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FACILE GREEN SYNTHESIS AND CHARACTERIZATION OF ZNO NANOPARTICLES AND THEIR BIOLOGICAL ACTIVITIES

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Abstract

The purpose of this research is to offer an environmentally friendly method for synthesising zinc oxide nanoparticles by utilising an extract from the fruit of Citrullus cooperi. The old methods of chemical synthesis are replaced by green strategy, which offers an environmentally benign and sustainable alternative. Fourier-transform infrared spectroscopy, ultraviolet-visible spectroscopy, scanning electron microscopy, and energy-dispersive X-ray analysis were utilised in order to acquire a comprehensive understanding of the ZnO nanoparticles that were created. Due to the fact that FTIR was able to confirm the presence of Zn-O bonds and organic components in the fruit extract, it was determined that the fruit extract served as a capping agent. However, scanning electron microscopy (SEM) revealed a crumpled and agglomerated look, whilst UV-Vis spectroscopy exhibited discrete absorption peaks. Both the elemental composition of the ZnO nanoparticles and their composition were validated by the EDX examination. The ZnO nanoparticles that were generated had a high antibacterial efficiency against Escherichia coli as well as a powerful antioxidant activity that was comparable to or even exceeded that of ascorbic acid. The findings demonstrated that the fruit extract of Citrullus colocynthis is effective as a reducing and capping agent in the environmentally friendly manufacture of zinc oxide nanoparticles, which may have significance for biological applications. In subsequent research, the focus should be on optimising the parameters of the synthesis process and investigating the safety and effectiveness of these ZnO nanoparticles in living organisms.

INTRODUCTION

The synthesis of nanoparticles has attracted considerable interest owing to their distinctive physicochemical features and many applications across multiple domains, including biomedicine, environmental remediation, and optoelectronics (Kalpana & Rajeswari, 2018)(Mumtaz et al., 2024). Traditional ZnO nanoparticles manufacturing methods frequently utilize toxic chemicals, require substantial energy, and entail intricate processes, prompting scientists to investigate more sustainable and environmentally friendly alternatives (Isa et al., 2021). A notable method is the green synthesis of ZnO nanoparticles, which employs natural resources, like plant extracts, microbes, or biomolecules, as reducing and capping agents (Isa et al., 2021)(Kalpana & Rajeswari, 2018). The eco-friendly synthesis of nanoparticles presents numerous benefits, such as gentle reaction conditions, diminished environmental effect, and the possibility of enhanced biocompatibility and biological activity (Kalpana & Rajeswari, 2018)(S. Ahmad, N. Ahmad, et al., 2024). This research report presents a simple green synthesis approach for the manufacture of ZnO nanoparticles and examines their structural, optical, and biological properties. ZnO nanoparticles have exhibited effectiveness against multiple bacterial species, such as *Escherichia coli* and *Staphylococcus aureus*, and have indicated potential in wound healing and cancer treatment. (Theivarasu and Thangavelu, 2017; Xie et al. 2023). The biological activity of ZnO nanoparticles is ascribed to multiple mechanisms, including the production of reactive oxygen species, the release of zinc ions, and interactions with cellular components. (Theivarasu and Thangavelu, 2017; Jiang et al. 2018) The generation of reactive oxygen species (ROS) can provoke oxidative stress in bacterial cells, resulting in membrane impairment and cellular mortality, hence enhancing the noted antibacterial efficacy. The liberation of zinc ions may potentially interfere with cellular mechanisms and enhance antibacterial properties. Moreover, ZnO nanoparticles can engage with biological membranes and penetrate cells, thereby affecting cellular signaling cascades and gene expression (Zakharova et al., 2019)(N. Ahmad et al., 2024).

This study seeks to provide an efficient green synthesis method for the production of ZnO nanoparticles with a natural plant extract and to assess their structural, optical, and biological characteristics.

