Phytogenic Fabrication of Zinc Oxide Nanoparticles Using Eclipta Alba and Evaluation of their In Vitro Antibacterial Activity

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**Abstract:** *Eclipta alba* is a well known medicinal plant which is used to treat gastrointestinal issues, skin conditions, fever, respiratory tract disorders, cuts and wounds. The plant contains a number of phytoconstituents, including luteolin, apigenin, ursolic acid, oleanolic acid, and saponins and have demonstrated antibacterial, anticancer, hepatoprotective, and neutralizing effects on snake venom. Zinc oxidenanoparticles is among one of the most researched studies conducted due to its ability to apply in varied downstream applications. These nanoparticles have been extensively used in various biological applications due to their nontoxic nature and the ability to change its physical and chemical properties. The current study aims to synthesize zinc oxide nanoparticles mediated by *Eclipta alba* and evaluate their in vitro antimicrobial activity against dental pathogens. Antimicrobial activity was evaluated by agar well diffusion assay,followed by characterization of synthesized nanoparticles by SEM, FTIR and EDX. This study concludes that ZnONPs mediated *Eclipta alba* showed significant antimicrobial activity against *S. mutans* (Zone of inhibition-13 mm at 100 µg/ml) and *C. albicans* (Zone of inhibition -14mm at 100 µg/ml).

**Keywords:** *Eclipta alba,* ZnO NPs, Antibacterial activity, SEM

# INTRODUCTION

*Eclipta alba* (L.) Hassk., typically called bhringoraj or bhringraj in Bangladesh and India, is a member of the Asteraceae family and is also known as Eclipta prostrata Roxb. The plant has a variety of medicinal uses, according to traditional medical systems in the Indian subcontinent and among tribal practitioners. It is frequently used to treat gastrointestinal issues, skin conditions, spleen enlargement, fever, respiratory tract disorders (including asthma), hair loss and graying of the hair, liver disorders (including jaundice), and cuts and wounds. The plant contains a number of phytoconstituents, including luteolin, apigenin, ursolic acid, oleanolic acid, and eclalba saponins. The pharmacological actions of plant extracts and certain phytoconstituents have demonstrated antibacterial, anticancer, hepatoprotective, and neutralizing effects on snake venom [(Leśniak et al., 2021)](https://paperpile.com/c/ZHryOn/aeTcq) [(Harsha & Subramanian, 2022)](https://paperpile.com/c/ZHryOn/9rDLW)

Phytoconstituents like wedelolactone and ursolic and oleanolic acids as well as luteolin and apigenin can form the basis of new drugs against cancer, arthritis, gastrointestinal disorders, skin diseases, and liver disorders [(Graf et al., 2023)](https://paperpile.com/c/ZHryOn/mYHD)[(Chawla, n.d.)](https://paperpile.com/c/ZHryOn/AlNZI)). Zinc oxide [nanoparticle](https://www.sciencedirect.com/topics/chemistry/nanoparticle) is among one of the most researched studies conducted due to its ability to apply in varied downstream applications [(Tiwari & Jain, 2023)](https://paperpile.com/c/ZHryOn/Drzt6). Physical and chemical behaviors of zinc oxide nanoparticle can be easily turned by changing the morphology by using different synthesis routes or different precursors or different materials to produce the nanomaterial.[(*Pharmaceutics*, 2013)](https://paperpile.com/c/ZHryOn/LnkHm). Among the metal oxide nanoparticles, zinc oxide nanoparticles have been extensively used in various biological applications due to their nontoxic nature, and they are also listed as “generally recognized as safe” (GRAS) by the U.S. FDA [(V. Sharma, 2011)](https://paperpile.com/c/ZHryOn/2NNG5).

Zinc oxide's ability to release reactive oxygen species (ROS) on its surface has been demonstrated in numerous tests to be the most effective antibacterial agent. Zinc oxide, on the other hand, has numerous uses in medication administration and biological fields and is recognized as being safe and biocompatible [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/ZHryOn/h2dR) . Because of their distinctive and varied properties, zinc oxide nanoparticles have been the focus of research over the past 20 years among metal oxide nanoparticles [(Katyal et al., 2021)](https://paperpile.com/c/ZHryOn/3eza) [(Mohan et al., 2018)](https://paperpile.com/c/ZHryOn/uR4n0) Owing to their biocompatibility, they are extensively employed as semiconductor nanomaterials in ethanol gas sensors, photocatalysis, pharmaceutical and cosmetic products, electronic and optoelectronic devices, and, most notably, the biomedicine industry [(Prabhu et al., 2013)](https://paperpile.com/c/ZHryOn/pWFL1).

