Eco Friendly Approach of Zno Nanoparticles Synthesis Using Tridax Procumbens and its Antibiofilm Antibacterial Activity Against Dental Caries Causing Pathogens

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**Abstract:** Dental caries is a significant oral health issue caused by biofilm formation and bacterial growth. The emergence of antibiotic resistance has necessitated the development of alternative antibacterial agents. This study explores the eco-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) using Tridax procumbens and evaluates their antibiofilm and antibacterial activities against dental caries causing pathogens. Zinc oxide (ZnO) nanoparticles have gained significant attention in various fields due to their unique properties and potential applications. However, the conventional methods of synthesising ZnO nanoparticles often involve the use of toxic chemicals and energy-intensive processes, which have detrimental effects on the environment. Therefore, there is a growing need to develop eco-friendly approaches for the synthesis of ZnO nanoparticles. The present study focused on the Eco-friendly Approach of ZnO Nanoparticles Synthesis using *Tridax procumbens* and its Antibiofilm and Antibacterial Activity against Dental Caries Causing Pathogens. *Tridax procumbens* bark collected from the local market used in the study. Further, a series of morphological, physiological, and conventional biochemical tests were performed to identify the selected microorganisms. In addition to this, the study conducted the following tests: Green synthesis of ZnO NPs from *Tridax procumbens* bark extract, XRD analysis of synthesised ZnO NPs, EDAX images of synthesised ZnoNPs, SEM pictures of synthesised ZnO NPs, Microtiter plates demonstrating the antibiofilm activity of ZnO NPs. Microtiter plate optical density (OD) 600nm reading (mean± standard error) of biofilm formation with ZnO NPs added at concentration of 25 to 100µg/mL. A) *Streptococcus mutants* andB). *Candida albicans.*In this study, the antibiofilm activity of ZnO NPs was evaluated by measuring biofilm growth with crystal violet in the presence of varying concentrations of ZnO NPs. ZnO NPs successfully reduced biofilm formation at concentrations of 100 μg/mL (*p <*0.05). ZnO NPs showed significant dose-dependent antibiofilm activity against *S. mutans*. The positive control without ZnO NPs exhibited growth at an OD of 1.123. Notable inhibition of colony growth would decrease OD. Bacterial growth was mildly inhibited by the addition of 50 μg/mL ZnO NPs (OD of 0.349) and was decreased further with the addition of increasing ZnO NP concentrations (OD of 0.3 at 100 μg/mL). Compared to previous study,Meroni et al analyzed the antibiofilm abilities of biological-derived AgNPs with a size of 11 nm. The abilities against *S.mutans* were not identified. In this study, we propose the use of *Tridax procumbens*, a medicinal plant known for its antimicrobial properties, as a green and sustainable source for the synthesis of ZnO nanoparticles. We also investigate the antibiofilm and antibacterial activity of these nanoparticles against dental caries-causing pathogens.

**Keywords:** ZnO Nanoparticles Synthesis, *Tridax procumbens*, Antibiofilm and Antibacterial Activity, Dental caries causing pathogens

# Introduction

Dental caries affect a large percentage of people worldwide and are particularly prevalent in Latin and Asian nations. According to Tandon et al. (2010), dental caries affect over 70% of younger generations. *Streptococcus mitis, including S. mutans, S. salivarius, S. sanguis, and S. sobrinus*, are significant dental caries-causing organisms [(Aparna et al., 2021; Durá-Travé & Gallinas-Victoriano, 2023)](https://paperpile.com/c/tIYIr5/X5sZ+PTym) The dental microorganisms that are acidogenic and responsible for carbohydrate fermentation. Dental caries are largely caused by these acidogenic bacterial strains fermenting different types of carbohydrates[(Kidd, 2005; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/tIYIr5/aVEE+WI6t). According to Islam et al. (2007), acid-producing bacteria contribute to demineralization and the development of cavities in teeth. By producing different polysaccharides extracellularly utilising sucrose, the bacterial strain S. mutans colonises the tooth's surface and easily encourages the development of plaque [(*Dental Caries: Diagnosis, Prevention and Management*, 2018; Poornima et al., 2021)](https://paperpile.com/c/tIYIr5/wytk+7tXs). Dental caries include tooth decay and the decalcification of calcium. Different Gram-negative anaerobic bacteria are implicated in periodontal disease. These include *Porphyromonas gingivalis*, *Actinobacillus* spp., *Prevotella* spp., Fusobacterium spp., and Prevotella sp. In most cases of periodontal disease, the area around the gingival crevice was infected, leading to a variety of inflammatory reactions, including cellular reactions in the gingival and connective tissues. It is primarily caused by the formation of biofilms on tooth surfaces, which consist of diverse bacterial communities [(Mann & Crabbe, 1996; Pandiyan et al., 2022)](https://paperpile.com/c/tIYIr5/nKYK+Cw7c). Conventional antibacterial agents are losing efficacy due to the rise of antibiotic resistance. Therefore, exploring novel eco-friendly approaches and alternative antibacterial agents is crucial.

