ANTIMICROBIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES USING SELECTED MEDICINAL PLANT EXTRACT

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Abstract

Zinc oxide nanoparticles (ZnO NPs) synthesized using extracts from medicinal plants were characterized and evaluated for their antimicrobial properties. Scanning electron microscopy (SEM) and Zeta sizer were used for characterization and to study the size. The nanoparticles exhibited significant antibacterial activity against a range of pathogens, including bacteria. These results suggest that ZnO NPs synthesized from medicinal plant extracts hold promise for applications in medicine and environmental remediation. Zinc oxide nanoparticles (ZnO NPs) synthesized via green synthesis using medicinal plant extracts have emerged as an eco-friendly and cost-effective alternative to traditional synthesis methods. Green synthesis involves using plant-based materials, which act as both reducing and stabilizing agents, eliminating the need for harsh chemicals and high energy inputs typically associated with chemical or physical synthesis processes.

Keywords: Zinc oxide nanoparticles (ZnO NPs), Medicinal plant extracts, antimicrobial activity, Scanning electron microscopy (SEM), Environmental remediation

Introduction:

The field of nanoparticle technology is making significant strides, with its applications in medicine expanding at an impressive pace. Scientists have discovered that nanoparticles (NP) can be used as a promising solution to combat bacterial multidrug resistance, which has become a global issue due to the misuse of antibiotic [1]. Utilizing nanoparticles as antimicrobial agents has the potential to overcome bacterial resistance by directly targeting the bacterial cell wall. This microbicidal effect occurs without the nanoparticles needing to penetrate into the cell [2]. Given their small size and expansive surface area, nanoparticles possess distinct chemical as well as physical characteristics that set them apart from their larger counterparts. These characteristics render them highly desirable for a wide range of applications, such as delivery of drugs, image processing, and antimicrobial action [3]. When it comes to antimicrobial activity, nanoparticles can interact with microbial cells in various ways, ultimately resulting in cell death. One mechanism includes the immediate contact of nanoparticles alongside the membrane of bacterial cells, causing its structure to be disrupted and resulting in cell lysis. Another mechanism involves the production of ROS, or reactive oxygen species, by tiny particles, which can lead to oxidative stress and harm to microbial cells. In addition, tiny particles can disrupt microbial cell processes, including the activity of enzymes and cell signaling, which enhances their antimicrobial capabilities [4].

Zinc oxide nanoparticles (ZnO NPs) are highly regarded for their exceptional antimicrobial properties, making them a prime contender for various antimicrobial applications. When moisture is present, ZnO NPs can release zinc ions (Zn2+), which can effectively disrupt microbial cell membranes and prevent the growth of microorganisms [5]. In addition, ZnO NPs have demonstrated photocatalytic activity when exposed to ultraviolet (UV) light, which enhances their antimicrobial properties. With its unique properties, ZnO NPs offer a promising solution to address the challenge of antimicrobial resistance.

Plant extracts have been utilized in conventional healthcare for their antimicrobial qualities for centuries. These extracts contain a diverse range of bioactive compounds, including flavonoids that are alkaloids, which and phenolic substances, which have demonstrated antimicrobial activity [6]. When plant extracts are combined with nanoparticles, they may improve the antimicrobial effects of nanoparticles via complementary interactions. Plant extracts contain bioactive compounds that can target various components within microbial cells, thereby impeding the development of resistance by microbes. Ultimately, the utilization of zinc oxide nanoparticles produced through plant extracts shows immense potential in addressing the issue of antimicrobial resistance. Through the utilization of nanoparticles and the potent properties found in plant extracts, scientists can create powerful antimicrobial agents to combat the escalating issue of antimicrobial resistance.

Over the past few years, the rise of AMR (antimicrobial resistance) has presented a major hurdle for global public health. Excessive and incorrect use of antibiotics has led to the emergence of resistant strains of microbes, resulting in more challenging infections to manage and placing a greater strain on the medical system [7]. Investigators have been investigating substitute antimicrobial agents, such as ceramic nanoparticles as well as extracts from plants, which have demonstrated encouraging antimicrobial activity in their studies [8]. Zinc oxide nanoparticles (ZnO NPs) are attracting significant interest because of their distinct physical characteristics, including a substantial surface area, their biocompatibility, and antimicrobial capabilities. ZnO NPs possess properties that make them a promising option over antimicrobial substances. In addition, plant extracts have been utilized in medical practices for their antimicrobial effects [9]. Recent studies have also demonstrated their ability to increase the antimicrobial agent activity of tiny particles. This study seeks to explore the antimicrobial properties of ZnO nanoparticles that have been created using specific extracts from medicinal plants [10]. The chosen plant extracts have well-established antimicrobial properties and consist of extracts from plants like neem (Azadirachta indica) and Ashwagandha. The combination of plant extracts and nanoparticles is intriguing because of their powerful antimicrobial effects when used together. Plant extracts contain a wide range of bioactive compounds, including flavonoids, alkaloids, and phenolic compounds. These compounds can boost the antimicrobial activity of nanoparticles by targeting various aspects of microbial cells [11-13]. In addition, incorporating plant extracts can help decrease the cytotoxicity of nanoparticles, enhancing their safety for various biomedical applications. Furthermore, it has been demonstrated that using green technology to synthesize NPs is more dependable and efficient than using physico-chemical methods [14].

