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Eco-Friendly Synthesis of ZnO Nanoparticles from Withania Somnifera and Targeting Oral Pathogens

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Background: Zinc oxide nanoparticles (ZnONPs) have garnered attention for their antimicrobial properties, making them promising agents in dentistry. This study focuses on the green synthesis of ZnONPs mediated by Withania somnifera and evaluates their effectiveness against oral pathogens Streptococcus mutans and Candida albicans.
Methods: ZnONPs were synthesized using an aqueous extract of Withania somnifera. The morphological characteristics of the nanoparticles were examined using Scanning Electron Microscopy (SEM), while Fourier Transform Infrared (FTIR) spectroscopy was utilized to identify functional groups involved in the reduction of Zn^{2+} ions. Antimicrobial activity was assessed through zone of inhibition assays against S. mutans and C. albicans at varying concentrations (50 µg/mL and 100 µg/mL).
Results: SEM images revealed that the synthesized ZnONPs exhibited a rough and irregular surface morphology, with a tendency to cluster and aggregate. FTIR analysis indicated a strong broad peak at 3481.35 cm^{-1}, signifying the presence of hydroxyl groups from phenolic compounds, which are crucial for the reduction process. The antimicrobial assay results demonstrated a zone of inhibition of 13.2 mm (control), 6.9 mm (50 µg/mL), and 16.4 mm (100 µg/mL) for S. mutans, while C. albicans showed 13.2 mm (control), 9.5 mm (50 µg/mL), and 12.8 mm (100 µg/mL). Notably, S. mutans exhibited the greatest inhibition at 100 µg/mL, while C. albicans showed reduced inhibition compared to the control.
Conclusion: The study highlights the effectiveness of Withania somnifera-mediated ZnONPs as potential antimicrobial agents against dental pathogens. The

findings suggest that these nanoparticles could be developed into novel therapeutic strategies for oral healthcare, addressing the increasing resistance to conventional antimicrobial agents.
Keywords: Zinc oxide nanoparticles, Fourier Transform Infrared, Scanning Electron Microscopy.

1. Introduction

Nanotechnology is the most important dynamic exploration region in current of material science (Islam et al.,2022). The significant growth in nanotechnology is best evidenced by the number of scientific articles and its many applications (Collares et al., 2020). Recently, the scientific research community worldwide expressed interest in synthesizing metal and metal oxide nanoparticles (NPs) (Amendola et al., 2020). ZnO-NPs are of great importance due to their wide variety of applications in photocatalysis, water purification, and antibacterial disinfection. ZnO-NPs display properties that are distinct from those of typical NPs (Llama et al, 2019). The biological activities of ZnO-NPs are size and morphology dependent, and are the subject of investigation by many researchers (Kubiak et al., 2022), (Hussien et al., 2022). ZnO-NPs are considered a multi-purpose option and in recent years, research has focused on these metallic NPs due to their remarkable antimicrobial properties (Shahi et al., 2018).

The antibacterial effects of these nanostructured agents is attributed to the high surface/volume ratio since it provides a greater contact area with agents in the environment. The ability to easily penetrate cell membranes disrupts various intracellular processes, resulting in high reactivity and antibacterial activity (Kaya et al., 2021), (Song et al., 2019). The incorporation of ZnO into dental components has received special attention, representing an effective alternative for biomedicine, specifically in oral health (Jiang et al., 2016). Although antimicrobial compounds have been reported to decrease the occurrence of dental disease, the use of antibiotics and chemical bactericides can have a negative impact on the bacterial flora of the oral cavity and intestinal tract (Chung et al., 2015), (Keskin et al., 2021). Since pathogens can acquire resistance against different antibiotics, agents that are characterized for having remarkable antibacterial activity and do not develop resistance are now in high deman (Sena et al., 2006), (Medina et al., 2021).

