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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 oxide nanoparticles 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 ZnO NPs 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*

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1. 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, Puk, and Guz 2021)

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 ((Chawla, n.d.)). Zinc oxide nanoparticle is among one of the most researched studies conducted due to its ability to apply in varied downstream applications . 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). 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))..

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. 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(Mohan et al. 2018) 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, Venkateswara Rao, and Sesha Sai Kumar 2013)).

Dental caries and dental plaque are two of the most common diseases in the world, caused by a combination of microorganisms and food debris. 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. 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(Martorano-Fernandes et al. 2023). 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). 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)(Chaudhary et al. 2023). 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. Nonetheless, the primary issues with green synthesis are the stability of the nanoparticles and our incomplete comprehension of their operations(Shukla and Iravani 2018). Green synthesis is based on an organism's well-documented ability to decrease metal ions in addition to stabilizing them into nanoparticles. 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) It is thought that plants have a diverse range of secondary metabolites that are worth studying.

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. These molecules served as both reducing agents and capping agents, which was necessary for the stability and biocompatibility of the nanoparticles (Subramanyam D et al. 2018). As a result, no additional chemical reducing or capping agents were needed. 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). Therefore the aim of the current study is to fabricate zinc oxide nanoparticles using Eclipta Alba and to evaluate its on vitro antibacterial activity.

2. 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. 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 clinical pathogenic strains of *S. mutans* and *C. albicans* were acquired from the Department of Cariology, Saveetha Dental College Tamil Nadu, India. 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

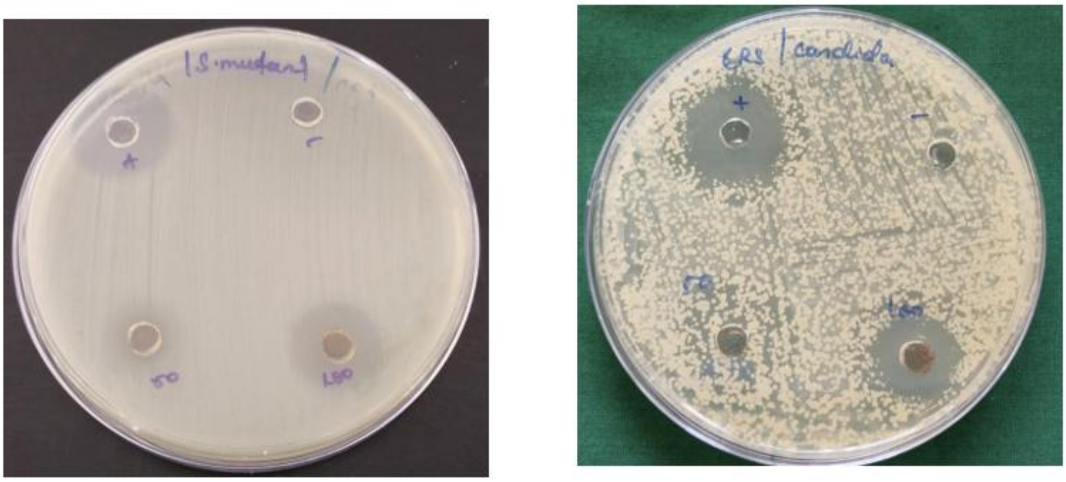

Fig 1: Antimicrobial activity of ZnO NPs tested against dental pathogens *S. mutants* and *C. albicans*

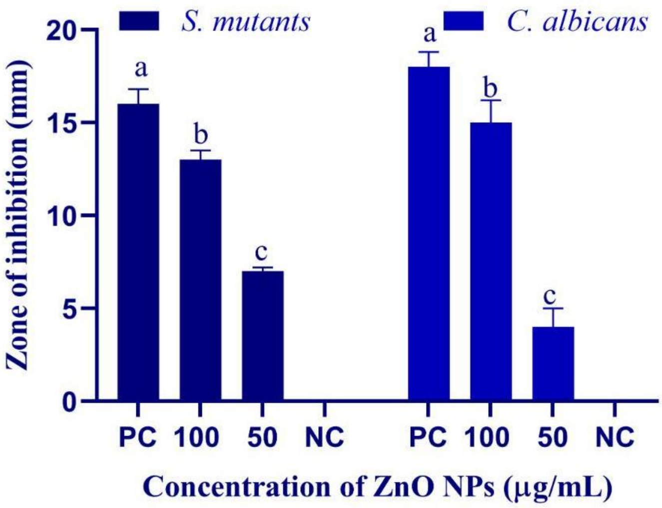

Fig 2: Antimicrobial activity zone of inhibition on ZnO NPs against dental caries pathogens (*S. mutants* and *C. albicans*). Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