Dental caries and dental plaque are two of the most common diseases in the world, caused by a combination of microorganisms and food debris [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/ZHryOn/1L4F) . In the presence of fermentable carbohydrates like sucrose and fructose, certain acid-producing bacteria, particularly *Streptococcus mutans,* colonize the dental surface and damage the hard tooth structure [(Jabin et al., 2021)](https://paperpile.com/c/ZHryOn/wjbaG).An opportunistic human fungal pathogen called *Candida albicans* commonly induces superficial infections of the mucosal surfaces most commonly mouth in vulnerable and disabled people. Nonetheless, the organism is frequently found in healthy individuals as a commensal, where it is a part of the typical microflora [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/ZHryOn/1axLq) [(Martorano-Fernandes et al., 2023)](https://paperpile.com/c/ZHryOn/lWmeb).

In a recent study, Akbar et al. produced 20 nm-sized zinc oxide nanoparticles and tested them against *Staphylococcus aureus* and *Salmonella typhimurium* and was found that the nanoparticles had strong antibacterial properties against the examined pathogens [(Akbar et al., 2023)](https://paperpile.com/c/ZHryOn/2SDd3) Salem et al. also examined the antimicrobial properties of zinc oxide nanoparticles against enterotoxic *Escherichia coli* and *Vibrio cholera*, whereas Chaudhary et al evaluated the antimicrobial properties of zinc oxide nanoparticles against the pathogenic organisms and fungi *Aspergillus* *niger* and *Aspergillus oryzae*, as well as *Staphylococcus epidermidis*, *Klebsiella pneumoniae, Escherichia coli,* and fungi [(Mokhtar et al., 2023)](https://paperpile.com/c/ZHryOn/G8uqK)[(Chaudhary et al., 2023)](https://paperpile.com/c/ZHryOn/33ogd). Green synthesis is a new field that uses biosafe and ecologically friendly reagents to create nontoxic compounds. It could be a good substitute for physical and chemical approaches [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/ZHryOn/9Hn9)[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/ZHryOn/7Bffb). Nonetheless, the primary issues with green synthesis are the stability of the nanoparticles and our incomplete comprehension of their operations[(Shukla & Iravani, 2018)](https://paperpile.com/c/ZHryOn/6t1bw). Green synthesis is based on an organism's well-documented ability to decrease metal ions in addition to stabilizing them into nanoparticles [(Katyal et al., 2021)](https://paperpile.com/c/ZHryOn/3eza). Because they create more stable forms of nanoparticles than microbes, plants are thought to be the greatest candidates for the green synthesis of these particles [(Awwad, 2013)](https://paperpile.com/c/ZHryOn/wxp9X) It is thought that plants have a diverse range of secondary metabolites that are worth studying [(Solanki et al., 2022)](https://paperpile.com/c/ZHryOn/0eyku).

Recent studies have focused on the phytoconstituents made by plants in an effort to better understand how these compounds work together to reduce metal nanoparticles through a process known as bio-reduction[(Chidambaram et al., 2022)](https://paperpile.com/c/ZHryOn/s3suw).. These molecules served as both reducing agents and capping agents, which was necessary for the stability and biocompatibility of the nanoparticles. As a result, no additional chemical reducing or capping agents were needed [(Deepika et al., 2022)](https://paperpile.com/c/ZHryOn/wKpj). Furthermore, these plant-derived compounds not only functioned as a growth terminator for zinc oxide nanoparticles but also served as a linker molecule to facilitate the self-assembly of two or more molecules of zinc oxide-formed ZnO NPs [(Al-Enazi et al., 2023)](https://paperpile.com/c/ZHryOn/uEzAn). Therefore the aim of the current study is to fabricate zinc oxide nanoparticles using Eclipta Alba and to evaluate its on vitro antibacterial activity.

# MATERIALS AND METHODS

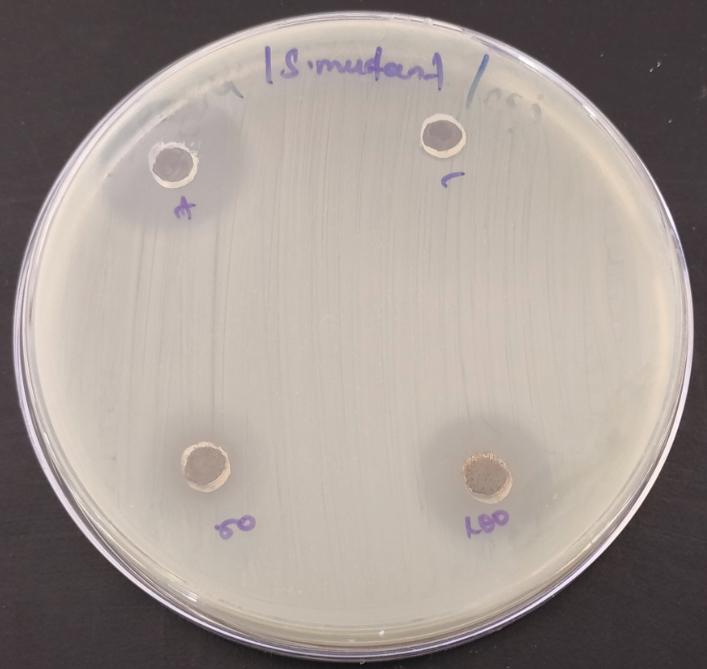
## Synthesis of Zinc oxide nanoparticles