Withania somnifera Dunal (Solanaceae), popularly called ashwagandha, is a medicinal plant and its extracts are com- monly used to cure various diseases. It possesses anti-tumor, anti-stress, anti-oxidant, anti-inflammatory, hematopoietic, anti-aging, immunomodulatory, anxiolytic and anti-depres- sive properties (Vinodhini et al., 2020). In this study, W. somnifera leaf extract-assisted ZnO NPs were synthesized (Ws-ZnO NPs) by green approach and its antibacterial effect was investigated.

2. Materials and methods

2.1 Preparation of plant extract

The plant leaves of W. somnifera collected and plant aqueous plant extract was prepared by mixing 5 g of washed and dried fine powdered leaves with 250 mL saline water wash and

ethanol wash twice and was boiled for 20 mins. After cooling to room temperature, the extract was filtered by Whatman no. 1 filter paper. The extract was stored at 4C for further applications.

2.2 Biological Synthesis of ZnO NPs from W. somnifera

Green synthesis of ZnO NPs were carried out using plant extract of W. somnifera by dropwise addition to the 0.5 g zinc acetate solution, which was stirred at 1200 rpm and 50C. The solution was kept under observation until the color changes to whitish yellow. The NPs were then extracted from the solution after it had been centrifuged for 15 minutes at 9000 rpm. After three rounds of deionized water washing, the NPs were collected and centrifuged once more. ZnONPs that were produced were stored at 4C for characterization investigations and additional testing. The synthesis was carried out in temperature tests at 60°C, 80°C, and 100°C. In order to conduct pH studies, 1 M sodium hydroxide was used to raise the pH of the zinc acetate solution to 7 or 12.

2.3 Characterization of WS-ZnONPs

2.3.1 FTIR Analysis

The functional groups present in the ZnONPs were characterized using a Vertex 70 ATR-FTIR spectrometer (Bruker). The analysis was performed over a spectral range of 4000–400 cm^{-1}, with the FTIR detector operating at a high resolution. The ZnONPs sample was prepared by placing a small amount of the synthesized material on the ATR crystal. The infrared radiation passed through the sample, and the absorbance at different wavelengths was recorded, revealing the specific vibrational frequencies corresponding to different chemical bonds. This helped in identifying the functional groups involved in the stabilization of ZnONPs (Kiani et al., 2022).

2.3.2 SEM Analysis

The morphology and particle size of the ZnONPs were examined using field-emission scanning electron microscopy (FESEM), specifically with a Hitachi SU-5000 microscope (Netherlands). A small amount of the sample was dispersed onto a carbon-coated copper grid. The grid was then mounted on the FESEM stub and sputter-coated with a thin layer of conductive material to prevent charging under the electron beam. The sample was scanned at high resolution, and the SEM images were captured, providing detailed information about the shape, size, and surface texture of the ZnONPs (Kiani et al., 2022).

2.4 Biological application

2.4.1 Antibacterial Activity

The antibacterial activity of ZnONPs was assessed using the agar diffusion method. The test organisms included the Gram-positive bacterium Staphylococcus mutans and the fungal strain Candida albicans. Mueller-Hinton agar (MHA) was sterilized by autoclaving at 121°C for 20 minutes and poured into sterile petri dishes, where it was allowed to solidify. Bacterial cultures were prepared by growing the organisms overnight in broth and adjusting their density to 1.5 × 10^8 cells/mL using the 0.5 McFarland turbidity standard. Spread plates were prepared by inoculating 100 µL of bacterial culture onto the surface of the solidified agar. Wells were then cut into the agar, and 100 µL of ZnONPs suspension was added to each well. Chloramphenicol

(10 µg/mL) served as the positive control, and the plates were pre-incubated at room temperature for 1 hour to allow proper diffusion of the ZnONPs into the agar. The plates were subsequently incubated at 37°C for 24 hours. After incubation, the zone of inhibition around the wells was measured to assess the antibacterial and antifungal potential of the ZnONPs. The diameter of the clear inhibition zones indicated the effectiveness of ZnONPs against the tested microbial strains (Kiani et al., 2022).

