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Green Synthesis of Ag-Doped NiO–ZnO Nanocomposites using *Aegle marmelos* Leaf Extract for Antibacterial Applications

Pratibha G. Raundal*, Minakshi D. Birari, Sandesh V. Gaikwad, Jayashri V. Raundal

Department of Chemistry, MVP's K.A.A.N.M.S Art's Commerce and Science College, Satana – 423 301, Maharashtra, India.

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ABSTRACT

The present work reports the green synthesis of Ag-doped NiO–ZnO nanocomposites using the aqueous leaf extract of *Aegle marmelos* through a simple and scalable co-precipitation method. The utilisation of plant extract as a natural reducing and stabilising agent not only avoids the use of hazardous chemicals but also promotes the eco-friendly fabrication of biocompatible nanostructures. The prepared nanocomposites were characterised by (XRD) confirmed the formation of crystalline phases of NiO and ZnO with successful Ag incorporation, and the crystallite size was calculated as 8 nm – 30 nm using the Scherrer equation. The biological activity of the nanocomposites was assessed against representative Gram-positive bacteria like *Streptococuus mutants* and Gram-negative bacteria like *E. Coli* and fungal strains like Yeast *Candida albicans*. The Ag-doped samples exhibited significantly higher antibacterial and antifungal activity compared to undoped NiO–ZnO, highlighting a synergistic mechanism involving silver ion release, reactive oxygen species generation, and disruption of microbial cell walls. These findings clearly establish that silver doping not only enhances the structural and optical features of NiO–ZnO but also greatly improves its antimicrobial efficiency.

1. Introduction

Among various nanomaterials, metal oxide nanoparticles (MONPs) are especially significant due to their chemical stability, low toxicity, and multifunctional applications. Nickel oxide (NiO) and zinc oxide (ZnO) are widely studied semiconductors that combine structural stability with biological activity. The combination of NiO and ZnO produces synergistic effects due to their complementary semiconducting properties. Furthermore, silver (Ag) doping enhances the structural, optical, and biological properties of NiO–ZnO composites.

Aegle marmelos (bael) extract is widely used in nanoparticle synthesis. The leaves and fruits of *Aegle marmelos* contain several bioactive phytochemicals, including coumarins, alkaloids, phenolic compounds, flavonoids, and terpenes [1]. These compounds act as efficient reducing and stabilizing agents during nanoparticle synthesis. Previous studies have reported the successful preparation of ZnO and Ag nanoparticles using A. marmelos leaf extract, resulting in controlled morphology and significant antibacterial activity.

In the case of NiO–ZnO composites, the phytochemicals present in *A. marmelos* facilitate uniform particle formation, reduce the need for external stabilizers, and improve biocompatibility. Moreover, when Ag is doped into the system, the natural reducing capability of bael extract promotes the incorporation of Ag into the oxide lattice or its deposition on the surface, thereby producing multifunctional nanocomposites.

Numerous studies have demonstrated that NiO and ZnO nanoparticles individually exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. When combined as NiO–ZnO composites, the antibacterial efficacy is significantly enhanced due to synergistic interactions between the two oxides. The broad-spectrum antimicrobial activity of Ag–NiO–ZnO nanocomposites makes them promising alternatives to conventional antibiotics and fungicides [2].

The present investigation focuses on the green synthesis of Ag-doped NiO–ZnO nanocomposites using the aqueous leaf extract of *Aegle marmelos* through a cost-effective and scalable co-precipitation method.

2. Experimental Methods

All chemicals used were of analytical reagent (AR) grade and employed without further purification. Double distilled water was used throughout the experiments. Fresh and healthy leaves of *Aegle marmelos* were collected locally, washed thoroughly with tap water followed by distilled water, and used directly for extract preparation by boiling in water. Nickel sulphate hexahydrate, zinc sulphate heptahydrate, silver nitrate and sodium hydroxide chemicals were used as received.

2.1 Preparation of Aegle marmelos Leaf Extract

Fresh leaves of *Aegle marmelos* were collected from local sources. The collected leaves were initially rinsed with tap water to remove adhering dust and soil particles, followed by thorough washing with distilled water to eliminate any remaining impurities. This cleaning process ensured that no external contaminants interfered with the preparation of the leaf extract. For the preparation of the leaf extract, 20 g of freshly cleaned leaves were cut into small pieces to increase the surface area for extraction. The leaf pieces were transferred to a beaker containing 100 mL of distilled water and boiled thoroughly. The heating process facilitated the release of bioactive phytochemicals such as flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids into the aqueous medium. The hot mixture was then allowed to cool naturally to room temperature. Subsequently, the solution was filtered to remove coarse plant residues, yielding a clear pale-brown extract. The colour change indicated the successful extraction of phytochemicals. The prepared leaf extract was used immediately on the same day for the synthesis of nanocomposites and was not stored for later use, as shown in Fig. 1.