The supplier of the zinc nitrate hexahydrate (Zn (NO,)2- 6H,O) was Sigma-Aldrich Chemicals in India. Fresh leaves were washed three times in the presence of distilled water to remove dust, chopped, and added to water (1:10) at 60°C while being continuously stirred for 30 min . After filtering, the mixture was cooled and kept at 40°C for additional use(Chehelgerdi et al., 2023). 24h spent shaking the leaf extract with 0.2M zinc nitrate (1: 9). The colour change of the liquid from brown into a semi-solid creamy colour indicated the formation of ZnO NPs. The phytochemicals found in biomaterials (such plant extract) can function as reducing agents, transforming the metal precursors into metal nanoparticles (NPs). The shape and size of NPs were estimated by SEM.The functional group was detected through Fourier transform infrared spectroscopy (FTIR) with 400-4000 cm-1.

## Antimicrobial activity

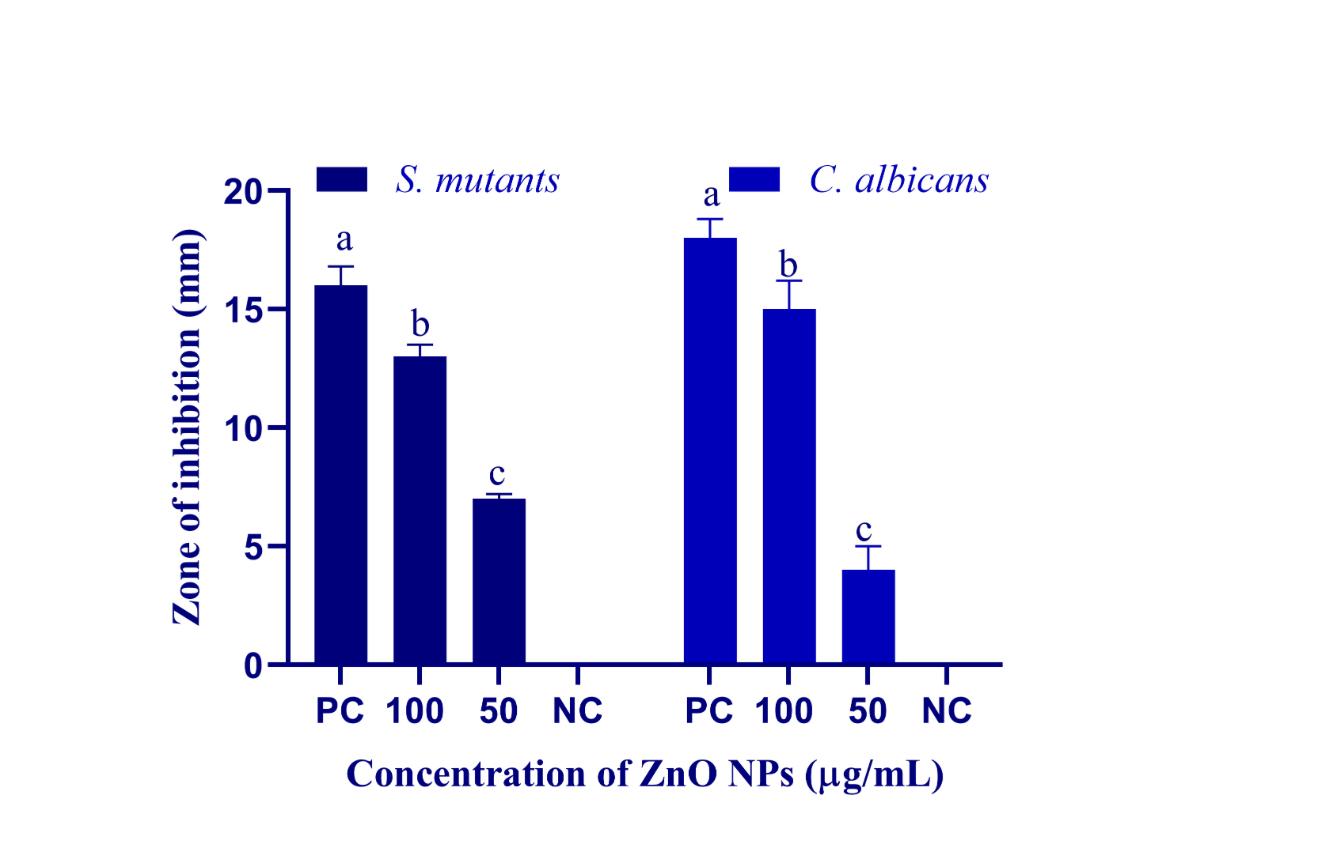
The *E. alba*-mediated silver nanoparticles were tested for antibacterial activity by employing the agar well diffusion method . The pathogenic cultures were properly sub-cultured and maintained in our laboratory. In the antibacterial assays, AgNPs (50 and 100 μg/mL) were poured into the wells of Mueller–Hinton agar (MHA) plates, respectively, after which they were incubated for 24 h at 37 °C and 25 °C, respectively. The antibiotic chloramphenicol was used as a positive control. The growth inhibition zones were measured by the zone inhibition scale (Hi-Media, India).