3 Results

3.1 Scanning Electron Microscopy (SEM) analysis

The SEM image of Withania somnifera-mediated ZnO nanoparticles reveals a rough and irregular surface morphology. The particles appear to be clustered and aggregated, indicating that the nanoparticles might form larger structures due to the inherent tendency of ZnO nanoparticles to agglomerate. The magnification shows particles in the nanoscale range, with individual nanoparticles appearing to have a granular structure. The average particle size seems to be below 100 nm, as evident from the scale bar, which is consistent with other reports on green-synthesized ZnONPs. This nanoscale size range is critical for the antimicrobial and other biological properties of ZnONPs, as smaller particles generally exhibit enhanced surface area-to-volume ratios, which facilitates better interaction with microbial cells. The clustered nature of the nanoparticles could be due to the presence of organic compounds from Withania somnifera extract acting as capping agents, stabilizing the nanoparticles but also leading to some degree of aggregation. The surface roughness observed in the SEM image further supports the hypothesis that bioactive molecules from the plant extract are coating the ZnONPs, potentially enhancing their stability and biological activity (Fig.1).

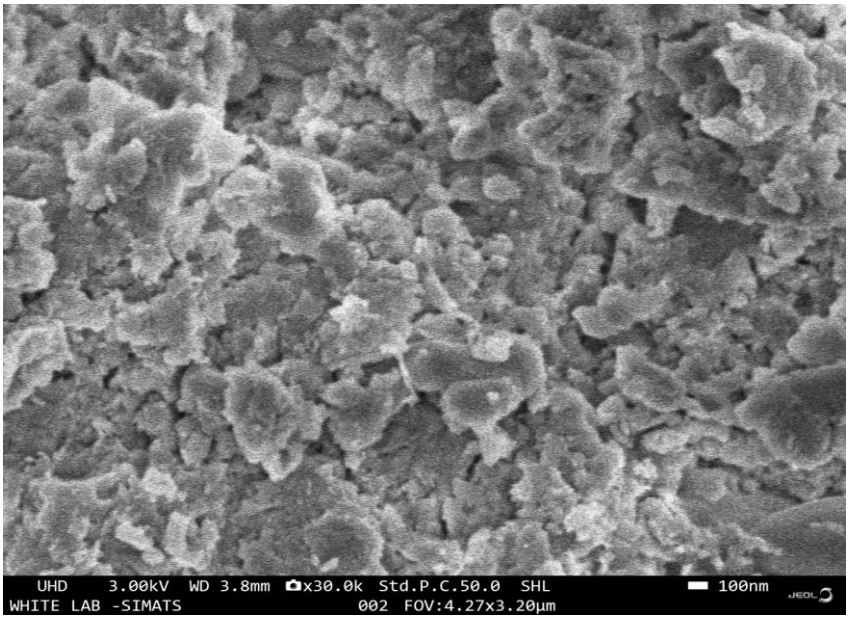

Fig.1. Scanning Electron Microscopy of WS- ZnONPs

3.2 FTIR analysis

The FTIR analysis of Withania somnifera-mediated ZnONPs revealed several distinct peaks corresponding to various functional groups, confirming the involvement of phytochemical constituents in the synthesis and stabilization of the nanoparticles (Fig.2). A strong broad peak at 3481.35 cm^{-1} was attributed to O-H stretching, indicating the presence of hydroxyl groups from phenolic compounds, which play a crucial role in reducing Zn^{2+} ions