Methodology

Synthesis and Optimization of ZnO NPs

ZnO nanoparticles were manufactured with a green technique that employed *Citrullus colocynthis* fruit extract as both a reducing and capping agent. Zinc acetate dihydrate served as the precursor salt. The synthesis procedure entailed combining 1.61g of zinc acetate with 150 mL of the fruit extract, thereafter heating and maintaining continuous agitation. A brownish precipitate signified the synthesis of ZnO nanoparticles. The precipitate was further subjected to centrifugation at 5000 rpm for 15 minutes, thoroughly rinsed with distilled water to eliminate contaminants, and dried in an oven at 80°C for 8 hours. The dried ZnO nanoparticles were calcined at 300°C for three hours (S. Ahmad, M. A. Ahmad, et al., 2024).

The synthesis settings were refined to attain excellent nanoparticle production. The reaction time was assessed at 30, 40 and 60-minute intervals, with absorbance readings obtained by a spectrophotometer. The influence of temperature was examined by performing the reaction at ambient temperature, 35°C, 50°C, 60°C and 80°C.

Antimicrobial Efficacy

The antibacterial efficacy of the produced ZnO nanoparticles was evaluated against *Escherichia coli*. The bacterial strain was acquired from the Biotechnology Department of Abdul Wali Khan University Mardan and cultivated in nutritional broth.

Evaluation of Antibacterial Activity

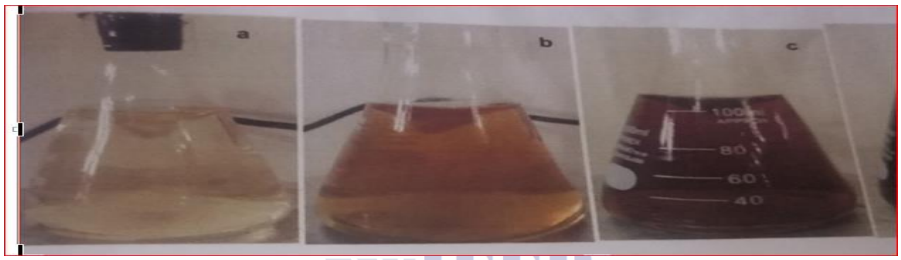
The antibacterial efficacy of the produced ZnO nanoparticles and *Citrullus colocynthis* fruit extract was assessed against *Escherichia coli* utilizing the well diffusion method. Stock solutions were formulated in distilled water at a concentration of 25 mg/mL. Two-fold dilutions were performed to achieve concentrations of 50 mg/mL and 100 mg/mL. Nutrient agar was autoclaved, dispensed into petri dishes and permitted to harden. A standardized

bacterial suspension was applied to the agar surface. Wells were created in the agar and the test samples were introduced. Streptomycin and distilled water functioned as positive and negative controls, respectively. The plates were incubated at 25°C for 24 hours and the zones of inhibition were quantified to evaluate antibacterial efficacy.

Results

Green Synthesis of ZnO NPs

Zinc oxide nanoparticles were manufactured via a green technique utilizing an aqueous extract of *Citrullus colocynthis* fruit. 1.79 g of zinc acetate solution, comprising approximately 1.61 g of zinc acetate dihydrate, was incorporated into 250 mL of the fruit extract. The solution was incessantly agitated using a magnetic stirrer. The reaction mixture's initial whitish hue progressively changed to dark brown after 30-40 minutes, with complete reaction occurring after 3 hours of constant agitation. The color alteration signified the synthesis of ZnO nanoparticles. The resultant nanoparticles were filtered through multiple centrifugation cycles at 5000 rpm for 15 minutes utilizing Eppendorf tubes. Three thousand milliliters of the ZnO nanoparticle solution was centrifuged, resulting in four grams of pure ZnO nanoparticles. The pure nanoparticles were retrieved from the bottom of the Eppendorf tubes following centrifugation and subsequently dehydrated in an oven.



Fig(1) a: Before solution, the result of plant extract always appear changes to plant extract.b: Plant extract and Zn (CH3CO2)2 are prepared after 30 to 40 minutes. C: plant extract and Zn $(CH_3CO_2)_2$, solution after 30 minutes.