# RESULTS

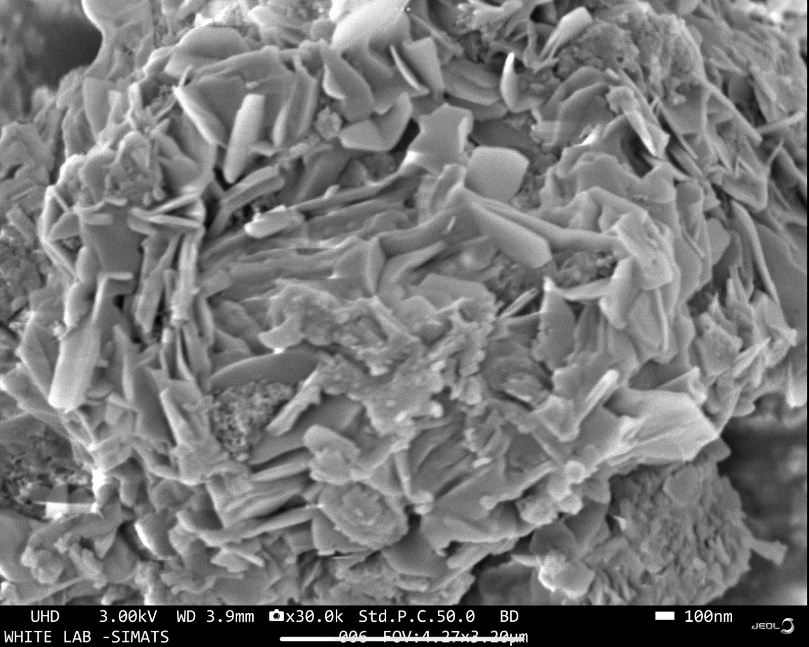


1. (b)

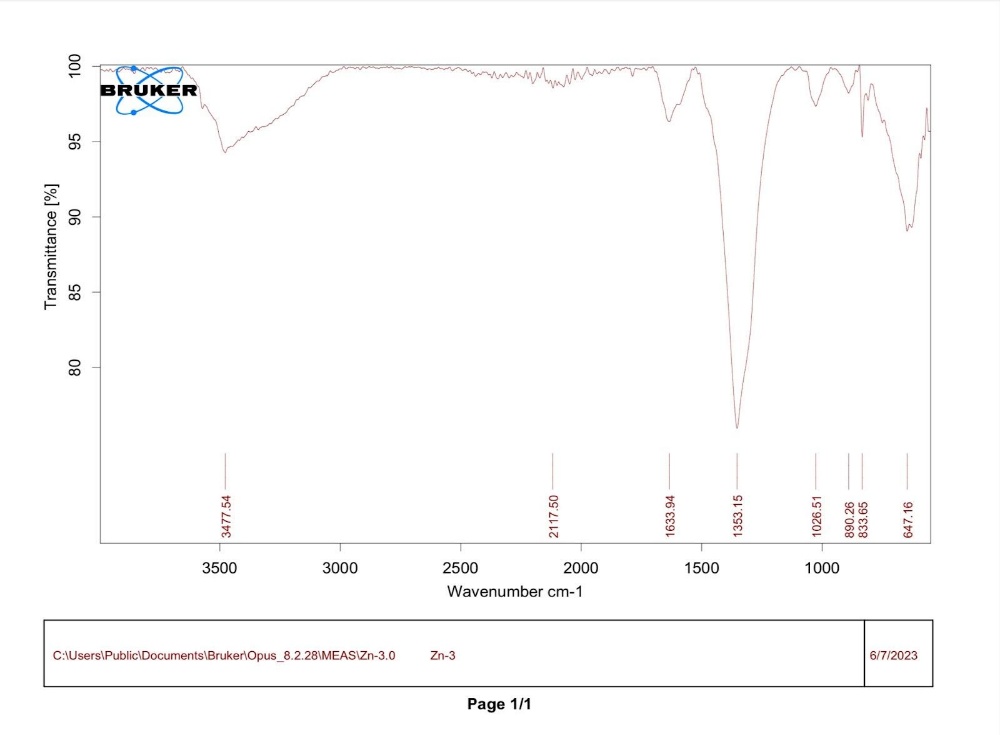
**Fig. 1.** Antimicrobial activity of ZnO NPs tested against dental pathogens *S. mutants* and *C. albicans*



**Fig. 2.** Antimicrobial activity zone of inhibition on ZnO NPs against dental caries pathogens (*S. mutants* and *C. albicans*). Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.