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Mechanical strength, chemical reactivity, biological characteristics, and diffusivity are all enhanced when a material's particle size drops from the micrometer to the nanometer region [15]. The synthesized ZnO NPs will be tested for their antimicrobial activity against a range of infectious microbes, including microbial and fungal pathogens. This study will offer helpful insights into the possible use of ZnO NPs synthesized using extracts from medicinal plants as antimicrobial agents. These findings could help with the establishment of innovative strategies for combating antimicrobial resistance.

Materials & Method:

Characteristics of synthesis ZnO nanoparticles: The dimensions and structure of the produced zinc oxide nanoparticles (ZnO NPs) were analyzed utilizing advanced imaging methods such as scanning electron microscopy (SEM) and Zeta sizer.

Bacterial growth conditions:

The growth conditions for Escherichia coli and Bacillus subtilis were optimized to ensure robust and reproducible results. E. coli cultures were grown in Luria-Bertani (LB) broth at 37°C with constant agitation at 180 rpm to promote aeration. For B. subtilis, growth was carried out in nutrient broth at 30°C under similar agitation conditions to support aerobic metabolism. Both organisms were cultured to the mid-logarithmic phase to ensure active cell division, with the optical density at 600 nm (OD600) monitored to assess growth. These conditions provided an ideal environment for the respective bacteria, enabling consistent experimental outcomes.

Antimicrobial activity: Testing the antimicrobial properties of zinc oxide nanoparticles (ZnO NPs) specifically for Escherichia coli (E. Coli) and B. Subtilis. As part of the procedure, ZnO NPs are prepared and then carefully analysed to determine their size, morphology, structure, and optical properties.

Nutrient agar plates are prepared to evaluate the effect of concentrations of zinc oxide nanoparticles (ZnO NPs) on bacterial growth, specifically to gram negative Escherichia coli (E. Coli) and gram positive Bacillus subtilis nonpathogenic bacterial strains. The nutrient agar powder is dissolved in distilled water to create a nutrient-rich agar solution. This solution is then poured into sterile Petri dishes and left to solidify. Various concentrations of ZnO NPs are incorporated into the discs of Whatman filter paper (6mm size) and placed to the agar media plates after the spreading of E. coli and B. Subtilis broth culture to agar plate surface, resulting plates having with different ZnO NP concentrations on discs. The growth of E. Coli and B. Subtilis are then closely monitored to examine the impact of ZnO NPs on bacterial growth and study the zone of inhibition.

Statistical analysis is employed to assess the significance of the findings, such as the minimum inhibitory concentration (MIC) of ZnO NPs. This assay offers helpful insights through the antimicrobial properties of ZnO NPs and its potential utilization in regulating the growth of bacteria.

Result & Discussion:

The results of the study provided a comprehensive characterization of zinc oxide (ZnO) nanoparticles and their antimicrobial activities. Scanning electron microscopy (SEM) images (Figure 1) revealed that the photosynthesized ZnO nanoparticles were uniformly distributed with an average size of approximately 20 nm, predominantly spherical in morphology, indicating successful synthesis. The Zeta sizer (Figure 2) confirmed the crystalline nature of the ZnO nanoparticles, showing an average size of around 90 nm.