Fourier-Transform Infrared Spectroscopy Analysis

FTIR study identified functional groups on produced ZnO NPs and examined their bonding properties. The large peak at 3284 cm^{-1} is likely caused by O-H stretching vibrations from hydroxyl groups on nanoparticle surfaces or leftover water molecules. Peaks in the 500-1000 cm^{-1} range indicate Zn-O stretching vibrations and the lattice structure. Additional C-H, C=C, and C-C bond peaks imply chemical substances from the *Citrullus colocynthis* fruit extract employed in the synthesis. Peaks detected above 2500 cm^{-1} may be caused by contaminants introduced during synthesis. More research and purification may be needed to reduce these contaminants.

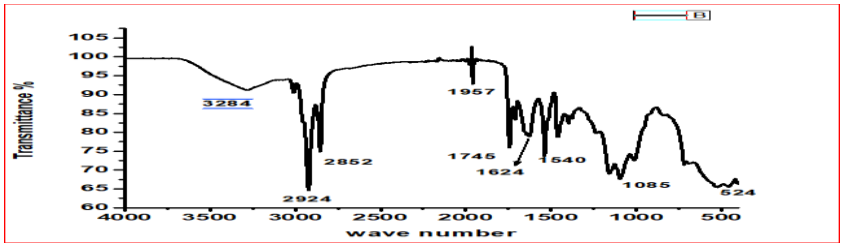
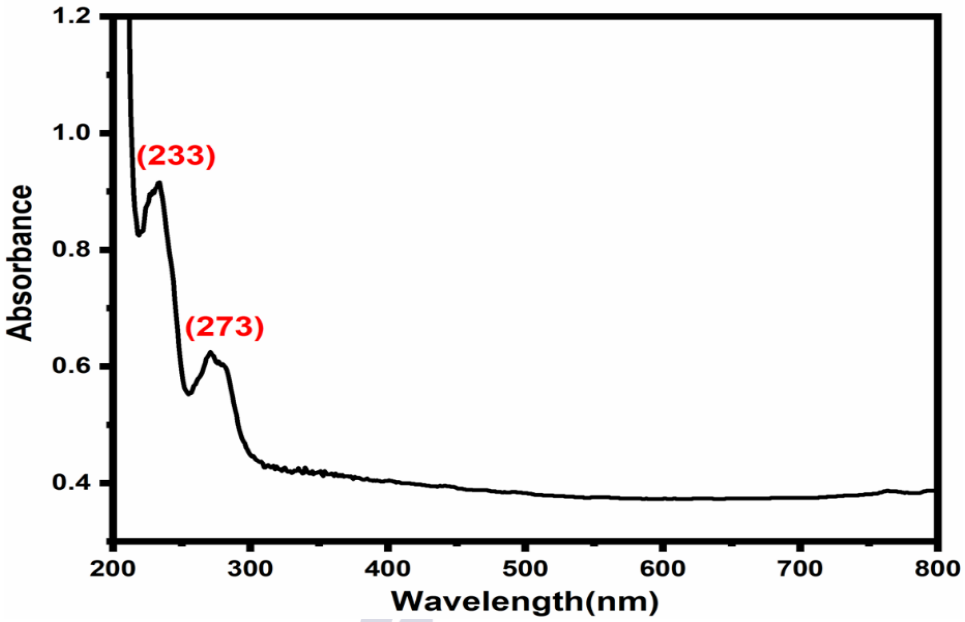


Fig (2) Fourier-transform infrared spectroscopy (FTIR) of (ZnO NPs)

UV-Visible ZnO Nanoparticles Analysis

The ZnO NPs' UV-visible absorption spectra was acquired using a PerkinElmer Lamda-25 UV-Vis spectrophotometer. In 100% ethanol, ZnO NPs were disseminated and absorbance measured from 200 to 800 nm. 233 and 273 nm are notable absorption peaks in the spectra.



Fig(3) UV-visible analysis of ZnO NPs

SEM and Energy-Dispersive X-ray Analysis

Synthesized ZnO NPs were wrinkled and agglomerated by SEM. The elemental composition of Fig 4 shows Zn and O from EDX analysis. The table shows the percentage composition, confirming ZnO NP production.