**Fig. 3.** SEM and FTIR analysis of synthesized nanoparticles to evaluate its morphological and topographical features.

To determine which functional groups in biomolecules are in charge of the bio-reduction of silver ions and the stability of Ag-NPs, FTIR analysis was performed (Saadh et al., 2024). Infrared radiation is absorbed by chemical groups, which then transform it into vibrational and/or rotational energy signals. The resulting signals manifest as a spectrum and serve as the sample's molecular fingerprint. Since every molecule or chemical structure has a distinct spectral fingerprint, FTIR analysis is an excellent method for identifying chemicals (Fig.4).

**Fig .4.** FTIR illustrates different functional groups present in the synthesised nanoparticles.

# DISCUSSION

From the current study, it is discovered that ZnONPs mediated *Eclipta alba* showed significant antimicrobial activity against *S.mutans* (Zone of inhibition-13 mm at 100 µg/ml) and *C. albicans* (Zone of inhibition -14mm at 100 µg/ml).Many reports on antimicrobial activity of ZnONPs against human dental pathogens are available including *S. mutans , S. aureus, E. coli,* and *Lactobacillus fermentum* [*(MOHAPATRA & Kumar, 2015)*](https://paperpile.com/c/ZHryOn/ssbH8) highlighting their potential as alternative antibiofilm treatments against *Rothia dentocariosa* and *Rothia mucilaginosa* [*(Mantaj et al., 2018)*](https://paperpile.com/c/ZHryOn/zyxDK). Phyto-Fabrication of ZnO Nanoparticles Using *Piper* betel Aqueous Extract showed good antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidphillus* [*(Bolla et al., 2023)*](https://paperpile.com/c/ZHryOn/o82YX). Several potential bactericidal mechanisms have been proposed by scientists. One of the suggestions is that smaller nanoparticles (NPs) have a higher surface reactivity and easier cell penetration, which releases the Zn2+. One of the key theories behind antibacterial mechanisms is the release of Zn2+ from ZnO NPs, which is known to block a variety of bacterial cell activities, including active transport, bacterial metabolism, and enzyme activity. Afterwards, the bacterial cell died as a result of Zn2+'s toxicity to its biomolecules [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/ZHryOn/eS4D)[(Sushanthi et al., 2021)](https://paperpile.com/c/ZHryOn/95xwT)[(Harsha et al., 2022)](https://paperpile.com/c/ZHryOn/Mf8xI). The attachment of ZnO NPs to the bacterial cell membrane through electrostatic forces is another potential mechanism for the antimicrobial activity of NPs. ZnO NPs' positive zeta potential facilitates their attachment to negatively charged bacterial cells, allowing for cell penetration[(Neha et al., 2021)](https://paperpile.com/c/ZHryOn/BT6OH)[(Maliael et al., 2021)](https://paperpile.com/c/ZHryOn/2K4Xw)[(Lakshmi, 2021)](https://paperpile.com/c/ZHryOn/dV1k5)[(Dharman et al., 2021)](https://paperpile.com/c/ZHryOn/Nx1xl)

. This interaction could cause the bacterial cell integrity to be compromised and the membrane plasma structure to be distorted, which would lead to intracellular contents leaking out and eventual cell death [(Younas et al., 2023)](https://paperpile.com/c/ZHryOn/YHUV8). Furthermore, the buildup of ZnO NPs in the cell disrupts the bacteria's metabolic processes, ultimately resulting in their demise. To assess the antimicrobial activity of the biologically synthesized ZnO NPs, a well-diffusion test was conducted against a range of Gram-positive and Gram-negative bacteria, as well as fungi. According to the findings, *Pseudomonas aeruginosa* and *Aspergillus flavus* each displayed a maximum zone of inhibition measuring 22 ± 1.8 mm and 19 ± 1.0 mm, respectively [(Subramaniyan et al., 2023)](https://paperpile.com/c/ZHryOn/OKXM)

Similar antimicrobial effects were observed against *P. aeruginosa* in the extracellular synthesis of ZnO NPs using the endophytic bacteria *Sphingobacterium thalpophilum* [*(Tamayo, 2009)*](https://paperpile.com/c/ZHryOn/v3aHk). Secondary metabolites, which have a variety of biological activities, are abundant in plants. The field of plant-based research has expanded in the modern era. The antimicrobial properties of a few of the phytoconstituents have already been demonstrated. Many studies have demonstrated the bacteriostatic, bactericidal, and fungicidal effects of phytoconstituents, including coumarin, flavonoids, phenolics, alkaloids, terpenoids, tannins, and polyacetylenes, against a range of human pathogens. Several other studies corroborated the idea that the inhibition of secondary metabolites' activity is caused by disruptions to protein synthesis, biochemical pathways, and outer membrane degradation. Study done by Rahul et al, the methanolic extract of *Eclipta alba* demonstrated antimicrobial activity against *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (sensitive), *E. coli* (sensitive), and *Pseudomonas aeruginosa* (sensitive), with the greatest effect observed at 200 mg/ml. The extract's antimycobacterial activity demonstrated its ability to cultivate M. tuberculosis (H37Rv) and *M. tuberculosis* (MDR). It was noted that during the study, the extract's antimicrobial activity against *Pseudomonas aeruginosa* (sensitive) at 200 mg/ml showed a larger zone of inhibition than that of the standard medication [(R. K. Sharma et al., 2022)](https://paperpile.com/c/ZHryOn/eUF08).