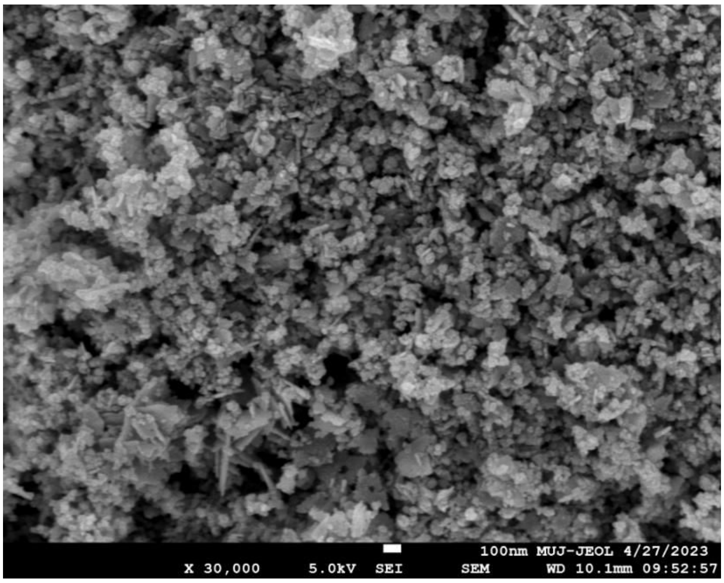

Figure 1 - SEM images of the Photosynthesized ZnO NPs

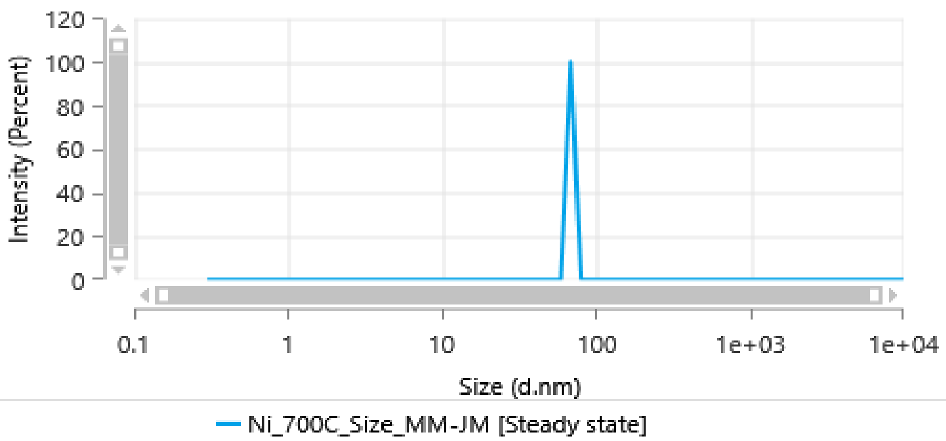

Figure 2 – ZETA sizer analysis of ZnO nanoparticles

The antimicrobial activity assessment demonstrated significant antibacterial properties of ZnO nanoparticles. In the disc diffusion method, ZnO nanoparticles exhibited notable antibacterial activity against Bacillus subtilis at the highest concentration, with zones of inhibition measuring 12 mm at a concentration of 2 mg/ml and no zone of inhibition less than 2 mg/ml concentration of ZnO nanoparticles (Figure 3). Similarly, effective antibacterial activity was observed against Escherichia coli, with inhibition zones of 13 mm at 1 mg/ml and 20 mm at 2 mg/ml (Figure 4).

Test Control

Figure 3 - Zone of Inhibition (Bacillus subtilis)

The disc diffusion assay for Escherichia coli (Figure 4) showed zones of inhibition around the disc containing ZnO nanoparticles, with more pronounced inhibition at higher concentrations.

Test Control

Figure 4 - Zinc Oxide Nanoparticles (disc diffusion, E. Coli A-Test and B control)

The antimicrobial activity of ZnO nanoparticles highlighted their broad-spectrum antibacterial properties, effectively inhibiting the growth of both Gram-positive and Gram-negative bacteria. This demonstrated the potential of ZnO nanoparticles as promising candidates for various antimicrobial applications.

The results of this study provide compelling evidence for the effective synthesis and characterization of zinc oxide (ZnO) nanoparticles and their significant antimicrobial properties. The successful synthesis of uniformly distributed ZnO nanoparticles with an average size of approximately 20 nm, as observed through TEM images, indicates the potential

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of phytosynthesis using medicinal plant extracts. This method is advantageous due to its environmentally friendly nature and the ability to produce nanoparticles with desirable morphological characteristics.

The antimicrobial activity assays revealed significant antibacterial properties of ZnO nanoparticles against both Bacillus subtilis and Escherichia coli. The zones of inhibition in the disc diffusion method demonstrated the effectiveness of ZnO nanoparticles in inhibiting bacterial growth. The larger inhibition zones at higher concentrations suggest a dose-dependent antibacterial activity, which is consistent with the production of reactive oxygen species (ROS) that damage bacterial cell components, ultimately leading to cell death. The activity against both Gram-positive and Gram-negative bacteria highlights the versatility of ZnO nanoparticles as antimicrobial agents.