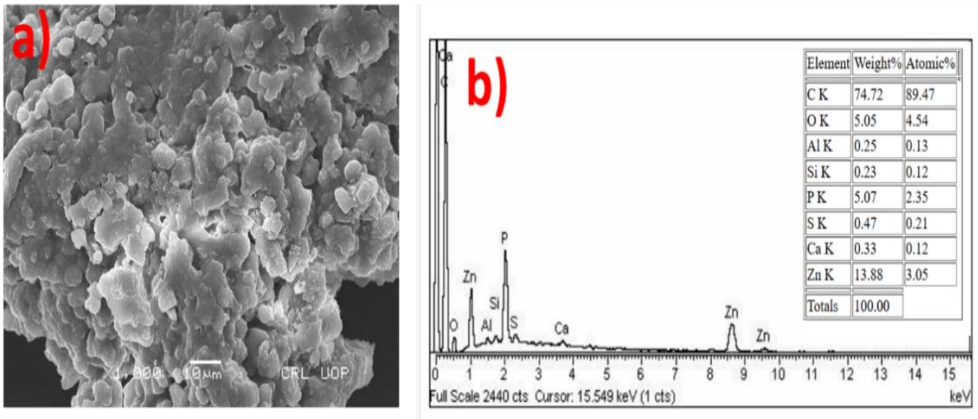


Fig 4 (a) (SEM) Result of (ZnO NPs) (b) (EDX) Result of (ZnO NPs)

Antibacterial Effect

ZnO NPs produced from *Citrullus colocynthis* fruit extract showed disc diffusion-based antibacterial action against *E. coli*. At 2 mg/mL extract concentration, a substantial inhibitory zone was found.

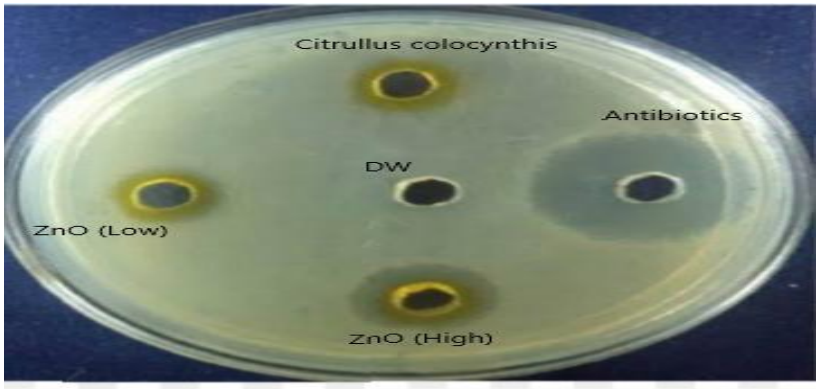


Fig 5 : Antibacterial activity of Zinc oxide nanoparticles from Citrullus colocynthis L. extracts

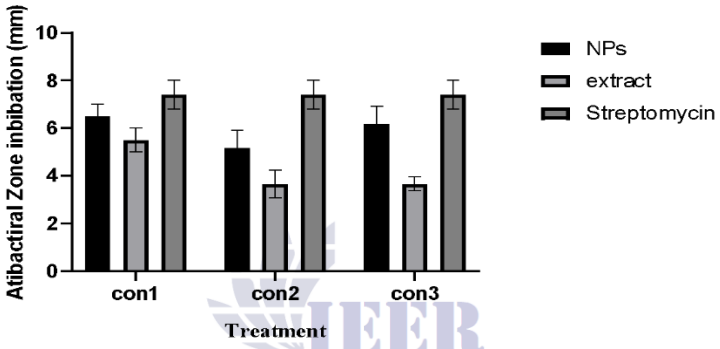


Fig 6 Antibacterial property of ZnO NPs

Antioxidant Capability

DPPH radical scavenging showed that ZnO NPs and *Citrullus colocynthis* fruit extract were antioxidants. The fruit extract effectively inhibited (97%) at 50 µg/mL, surpassing ascorbic acid (71%). Both NPs and extract showed significant inhibition (95.5%) at 100 and 150 µg/mL. These data imply that the extract and ZnO NPs may be free radical scavengers, warranting further in vivo safety and efficacy studies.