to ZnONPs. The peak at 2352.24 cm^{-1} corresponds to O=C=O stretching, suggesting the involvement of carbonyl groups, potentially from organic acids in the plant extract. Additionally, the C=C=O stretching at 2189.6 cm^{-1} and N=C=O stretching at 2086.09 cm^{-1} indicate the presence of unsaturated and isocyanate compounds, which might contribute to nanoparticle formation. The peaks observed at 1994.68 cm^{-1} (N=C=S stretching) and 1641.79 cm^{-1} (C=N stretching) suggest the presence of amides or similar nitrogen-containing compounds, which may contribute to both the reduction and stabilization of ZnONPs. The S=O stretching observed at 1348.88 cm^{-1} indicates the possible involvement of sulfonate groups, which might enhance the stability of the nanoparticles. Peaks at 1027.68 cm^{-1} (C-F stretching), 892.98 cm^{-1} (C=C bending), and 834.41 cm^{-1} (C-H bending) demonstrate the participation of aliphatic and aromatic compounds, contributing further to nanoparticle stabilization. The presence of halogen-containing functional groups, as evidenced by C-Br (628.93 cm^{-1}) and C-I stretching (593.16 cm^{-1} and 575.95 cm^{-1}), suggests that bioactive compounds in Withania somnifera may impart unique properties to the ZnONPs. These findings highlight the role of various phytochemicals in the green synthesis of ZnONPs, confirming that Withania somnifera extract acts as both a reducing and capping agent during the synthesis process (Table.1).

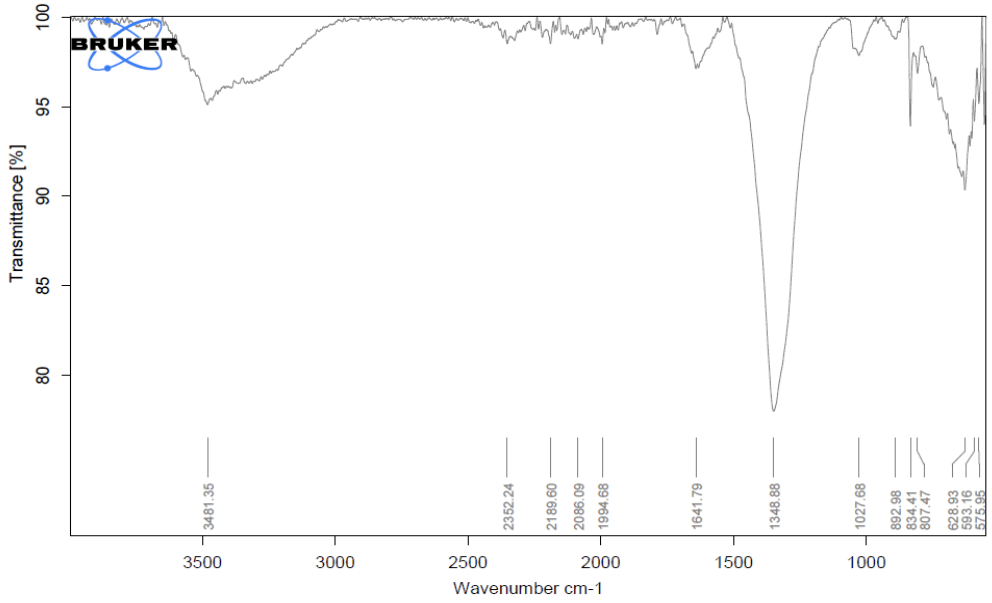

Fig.2. FTIR analysis of WS-ZnONPs

PEAK VALUE	GROUP
3481.35	O-H stretching
2352.24	O=C=O stretching
2189.6	C=C=O stretching
2086.09	N=C=O stretching
1994.68	N=C=S stretching
1641.79	C=N stretching
1348.88	S=O stretching
1027.68	C-F stretching
892.98	C=C bending
834.41	C-H bending
807.47	C-H bending
628.93	C-Br stretching
593.16	C-I stretching
575.95	C-I stretching