# CONCLUSION

This study concludes that ZnO NPs showed significant antimicrobial activity against dental pathogens.Further more research is encouraged for drug formulation to treat oral infections. Mostly plant-based extracts are used in green nanoparticle development strategies. Concerning the easy, large-scale, and environmentally friendly aspects of producing nanomaterials, phyto-mediated syntheses ought to be promoted. In recent years, ZnO NPs have been developed extensively using plant extracts. Zinc oxide nanoparticles (ZnO NPs) are a very versatile material. Interestingly, some phytogenic ZnO NPs show potentials that are comparable to or even higher than those of chemically derived samples.

# REFERENCES

1. [Ajay, R., Rakshagan, V., Queenalice, A., Vinothkumar, S., Ravivarman, C., & Saravanadinesh, P. (2022). Effect of triazine comonomer substitution on the structure and glass transition temperature of monomethacrylate-based resin polymer: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 202–207.](http://paperpile.com/b/ZHryOn/7Bffb)
2. [Ajay, R., Sasikala, R., Rakshagan, V., Raghunathan, J., LalithaManohari, V., & Baburajan, K. (2022). Evaluation of cytocompatibility of thermopolymerized denture base copolymer containing a novel ring-opening oxaspiro comonomer. *World Journal of Dentistry*, *13*(2), 127–132.](http://paperpile.com/b/ZHryOn/9Hn9)
3. [Ajay, R., Suma, K., Sasikala, R., Rakshagan, V., Baburajan, K., & Kalarani, G. (2022). Evaluation of linear dimensional stability of monomethacrylate-based dental polymer containing a novel tricyclic diacrylate cross-linker using a novel surface-level index technique. *World Journal of Dentistry*, *13*(6), 568–573.](http://paperpile.com/b/ZHryOn/1axLq)
4. [Akbar, N., Javed, M., Arif Khan, A., Masood, A., Ahmed, N., Mehmood, R. Y., Khisro, S. N., Abdul, M. A. S., Mohammad Haniff, M. A. S., & Shah, A. (2023). Erratum: Zircon-Type CaCrO Chromite Nanoparticles: Synthesis, Characterization, and Photocatalytic Application for Sunlight-Induced Degradation of Rhodamine B. *ACS Omega*, *8*(38), 35441.](http://paperpile.com/b/ZHryOn/2SDd3)
5. [Al-Enazi, N. M., Alsamhary, K., Ameen, F., & Kha, M. (2023). Plant extract-mediated synthesis Cobalt doping in zinc oxide nanoparticles and their cytotoxicity and antibacterial performance. *Heliyon*, *9*(9), e19659.](http://paperpile.com/b/ZHryOn/uEzAn)
6. [Awwad, A. (2013). *Green Synthesis of Zinc Oxide Nanoparticles at Ambient Temperature*. LAP Lambert Academic Publishing.](http://paperpile.com/b/ZHryOn/wxp9X)
7. [Balaji Ganesh S, & Sugumar, K. (2021). Internet of Things—A novel innovation in dentistry. *Journal of Advanced Oral Research*, *12*(1), 42–48.](http://paperpile.com/b/ZHryOn/1L4F)
8. [Bolla, V. L., Jyothi, M., Mettu, S. R., Manoj Kumar, M. G., Rao, K. N., Reddy, M. S., & Koppolu, P. (2023). Effectiveness of three mouth rinsing agents against mutans and species - A comparative study. *Annals of African Medicine*, *22*(3), 365–372.](http://paperpile.com/b/ZHryOn/o82YX)
9. [Chaudhary, S., Ali, Z., Tehseen, M., Haney, E. F., Pantoja-Angles, A., Alshehri, S., Wang, T., Clancy, G. J., Ayach, M., Hauser, C., Hong, P.-Y., Hamdan, S. M., Hancock, R. E. W., & Mahfouz, M. (2023). Efficient in planta production of amidated antimicrobial peptides that are active against drug-resistant ESKAPE pathogens. *Nature Communications*, *14*(1), 1464.](http://paperpile.com/b/ZHryOn/33ogd)
10. [Chawla, R. K. (n.d.). *Phytochemical Study and Antimicrobial Activity of Bhangra ( Eclipta Alba (L.) Hassk) [with CD Copy]*.](http://paperpile.com/b/ZHryOn/AlNZI)
11. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
12. [Chidambaram, S. R., George, A. M., Muralidharan, N. P., Prasanna Arvind, T. R., Subramanian, A., & Rahaman, F. (2022). Current overview for chemical disinfection of dental impressions and models based on its criteria of usage: A microbiological study. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *33*(1), 30–36.](http://paperpile.com/b/ZHryOn/s3suw)