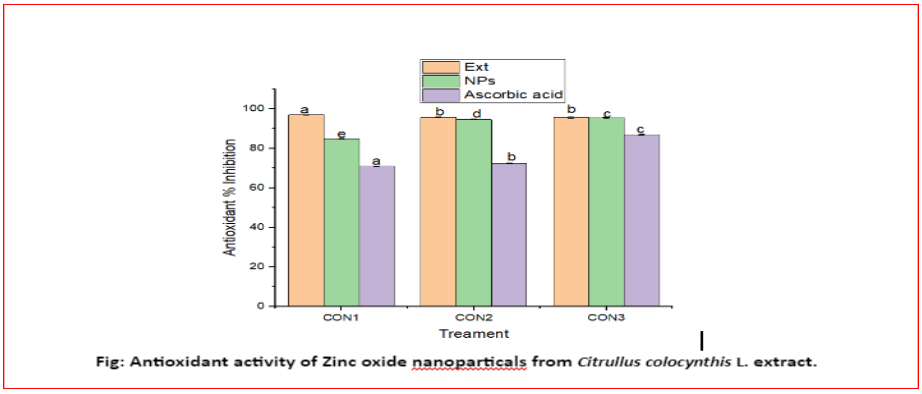


Fig 7: Antioxidant activity of Zinc oxide nanoparticles from *Citrullus colocynthis* L. extract.

Discussion

Green ZnO NP production utilizing *Citrullus colocynthis* fruit extract was successful and eco-friendly. The color change from pale to dark brown throughout synthesis showed ZnO NP production, consistent with prior reports (S. Ahmad, M. A. Ahmad, et al., 2024). ZnO bonds and O-H stretching vibrations, likely from surface hydroxyl groups or residual water, were detected by FTIR. C-H, C=C, and C-C linkages suggest the fruit extract caps nanoparticles, stabilizing them and possibly affecting their characteristics. Some contaminants were found, although purifying processes could reduce them. ZnO NPs absorb at 233 and 273 nm in the UV-Vis spectrum. These peaks were from ZnO electronic transitions and demonstrate nanoparticle production. Due to biomolecules from the plant extract, green produced nanoparticles were agglomerated. The EDX examination validated Zn and O elemental composition, verifying ZnO NP production. To assess nanoparticle stoichiometry, EDX quantitative analysis could be used (N. Ahmad et al., 2024).

The produced ZnO NPs showed promising *E. coli* antibacterial activity. These methods include the release of zinc ions, which break bacterial cell membranes and disturb cellular processes (Ghaffari Yaichi et al., 2025). ZnO NPs may potentially connect with bacterial cell walls and internalize, causing antimicrobial effects (Sadeghi-avalshahr et al., 2025). Further research could determine the mechanisms of action and assess antibacterial effectiveness against more microorganisms. ZnO NPs and *Citrullus colocynthis* fruit extract had significant antioxidant activity, with the extract outperforming ascorbic acid. This significant antioxidant capacity shows that the extract and ZnO NPs can scavenge free radicals, which cause oxidative stress (El-Shazoly et al., 2025). Further research is needed to determine the mechanisms of antioxidant activity and their therapeutic potential. This study shows the green production of ZnO NPs utilizing *Citrullus colocynthis* fruit extract, indicating biomedical uses. To improve synthesis parameters, examine biological activity processes, and assess in vivo safety and efficacy, more research is needed.

Conclusion

This work showed green ZnO NPs production utilizing *Citrullus colocynthis* fruit extract. FTIR, UV-Vis, SEM, and EDX confirmed the production and elemental composition of produced ZnO NPs. ZnO NPs also showed potential antibacterial action against *E. coli* and antioxidant activity comparable to or greater than ascorbic acid. These findings suggested *Citrullus colocynthis* fruit extract could be a sustainable and eco-friendly reducing and capping agent for ZnO NPs with interesting biomedical applications. Optimizing synthesis parameters, understanding biological activity pathways and testing ZnO NPs' in vivo safety and efficacy are priorities for future research.

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