Table.1. FTIR peak analysis for WS-ZnONPs

3.3 Biological application

3.3.2 Antimicrobial activity (Well Diffusion Method)

The antimicrobial efficacy of Withania somnifera-mediated ZnO nanoparticles was assessed against the oral pathogens Streptococcus mutans and Candida albicans using the agar diffusion method, with chloramphenicol as a positive control (Fig.3). For S. mutans, the control exhibited a zone of inhibition of 13.2±0.3 mm (Fig.4). WS-ZnONPs at a concentration of 50 µg/mL showed a zone of 6.9±0.5 mm, which increased significantly to 16.4±0.3 mm at 100 µg/mL, surpassing the control and demonstrating strong antibacterial activity. In the case of C. albicans, the control produced a zone of inhibition of 13.2±0.2 mm. At 50 µg/mL, WS-ZnONPs achieved a zone of 9.5±0.7 mm, which improved to 12.8±0.4 mm at 100 µg/mL, approaching the efficacy of the control. These results highlight the concentration-dependent antimicrobial activity of WS-ZnONPs, with higher concentrations displaying superior efficacy, particularly against S. mutans, a major contributor to dental caries. The observed inhibition may be attributed to the nanoparticles' ability to disrupt microbial cell membranes and induce oxidative stress, leading to cell death. Given these promising results, WS-ZnONPs offer great potential as a natural, effective alternative to conventional antibiotics in oral care products such as toothpaste or mouthwash, particularly for combating oral pathogens like S. mutans and C. albicans.