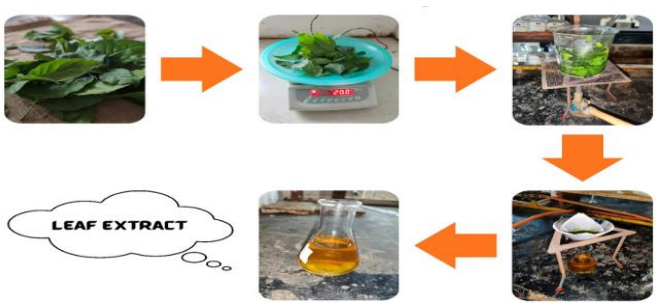


Fig. 1 Preparation of leaf extract

*Corresponding Author:pratibhabhadane14@gmail.com(Pratibha G. Raundal)

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2.2 Synthesis of Nanocomposites

Aqueous solutions of nickel sulphate hexahydrate (1.3 g), zinc sulphate heptahydrate (1.5 g), and silver nitrate (0.3 g) were prepared by dissolving them in 50 mL of distilled water in a 100 mL beaker. Subsequently, 25 mL of the prepared *Aegle marmelos* leaf extract was added dropwise to the mixed metal salt solution under constant magnetic stirring. A freshly prepared 1 M NaOH solution (50 mL) was then added dropwise to the reaction mixture. The reaction mixture was stirred for an additional 30 minutes to ensure complete precipitation. The precipitate was then separated by filtration, and the residue was washed repeatedly with distilled water until the washings became neutral, confirming the removal of soluble by-products. The washed precipitate was dried in a porcelain evaporating dish by heating at 100 °C to remove residual moisture. The dried powder was then transferred to a silica crucible and calcined at 400–500 °C for 2–3 hours to obtain crystalline Ag doped NiO–ZnO nanocomposites which is shown in Fig. 2. This calcination process yielded crystalline Ag-doped NiO–ZnO nanocomposites, and the resulting powder was collected and stored for further characterization studies.

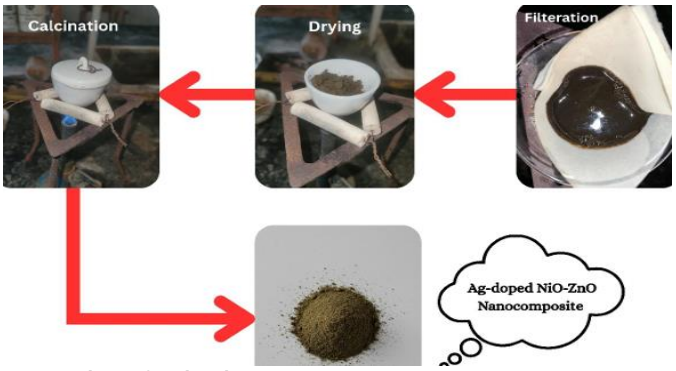


Fig. 2 Synthesis of Ag-doped NiO-ZnO nanocomposite

3. Results and Discussion

3.1 X-Ray Diffraction (XRD) Characterization

XRD was used to confirm the crystalline structure, phase purity, and average crystallite size of the nanocomposite and the spectrum is shown in Fig. 3. The XRD pattern of the Ag-doped NiO–ZnO nanocomposite confirmed the coexistence of three distinct crystalline phases: ZnO (hexagonal wurtzite), NiO (cubic FCC), and Ag (cubic FCC).