*Tridax procumbens* L. (T. procumbens), an Asian Ayurvedic plant with a long history of use, is a member of the Asteraceae family. T. procumbens has been used in traditional medicine for centuries to treat wounds, skin conditions, and to prevent blood clotting[(White, 1936)](https://paperpile.com/c/tIYIr5/JpL2). It has antileishmanial, antioxidant, anticancer, immunomodulatory, insecticidal, anthelmintic, cardiovascular, antiseptic, antibacterial, and insecticidal effects. It also has anticoagulant and antileishmanial characteristics [(D. K. Jain et al., 2012)](https://paperpile.com/c/tIYIr5/lnWG). Nanoparticle drug delivery systems are engineering innovations that use nanoparticles to deliver therapeutic medications to specific locations and regulate drug synthesis. The negative effects of a drug delivery method should be minimised, and dosage should be optimised[(Tahir et al., 2017)](https://paperpile.com/c/tIYIr5/1Zqb). Nanoparticles have drawn interest recently as a result of their possible use in efficient medication delivery. Compared to their larger equivalents, nanomaterials have various chemical, physical, or biological characteristics that can be advantageous for drug delivery systems[(Zeidan et al., 2022)](https://paperpile.com/c/tIYIr5/kD3K). The high surface-to-volume ratio, the ability to respond to chemical and geometric stimuli, and the capacity of nanoparticles to interact with biomolecules to enhance absorption into cell membranes are a few of the key benefits of nanoparticles. A broad range of organic and inorganic materials are included in the category of nanoparticles, each of which possesses special tunable properties that can be selectively customised for particular uses.

Zinc oxide (ZnO) nanoparticles have gained significant attention in various fields due to their unique properties and potential applications. However, the conventional methods of synthesising ZnO nanoparticles often involve the use of toxic chemicals and energy-intensive processes, which have detrimental effects on the environment. [(Chokkattu et al., 2023; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/tIYIr5/Idef+wTAV) Therefore, there is a growing need to develop eco-friendly approaches for the synthesis of ZnO nanoparticles. In this study, we propose the use of Tridax procumbens, a medicinal plant known for its antimicrobial properties, as a green and sustainable source for the synthesis of ZnO nanoparticles[(Elekhnawy et al., 2023)](https://paperpile.com/c/tIYIr5/F2tj). We also investigate the antibiofilm and antibacterial activity of these nanoparticles against dental caries-causing pathogens. The synthesis ZnO nanoparticles are characterised using various techniques such as UV-Visible spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM)[(Joshi et al., 2022)](https://paperpile.com/c/tIYIr5/AD2L). These analyses provide information about the size, structure, morphology, and functional groups of the nanoparticles. The Synthesis ZnO nanoparticles' antibiofilm ability is assessed against microorganisms that cause tooth cavities.[(*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d.)](https://paperpile.com/c/tIYIr5/fKdM) ZnO NPs' ability to limit the growth of biofilms and dislodge existing ones is tested using techniques including crystal violet staining and confocal laser scanning microscopy (CLSM). ZnO nanoparticles are examined for their antibacterial properties against microorganisms known to cause dental caries, such as Streptococcus mutans and Lactobacillus acidophilus.[(Laghari et al., 2023)](https://paperpile.com/c/tIYIr5/shnW) To ascertain the antibacterial properties of ZnO NPs, agar well diffusion assay and minimum inhibitory concentration (MIC) determination are performed. To describe the research's findings on the development of eco-friendly ZnO nanoparticles, characterise them, and evaluate their antibiofilm and antibacterial properties against microorganisms that cause tooth caries, with a particular emphasis on their potential in dental healthcare.

# Materials and methods

## Collection of Tridax procumbens

On their collection, the Tridax procumbens leaves were carefully cleaned under running water, allowed to dry at room temperature, and then chopped into little pieces in order to get rid of any last bits of dust.