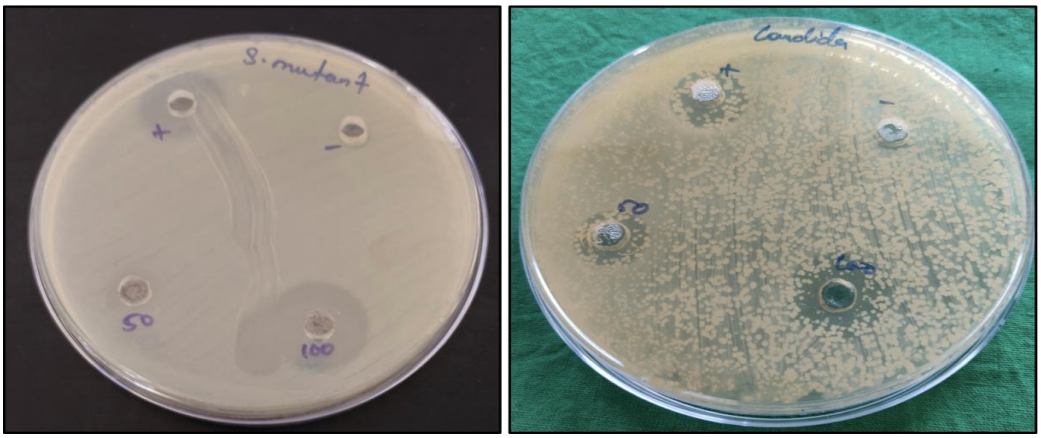

Fig.3. Antibacterial activity of synthesized WS-ZnONPs against oral pathogens

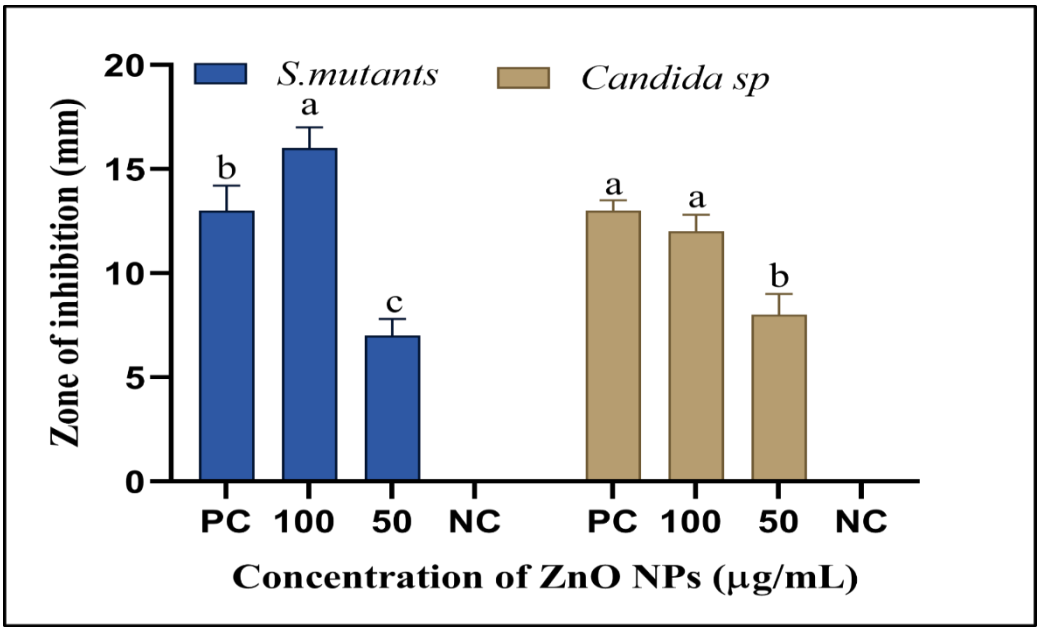

Fig.4. Zone of inhibition on S. mutant and Candida albicans Mean values within the column followed by the same letter in superscript are not significantly different at $p< 0.05$ level.

4 Discussion

Herbal products have gained global popularity due to their appealing qualities, such as being safe, non-toxic, and readily available at a low cost. The limitations and side effects associated with modern pharmaceuticals have driven the growing interest in the herbal drug industry worldwide. Researchers are particularly drawn to ZnONPs for their versatile applications in wound healing, antibacterial, antifungal, anti-inflammatory, antioxidant, and optical fields. Nanotechnology has rapidly advanced, and ZnONPs have emerged as a promising tool in this domain (Bisht et al., 2014).

Kumari et al. (2015) conducted a toxicity study on green-synthesized ZnONPs using Amaranthus caudatus leaf extract and demonstrated notable antibacterial activity against Staphylococcus epidermidis and Enterobacter aerogenes. Another study by Alam et al., (2012) highlighted that methanolic leaf extract of Withania somnifera effectively inhibited methicillin-resistant Staphylococcus aureus and Enterococcus species, with inhibition zones of 20.6 mm and 19.4 mm, respectively, at a concentration of 2 mg/ml (100 μL).

Further research showed that Withania somnifera-mediated ZnO NPs exhibited antibacterial activity comparable to ciprofloxacin, particularly against E. faecalis and S. aureus, suggesting that Ws-ZnONPs could serve as potential alternatives to conventional antibiotics. The enhanced antibacterial effect of Ws-ZnONPs may be attributed to the presence of somniferin in the leaves and the small particle size (Alam 2012).

Additionally, Mamta Kumari et al. (2015) demonstrated that aqueous root extract of W. somnifera holds significant antibacterial potential against E. coli O78, supporting its use in traditional Ayurvedic medicine and providing a basis for alternative drug development. In another study, W. somnifera leaf extract showed the highest antibacterial activity against Salmonella typhi (32.00 ± 0.75 mm zone of inhibition) and the lowest against Klebsiella pneumoniae (19.00 ± 1.48 mm zone of inhibition), further confirming its efficacy against Gram-negative bacteria, particularly S. typhi.

5 Conclusion

The ZnO nanoparticles synthesized using Withania somnifera demonstrated significant antibacterial potential against Streptococcus mutans and Candida albicans. These ZnONPs show promise as effective agents against multidrug-resistant microorganisms, offering a potential alternative to conventional antibiotics. This study lays the foundation for further exploration of innovative methodologies and clinical applications in antimicrobial treatments. Future studies should focus on cytotoxicity assessments and evaluate the application of ZnO NPs in products such as toothpaste and mouthwash. Additionally, the binding capacity of ZnO NPs to the surface of Candida albicans warrants further investigation.

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