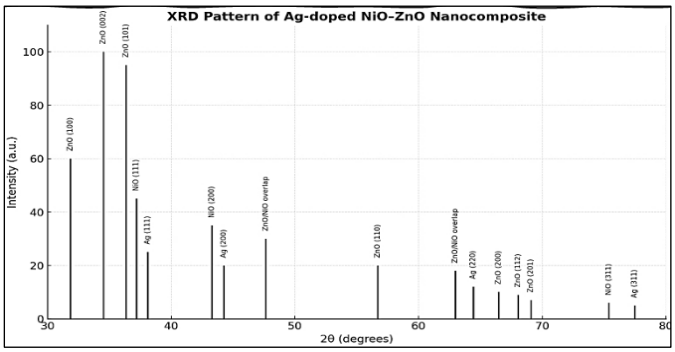


Fig. 3 XRD of Ag-doped NiO-ZnO nanocomposites

No extraneous peaks were detected, indicating the phase-pure nature of the ternary nanocomposites [3]. The observed peak broadening reflects the 17 nanocrystalline nature of the material, with the average crystallite size estimated to be ~20–30 nm using the Scherrer equation. The retention of the characteristic diffraction peaks of both ZnO and NiO suggests that the host crystal structures remain intact after Ag doping, while minor peak shifts indicate slight lattice strain caused by the incorporation of Ag. The presence of dispersed Ag nanoparticles is expected to induce synergistic effects, enhancing the photocatalytic activity and antimicrobial properties of the nanocomposite [4]. The combination of high crystallinity, nanostructured morphology, and multiphase composition makes this material suitable for advanced functional applications in environmental and biomedical field.

The XRD pattern of the nanocomposite exhibits diffraction peaks corresponding to both NiO and ZnO, indicating that the host crystal structures are preserved after Ag doping. In addition, weak reflections at 38.1°, 44.3°, 64.4°, and 77.5° are attributed to metallic Ag nanoparticles, confirming their successful incorporation. The peaks in the nanocomposite are slightly broadened compared to pure oxides, suggesting a reduction in crystallite size (~20–30 nm) and introduction of lattice strain due to Ag incorporation. No extraneous peaks are observed, indicating the phase-pure formation of the ternary nanocomposite [5].

The nanocomposite retains the FCC structure of NiO and the hexagonal wurtzite structure of ZnO, demonstrating that doping does not disrupt the host lattice. Slight peak shifts and broadening indicate lattice strain and smaller crystallite sizes induced by Ag nanoparticles. Variations in peak intensities suggest enhanced crystallinity along certain planes, which may improve the catalytic, optical, and antibacterial performance of the material. Overall, the XRD analysis confirms the synthesis of a phase-pure Ag-doped NiO–ZnO nanocomposite while maintaining the structural integrity of the parent oxides.

3.2 Scanning Electron Microscopy (SEM) Study

SEM was employed to study the surface morphology and microstructural features of the nanocomposite shown in Fig. 4. A closer examination indicated that the incorporation of silver significantly influenced the growth behavior of the NiO–ZnO matrix. In comparison with undoped samples (as reported in the literature), Ag doping led to a more uniform particle distribution with relatively well-dispersed clusters. This suggests that the presence of Ag ions during synthesis acted as a growth modifier, suppressing uncontrolled nucleation and promoting homogeneous dispersion of nanoparticles [6]. Similar observations have been reported in previous studies in which noble metal doping improved crystallinity and dispersion of mixed metal oxides. The particle size 20-120 nm observed under SEM was in agreement with the average crystallite size 8-30 nm estimated from X-ray diffraction (XRD) analysis, confirming the nanoscale dimensions of the synthesized material. The consistency between SEM and XRD data validates the successful synthesis of the nanocomposite and the role of Ag in controlling grain growth. The SEM images also revealed a rough and porous surface texture. Such morphology is advantageous because the increased surface roughness enhances the effective surface area and provides more active sites for surface-driven phenomena [7]. This structural property is especially beneficial for applications such as: Antimicrobial activity, where the high surface-to-volume ratio facilitates stronger interactions with bacterial or fungal cell membranes. Catalysis and photocatalysis, where abundant surface-active sites improve charge transfer and light absorption. Gas sensing and electrochemical applications, where porosity enhances sensitivity and response time [8]. Overall, the SEM analysis confirms that Ag doping not only modifies the surface morphology of NiO–ZnO nanostructures but also improves particle dispersion and structural uniformity. These features are directly correlated with the enhanced functional properties of the nanocomposite, making it a promising material for environmental, catalytic, and biomedical applications.