The comparison of two strains of bacteria revealed slight variations in susceptibility, with Escherichia coli showing larger inhibition zones. This could be attributed to differences in cell wall composition and permeability, which affect the uptake and interaction of ZnO nanoparticles with bacterial cells. The crystallite analysis using zeta sizer patterns confirmed the high purity and quality of the synthesized nanoparticles, which is crucial for ensuring consistent antimicrobial performance.

Table 1- Antimicrobial Activity of ZnO

S.no	Compounds	Concentration of compounds	Zone of Inhibition (Bacillus subtilis) In mm	Antimicrobial activity of compound	Zone of Inhibition (Escherichia coli) In mm	Disc Diameter (in mm)	Antimicrobial activity of compound	Control (DMSO)
1	ZnO Green Nanoparticles	2 mg	12	+++	13	6	++++	No activity found
		1 mg	--	---	12	6	+++	
		.5 mg	--	---	9	6	++	
		.125 mg	--	---	7	6	+	

The antimicrobial activity of ZnO nanoparticles against Bacillus subtilis and E. Coli was tested. With millimeter-measured inhibition zones, compound ZnO was tested at 0.25 mg, 0.5 mg, 1mg and 2 mg. ZnO nanoparticle zone of inhibition at 2 mg was 12 mm for Bacillus subtilis indicating moderate antimicrobial activity (++). At 2 mg, 1 mg, 0.5 mg and 0.25mg for E. coli zone of inhibition was 13 mm, 12 mm, 9 mm and 7 mm respectively for the concentration of ZnO nanoparticles concentrations.

ZnO nanoparticles showed slight antimicrobial activity against Bacillus subtilis and high for Escherichia coli at 2 mg, with zones of inhibition of 12 mm and 13 mm, respectively. Maintaining antimicrobial activity for E. coli but not for Bacillus subtilis. zone of inhibition decreased to 7 mm for Escherichia coli but zones of inhibition against Bacillus subtilis at 1 mg and below no activity observed.

No DMSO-treated control group had antimicrobial activity against either bacterium. Compounds ZnO have varying degrees of antimicrobial activity against Bacillus subtilis and E. Coli.

The study demonstrates that ZnO nanoparticles synthesized using medicinal plant extracts possess significant antimicrobial properties, with numerous applications across various sectors [16] such as in medicine, environmental settings, and act as effective photocatalyst in water purification applications. Zinc oxide nanoparticles are advantageous for agricultural applications as well due to their antibacterial characteristics. The findings demonstrated the superior antibacterial activity of the smaller ZnO NP [17-19]. The environmentally friendly synthesis method, coupled with the nanoparticles' broad-spectrum antibacterial activity, offers a sustainable and effective solution to combat bacterial multidrug resistance. Additionally, targeted metal nanoparticles can help stop the virus and infections from spreading to essential organs [20]. Future research could explore the synergistic effects of combining ZnO nanoparticles with other antimicrobial agents, as well as their potential applications in real-world scenarios to further validate their efficacy and safety.

Conclusion:

Zinc oxide nanoparticles (ZnO NPs) made from extracts of Azardica indica shows a great promise as an efficient and sustainable substitute for traditional nanoparticle manufacturing techniques. Utilizing biomolecules obtained from Azardica indica as stabilizing and reducing agents, the green synthesis approach lowers manufacturing costs and its impact on the environment. SEM and Zeta sizer were used for characterization, which verified the nanoparticles' effective production and desired morphological characteristics. With encouraging implications for both medicinal applications and environmental clean-up, their noteworthy antibacterial activity demonstrates their suitability in the fight against infections. These results highlight the potential of green-synthesized ZnO NPs as a flexible and environmentally benign nanomaterial to tackle present and upcoming issues in environmental sustainability and healthcare.

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Author contribution:

MIN: Conceptualization, data analysis, writing
SC: drafting & Editing
SZ: Analysis & Writing
FS: Editing
KKA: Supervision & Editing

Conflict of Interest:

Authors declare that there is no conflict of interest among the authors.

