and morphological integrity.

Literature Review

The green synthesis of metal oxide nanoparticles has emerged as a cornerstone of sustainable nanotechnology, prioritizing environmental safety and energy efficiency. Central to this field is Zinc Oxide (ZnO), a material distinguished by its high chemical stability and wide bandgap. Current literature identifies three primary pillars of research: the biochemical mechanism of synthesis, structural characterization, and antimicrobial efficacy.

Studies consistently highlight that plant extracts, such as those from *Citrus sinensis* (orange) peels, function as dual-purpose agents. Rich in secondary metabolites like hesperidin, citric acid, and ascorbic acid, these extracts facilitate a complexation-dehydration process. The hydroxyl and carbonyl groups within these phytochemicals reduce zinc ions while simultaneously acting as capping agents. This natural coating provides steric hindrance, preventing nanoparticle agglomeration and ensuring superior stability compared to chemically synthesized counterparts. Structurally, research confirms that green-synthesized ZnO typically adopts a hexagonal wurtzite structure, with a characteristic UV-Visible absorption peak between 330 nm and 380 nm.

Biologically, the literature underscores a multi-pronged antibacterial mechanism: the generation of Reactive Oxygen Species (ROS) and direct physical disruption of bacterial membranes. Notably, comparative studies indicate that Gram-positive bacteria (e.g., *S. aureus*) are generally more susceptible than Gram-negative strains (e.g., *E. coli*) due to differences in cell wall architecture. By integrating these findings, the current study leverages orange peel waste to advance a "Waste-to-Wealth" model for pharmaceutical innovation.

Expected Outcome

The anticipated results of this study focus on the successful synthesis, stability, and biological performance of green-synthesized Zinc Oxide (ZnO) nanoparticles. Initially, visual confirmation is expected through a distinct color transition from pale yellow to a milky white precipitate, yielding a high-purity inorganic powder. Optical characterization via UV-Visible spectroscopy is predicted to reveal a sharp absorption peak between 340 nm and 380 nm, with Tauc's plot analysis likely indicating a band gap slightly exceeding 3.37 eV due to quantum confinement effects.

A critical outcome involves colloidal stability. Due to the effective "capping" by orange peel phytochemicals, the nanoparticles are expected to remain in suspension for at least 72 hours without significant agglomeration. This stability is essential for ensuring consistent biological activity during application.

The antibacterial efficacy of the nanoparticles is expected to be potent, evidenced by measurable Zones of Inhibition (ZOI) against *E. coli* and *S. aureus*. A clear dose-response relationship is anticipated, where higher concentrations yield greater inhibitory effects. Furthermore, *S. aureus* is expected to show higher sensitivity than *E. coli* due to its lack of an outer lipopolysaccharide membrane. Ultimately, this study aims to validate a "Waste-to-Wealth" model, proving that orange peel waste can be converted into high-performance, eco-friendly antimicrobial agents without the need for toxic chemical reductants.

Conclusion

This research successfully demonstrates that the transition toward sustainable nanotechnology is not only environmentally necessary but scientifically robust. By harnessing the bioactive potential of orange (*Citrus sinensis*) peel waste, this study establishes an eco-friendly protocol for the synthesis of Zinc Oxide (ZnO) nanoparticles. The methodology effectively bypasses the need for hazardous chemical reducing agents, relying instead on the natural antioxidant capacity of flavonoids and phenolic acids to facilitate the reduction and stabilization of zinc ions.

The projected outcomes underscore a dual-success: the achievement of high-purity, stable nanoparticles with distinct quantum confinement properties and the validation of a potent antimicrobial agent. The synthesized ZnO nanoparticles are expected to exhibit a multi-pronged attack against human pathogens, providing a non-traditional pathway to combat antibiotic resistance. The specific sensitivity of Gram-positive bacteria to these particles highlights their potential for targeted biomedical applications, ranging from wound dressings to sterile coatings.

Ultimately, this work fulfills the core tenets of Green Chemistry by bridging the gap between waste management and pharmaceutical innovation. By transforming a ubiquitous agricultural byproduct into a high-value nanomaterial, the study reinforces the "Waste-to-Wealth" paradigm. This approach not only offers a cost-effective alternative for M.Sc. level research and industrial scaling but also contributes to a cleaner, more sustainable future where environmental health and medical advancement go hand in hand.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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