13. [Deepika, B. A., Ramamurthy, J., Girija, S., & Jayakumar, N. D. (2022). Evaluation of the antimicrobial effect of Ocimum sanctum L. oral gel against anaerobic oral microbes: An in vitro study. *World Journal of Dentistry*, *13*(S1), S23–S27.](http://paperpile.com/b/ZHryOn/wKpj)
14. [Dharman, S., (2021). Ecofriendly Synthesis, Characterisation and Antibacterial Activity Of Curcumin Mediated Silver Nanoparticles. *International Journal of Dentistry and Oral Science*, 2314–2318.](http://paperpile.com/b/ZHryOn/Nx1xl)
15. [Govindaraj, A., & Dinesh, S. P. S. (2021). Effect of chlorhexidine varnish and fluoride varnish on White Spot Lesions in orthodontic patients- a systematic review. *The Open Dentistry Journal*, *15*(1), 151–159.](http://paperpile.com/b/ZHryOn/h2dR)
16. [Graf, S., Thakkar, D., Hansa, I., Pandian, S. M., & Adel, S. M. (2023). 3D metal printing in orthodontics current trends, biomaterials, workflows and clinical implications. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/ZHryOn/mYHD)[10.1053/j.sodo.2023.01.001](http://dx.doi.org/10.1053/j.sodo.2023.01.001)
17. [Harsha, L., Navaneethan, R., Acid, T., & Acid, C. A.-A. (2022). CITRIC ACID-AN VITRO STUDY. *International Journal Clinical Dentistry*, *15*(3), 413–419.](http://paperpile.com/b/ZHryOn/Mf8xI)
18. [Harsha, L., & Subramanian, A. K. (2022). Comparative assessment of pH and degree of surface roughness of enamel when etched with five commercially available etchants: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 181–185.](http://paperpile.com/b/ZHryOn/9rDLW)
19. [Jabin, Z., Nasim, I., Vishnu Priya, V., & Agarwal, N. (2021). Quantitative Analysis and Effect of SDF, APF, NaF on Demineralized Human Primary Enamel Using SEM, XRD, and FTIR. *International Journal of Clinical Pediatric Dentistry*, *14*(4), 537–541.](http://paperpile.com/b/ZHryOn/wjbaG)
20. [Katyal, D., Subramanian, A. K., Venugopal, A., & Marya, A. (2021). Assessment of Wettability and Contact Angle of Bonding Agent with Enamel Surface Etched by Five Commercially Available Etchants: An In Vitro Study. *International Journal of Dentistry*, *2021*, 9457553.](http://paperpile.com/b/ZHryOn/3eza)
21. [Lakshmi, T. (2021). Medicinal value oral health aspects acacia catechu-an update. *International Journal Dentistry Oral ScienceVolume*, *8*, 1399–1401J.](http://paperpile.com/b/ZHryOn/dV1k5)
22. [Leśniak, P., Puk, K., & Guz, L. (2021). Parasiticidal effects of Eclipta alba and Arctium lappa extracts against Ichthyophthirius multifiliis. *Polish Journal of Veterinary Sciences*, *24*(4), 547–552.](http://paperpile.com/b/ZHryOn/aeTcq)
23. [Maliael, M. T., Subramanian, A. K., & Srirengalakshmi. (2021). Effectiveness of a fluoride-releasing orthodontic primer in reducing demineralization around brackets – a systematic review. *Orthodontic Waves (English Ed.)*, *80*(4), 218–223.](http://paperpile.com/b/ZHryOn/2K4Xw)
24. [Mantaj, J., Abu-Shams, T., Enlo-Scott, Z., Swedrowska, M., & Vllasaliu, D. (2018). Role of the Basement Membrane as an Intestinal Barrier to Absorption of Macromolecules and Nanoparticles. *Molecular Pharmaceutics*, *15*(12), 5802–5808.](http://paperpile.com/b/ZHryOn/zyxDK)
25. [Martorano-Fernandes, L., Brito, A. C. M., Araújo, E. C. F. de, Almeida, L. de F. D. de, Wei, X.-Q., Williams, D. W., & Cavalcanti, Y. W. (2023). Epithelial responses and Candida albicans pathogenicity are enhanced in the presence of oral streptococci. *Brazilian Dental Journal*, *34*(3), 73–81.](http://paperpile.com/b/ZHryOn/lWmeb)
26. [Mohan, S., Oluwafemi, S. O., Kalarikkal, N., & Thomas, S. (2018). *Characterization of Nanomaterials: Advances and Key Technologies*. Woodhead Publishing.](http://paperpile.com/b/ZHryOn/uR4n0)