References:

1. Fernando, S., Gunasekara, T., & Holton, J. (2018, May 6). Antimicrobial Nanoparticles: applications and mechanisms of action. Sri Lankan Journal of Infectious Diseases, 8(1), 2. https://doi.org/10.4038/sljid.v8i1.8167
2. Wang, L., Hu, C., & Shao, L. (2017, February). The antimicrobial activity of nanoparticles: present situation and prospects for the future. International Journal of Nanomedicine, Volume 12, 1227–1249. https://doi.org/10.2147/ijn.s121956
3. Altammar, K. A. (2023, April 17). A review on nanoparticles: characteristics, synthesis, applications, and challenges. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1155622
4. Abdal Dayem, A., Hossain, M., Lee, S., Kim, K., Saha, S., Yang, G. M., Choi, H., & Cho, S. G. (2017, January 10). The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles. International Journal of Molecular Sciences, 18(1), 120. https://doi.org/10.3390/ijms18010120
5. Awasthi, A., Sharma, P., Jangir, L., Awasthi, G., Awasthi, K. K., & Awasthi, K. (2020). Dose dependent enhanced antibacterial effects and reduced biofilm activity against Bacillus subtilis in presence of ZnO nanoparticles. Materials Science and Engineering: C, 113, 111021.
6. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021, September 27). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms, 9(10), 2041. https://doi.org/10.3390/microorganisms9102041
7. Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015, September 7). Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health, 109(7), 309–318. https://doi.org/10.1179/2047773215y.0000000030
8. Wang, L., Hu, C., & Shao, L. (2017, February). The antimicrobial activity of nanoparticles: present situation and prospects for the future. International Journal of Nanomedicine, Volume 12, 1227–1249. https://doi.org/10.2147/ijn.s121956
9. Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., & Mohamad, D. (2015, April 19). Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters, 7(3), 219–242. https://doi.org/10.1007/s40820-015-0040-x
10. Pino, P., Bosco, F., Mollea, C., & Onida, B. (2023, March 17). Antimicrobial Nano-Zinc Oxide Biocomposites for Wound Healing Applications: A Review. Pharmaceutics, 15(3), 970. https://doi.org/10.3390/pharmaceutics15030970
11. Habeeb Rahuman, H. B., Dhandapani, R., Narayanan, S., Palanivel, V., Paramasivam, R., Subbarayalu, R., Thangavelu, S., & Muthupandian, S. (2022, April 15). Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. IET Nanobiotechnology, 16(4), 115–144. https://doi.org/10.1049/nbt2.12078
12. Awasthi, A., Bansal, S., Jangir, L. K., Awasthi, G., Awasthi, K. K., & Awasthi, K. (2017, December). Effect of ZnO nanoparticles on germination of Triticum aestivum seeds. In Macromolecular symposia (Vol. 376, No. 1, p. 1700043).
13. Mendes, C. R., Dilarri, G., Forsan, C. F., Sapata, V. D. M. R., Lopes, P. R. M., de Moraes, P. B., Montagnolli, R. N., Ferreira, H., & Bidoia, E. D. (2022, February 16). Antibacterial

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action and target mechanisms of zinc oxide nanoparticles against bacterial pathogens. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-06657-y

14. Awasthi, G., Maheshwari, T., Sharma, R., Kumawat, T. K., Singh, G. P., & Lodha, P. (2023). Actions and reactions of plants derived zinc-oxide nano-particles. Materials Today: Proceedings.
15. Shi, L. E., Li, Z. H., Zheng, W., Zhao, Y. F., Jin, Y. F., & Tang, Z. X. (2014). Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: a review. Food Additives & Contaminants: Part A, 31(2), 173-186.
16. Abebe, B., Zereffa, E. A., Tadesse, A., & Murthy, H. A. (2020). A review on enhancing the antibacterial activity of ZnO: Mechanisms and microscopic investigation. Nanoscale research letters, 15, 1-19.
17. Aldeen, T. S., Mohamed, H. E. A., & Maaza, M. (2022). ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. Journal of Physics and Chemistry of Solids, 160, 110313.
18. Sharma, R., Agrawal, A., Sharma, A., Kumar, S., Sharma, P. K., Awasthi, K. K., ... & Awasthi, A. (2022). Effect of biosynthesized ZnO nanoparticles on wheat seedlings. Materials Today: Proceedings, 69, 1587-1595.
19. da Silva, B. L., Caetano, B. L., Chiari-Andréo, B. G., Pietro, R. C. L. R., & Chiavacci, L. A. (2019). Increased antibacterial activity of ZnO nanoparticles: Influence of size and surface modification. Colloids and Surfaces B: Biointerfaces, 177, 440-447.
20. Ulker, D., Abacioglu, N., & Sehirli, A. O. (2022). Cerium oxide (CeO2) nanoparticles could have protective effect against COVID-19. Lett. Appl. NanoBioScience, 12, 12.