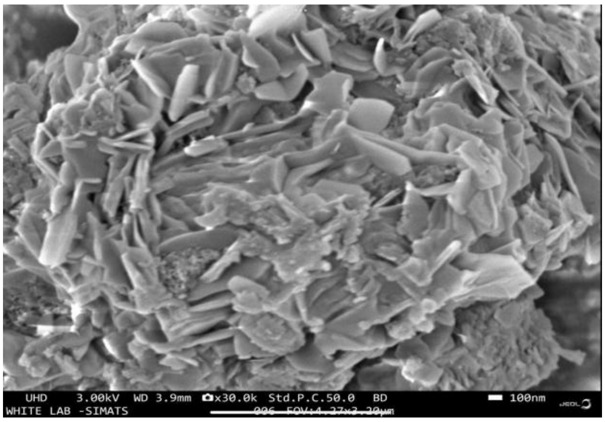

Fig 3: SEM analysis of synthesized nanoparticles to evaluate its morphological and topographical features.

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. 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).

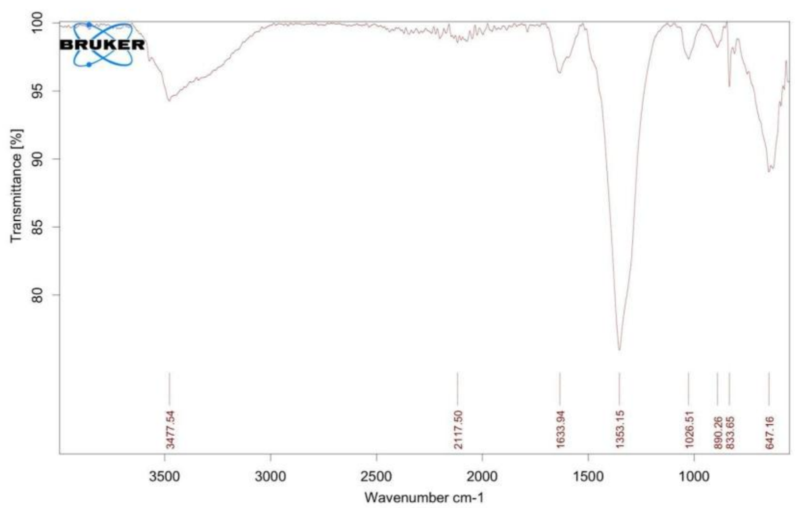

Fig .4: FTIR illustrates different functional groups present in the synthesized nanoparticles.

3. 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 and Kumar 2015) highlighting their potential as alternative antibiofilm treatments against *Rothia dentocariosa* and *Rothia mucilaginosa* ((Mantaj et al. 2018). Phyto-Fabrication of ZnO Nanoparticles Using *Piper* betel Aqueous Extract showed good antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidphillus* (Bolla et al. 2023). 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. The attachment of ZnO NPs to the bacterial cell membrane through electrostatic forces is another potential mechanism for the antimicrobial activity of NPs (Priyadharsini JV, et al. 2018) ZnO NPs' positive zeta potential facilitates their attachment to negatively charged bacterial cells, allowing for cell penetration. 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). 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)

Similar antimicrobial effects were observed against P. aeruginosa in the extracellular synthesis of ZnO NPs using the endophytic bacteria *Sphingobacterium thalpophilum (Tamayo 2009)*. 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 (Uma Maheswari TN et al. 2020).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 (Karthik EVG et al. 2021). 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 (Sharma et al. 2022).

4. 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.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTERESTS

All authors declare that there are no potential conflicts of interests.

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