27. [MOHAPATRA, & Kumar, R. (2015). *ENGINEERING CHEMISTRY WITH LABORATORY EXPERIMENTS*. PHI Learning Pvt. Ltd.](http://paperpile.com/b/ZHryOn/ssbH8)
28. [Mokhtar, L. M., Salim, I. A., Alotaibi, S. N., Awaji, E. A., Alotaibi, M. M., & Doman, A. O. (2023). Phytochemical Screening and Antimicrobial Activity of Methanolic Extract of (L.) (azkhar) Collected from Afif City, Saudi Arabia. *Life* , *13*(7). https://doi.org/](http://paperpile.com/b/ZHryOn/G8uqK)[10.3390/life13071451](http://dx.doi.org/10.3390/life13071451)
29. [Neha, N., Maiti, S., & Jessy, P. (2021). Adhesion microflora role denitrifies colour stability provisional crowns: in-vitro study. *Int J Dentistry Oral Sci*, *8*(8), 3805–3809.](http://paperpile.com/b/ZHryOn/BT6OH)
30. [*Pharmaceutics*. (2013). MDPI AG. https://doi.org/](http://paperpile.com/b/ZHryOn/LnkHm)[10.3390/pharmaceutics](http://dx.doi.org/10.3390/pharmaceutics)
31. [Prabhu, Y. T., Venkateswara Rao, K., & Sesha Sai Kumar, V. (2013). *Novel Synthesis of Fe Doped Zinc Oxide Nano Particles & Properties: Basics, Concepts, Methods, Characterization*. LAP Lambert Academic Publishing.](http://paperpile.com/b/ZHryOn/pWFL1)
32. Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.
33. [Sabarathinam, J., & Madhulaxmi, R. (2021). Development anti inflammatory antimicrobial silver nanoparticles coated suture materials. *Int J Dentistry Oral Sci*, *8*(3), 2006–2013.](http://paperpile.com/b/ZHryOn/eS4D)
34. [Sharma, R. K., Bibi, S., Chopra, H., Khan, M. S., Aggarwal, N., Singh, I., Ahmad, S. U., Hasan, M. M., Moustafa, M., Al-Shehri, M., Alshehri, A., & Kabra, A. (2022). In Silico and In Vitro Screening Constituents of Leaf Extract to Reveal Antimicrobial Potential. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2022*, 3290790.](http://paperpile.com/b/ZHryOn/eUF08)
35. [Sharma, V. (2011). *An Investigation Into the Mechanism of Toxicity of Zinc Oxide Nanoparticles*.](http://paperpile.com/b/ZHryOn/2NNG5)
36. [Shukla, A. K., & Iravani, S. (2018). *Green Synthesis, Characterization and Applications of Nanoparticles*. Elsevier.](http://paperpile.com/b/ZHryOn/6t1bw)
37. [Solanki, L., Shantha Sundari, K. K., Muralidharan, N. P., & Jain, R. (2022). Antimicrobial effect of novel gold nanoparticle oral rinse in subjects undergoing orthodontic treatment: An ex-vivo study. *Journal of International Oral Health: JIOH*, *14*(1), 47.](http://paperpile.com/b/ZHryOn/0eyku)
38. [Subramaniyan, Y., Khan, A., Fathima, F., & Rekha, P. D. (2023). Differential expression of urease genes and ureolytic activity of uropathogenic Escherichia coli and Pseudomonas aeruginosa isolates in different nutritional conditions. *Archives of Microbiology*, *205*(12), 383.](http://paperpile.com/b/ZHryOn/OKXM)
39. [Sushanthi, S., Doraikannan, S., Indiran, M., & Rathinavelu, P. (2021). *Rajeshkumar S. Vernonia Amygdalina*. 3330–3334.](http://paperpile.com/b/ZHryOn/95xwT)
40. [Tamayo, E. J. N. (2009). *Bacteriemia y diagnóstico convencional de Sphingobacterium spiritivorum*.](http://paperpile.com/b/ZHryOn/v3aHk)
41. [Tiwari, A., & Jain, R. K. (2023). Comparative evaluation of White Spot lesion incidence between NovaMin, probiotic, and fluoride containing dentifrices during orthodontic treatment using laser fluorescence - A prospective randomized controlled clinical trial. *Clinical and Investigative Orthodontics*, 1–8.](http://paperpile.com/b/ZHryOn/Drzt6)
42. [Younas, Z., Mashwani, Z. U. R., Ahmad, I., Khan, M., Zaman, S., Sawati, L., & Sohail. (2023). Mechanistic Approaches to the Application of Nano-Zinc in the Poultry and Biomedical Industries: A Comprehensive Review of Future Perspectives and Challenges. *Molecules* , *28*(3). https://doi.org/](http://paperpile.com/b/ZHryOn/YHUV8)[10.3390/molecules28031064](http://dx.doi.org/10.3390/molecules28031064)