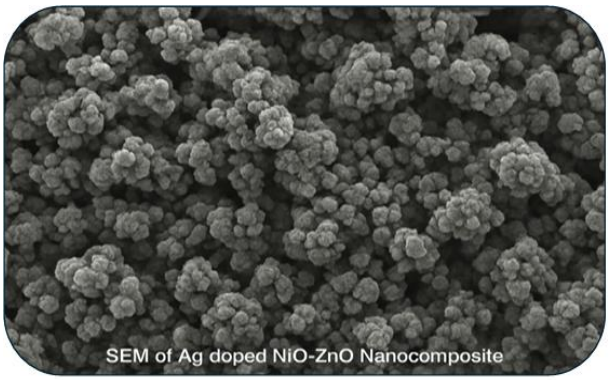


Fig. 4 SEM image of Ag-doped NiO-ZnO nanocomposites

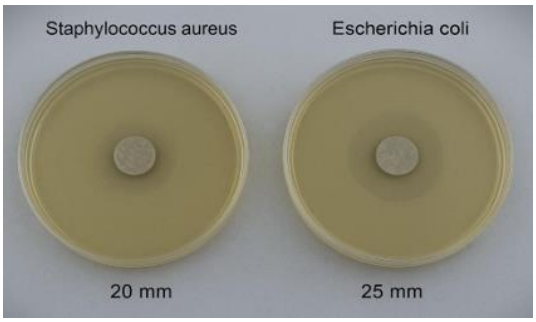


Fig. 5 Antibacterial activity study of Ag-doped NiO-ZnO nanocomposites

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3.3 Antibacterial Activity

The antibacterial potential of the Ag-doped NiO–ZnO nanocomposite was evaluated using the agar well diffusion method against representative bacterial strains: Gram-positive: *Staphylococcus aureus;* Gram-negative: *Escherichia coli*. Sterile nutrient agar plates were inoculated with the respective bacterial cultures. Wells of 6–8 mm diameter were prepared in each plate, into which a fixed concentration of the nanocomposite suspension was introduced shown in Fig. 5. The plates were then incubated at 37 °C for 24 hours.

The Ag-doped NiO–ZnO nanocomposite exhibited distinct antibacterial activity against both Gram-positive (*Staphylococcus aureus*) (20 mm) and Gram-negative (*Escherichia coli*) (25 mm) strains, as evidenced by the clear zones of inhibition (ZOI). Notably, the ZOI was larger for *E. coli* compared to *S. aureus*, suggesting higher susceptibility of Gram-negative bacteria under the tested conditions. This difference may be attributed to the enhanced interaction of Ag^+ ions and reactive oxygen species (ROS) with the outer membrane of *E. coli*, which facilitates nanoparticle penetration and oxidative stress [9].

The strong antibacterial activity of the nanocomposite can be ascribed to primary mechanisms of release of Ag^+ ions – silver ions attach to bacterial membranes, penetrate into cells, and interfere with DNA replication and protein function [10]. Overall, these findings confirm that Ag-doped NiO–ZnO nanocomposites possess potent antibacterial properties, with Gram-negative *E. coli* displaying greater sensitivity than Gram-positive *S. aureus*.

4. Conclusion

The present study successfully demonstrated the green co-precipitation synthesis of silver (Ag)-doped NiO–ZnO nanocomposites using *Aegle marmelos* (bael) leaf extract. Comprehensive characterisation confirmed the successful synthesis and functional enhancement of the nanocomposites. XRD analysis indicated the coexistence of NiO (FCC), ZnO (wurtzite), and metallic Ag phases, demonstrating phase purity and nanoscale crystallite sizes (~20–30 nm). SEM imaging revealed spherical to irregular nanoparticles with porous, rough surfaces, providing a high surface-to-volume ratio and abundant active sites. Collectively, these results show that Ag doping preserves the parent oxide structures while improving particle dispersion and crystallinity. Optical studies further highlighted enhanced material functionality. The nanocomposites exhibited clear inhibition zones against Gram-positive and Gram-negative bacteria, with *E. coli* showing greater susceptibility due to improved nanoparticle penetration and ROS-mediated oxidative stress. Overall, the Ag-doped NiO–ZnO nanocomposites synthesised via *Aegle marmelos* extract combine nanoscale morphology, high crystallinity, enhanced visible-light response, and broad-spectrum antimicrobial activity, making them suitable for antimicrobial coatings, biomedical devices, wound dressings, water purification, environmental remediation, and photocatalysis. In conclusion, this study validates an eco-friendly, green synthesis approach for producing advanced multifunctional nanomaterials and highlights its potential contribution to sustainable science and societal benefit. The successful incorporation of silver into NiO-ZnO via a biogenic route paves the way for the development of eco-friendly, multifunctional nanocomposites capable of addressing challenges in healthcare, environmental protection.

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