## Leaf Extract Preparation

To make an aqueous extract of *Tridax procumbens*, 10 g of chopped leaves and 100 mL of double-distilled water were mixed in a 500 mL Erlenmeyer flask. The mixture was filtered through Whatman no. 1 filter paper after being left to stand at 60 °C for 20 minutes. The zinc oxide nanoparticles were created using the filtered bark extract. Extra leaf solution was kept at -20 °C until needed again.

## Biosynthesis of Zinc oxide Nanoparticles

Ten millilitres of the leaf extract and ninety millilitres of a 1.0 mM solution in distilled water were combined. There was a noticeable colour shift from yellow to brown in less than a day. This demonstrated the creation of colloidal ZnO nanoparticles. ZnO NPs were produced following a 15-minute centrifugation at 10,000 rpm. The particle underwent three centrifugation cycles, freeze-drying, and grinding into a powder prior to its characterization. It wasn't placed back into deionized water till then.

## Green Synthesis of ZnO NPs

Zinc nitrate hexahydrate (Zn (NO3)2. 6H2O) was supplied by Sigma-Aldrich Chemicals, an Indian company. After being thoroughly cleaned three times with distilled water to get rid of dust, fresh leaves were cut and introduced to water (1:10) at 60ºC, stirring constantly for thirty minutes. The mixture was filtered, then chilled and stored for later use at 40ºC. Shaking the leaf extract with 0.2M zinc nitrate (1:9) took place for a full day. The liquid's color changed from brown to a creamy, semi-solid hue, signifying the production of ZnO NPs. Plant extracts and other phytochemicals present in biomaterials can operate as reducing agents, converting metal precursors into metal nanoparticles (NPs). Because phytochemical-containing materials contain antioxidants, they can function as both stabilising and reducing agents.

## Anti Biofilm Susceptibility Screening by 96-microtiter Well Plate Method

A quantitative study on the formation of biofilms was carried out using a 96-microtiter well plate.[(Konwar et al., 2021)](https://paperpile.com/c/tIYIr5/n73S) Brain Heart Infusion (BHI) broth was mixed with freshly generated bacteria, and the mixture was cultured for 72 hours at 37 degrees Celsius. After 24 hours, the cell suspensions were diluted 1:100 in the freshly prepared BHI broth medium. The positive control in this case was defined as bacterial cells that were not exposed to ZnO NPs. Additionally, ZnO NPs were introduced at concentrations ranging from 25, 50, 75, and 100 µg/mL to the bacterial cultures that had been treated. There was nothing in the sterile BHI broth medium. After that, 200 µL culture suspensions were added to the sterile 96-well microplates, both with and without ZnO NPs treatment. The plates were then incubated for an additional 24 hours at 37 ºC without shaking. Every bacterial suspension was kept in three duplicates. All of the treated and untreated cells in the microtiter wells were thrown out by turning the plates over. After that, unwanted material and free-floating cells were eliminated by thoroughly cleaning the plates three times in phosphate buffered saline (PBS, pH 7.2).

## Minimal Inhibitory Concentrations

A modified broth microdilution method was used to calculate the minimum inhibitory concentrations (MICs) of ZnONps against various bacterial strains [40, 41]. The stock solutions of ZnONps (0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 25, 50, 100, and 200 μg/mL) were prepared for the MIC estimation. Two sets of 2.0 mL of bacterial inoculum (culture density of 5×105 CFU/mL) were added to a 13×100 mm sugar test tube containing the sterile Mueller–Hinton broth for each strain of bacteria. Following a 1:2 dilution and a 24-hour incubation period at 37 °C, 2.0 mL individual concentrations of ZnONps were added to each test tube, bringing the total tube volume down to 4.0 mL. Microbiological growth's optical density (O.D.) was calculated at 600 nm. The MIC endpoint was the lowest dose of ZnONps that showed no growth during incubation. By swabbing the bacterial culture on MHA plates and then incubating it at 37 °C for 24 hours, the turbidity of the bacterial growth and the MBC values were ascertained. The MBC is regarded as the extract concentration at which all bacteria are eliminated.

## Antibacterial Activity

The agar well diffusion method was utilised to evaluate the antibacterial activity of ZnO nanoparticles mediated by S. mutans and Candida albicans. The clinical pathogenic strains of Candida albicans and S. mutans were obtained. In our lab, the pathogenic cultures were appropriately subcultured and cared for. ZnONps (50 and 100 μg/mL) were added to the Mueller-Hinton agar (MHA) plate wells, respectively, for the antibacterial assays. The plates were then incubated for 24 hours at 37 °C and 25 °C, respectively. As a positive control, the antibiotic chloramphenicol was employed. The zone inhibition scale (Hi- Media, India) was used to measure the growth inhibition zones.

# Results

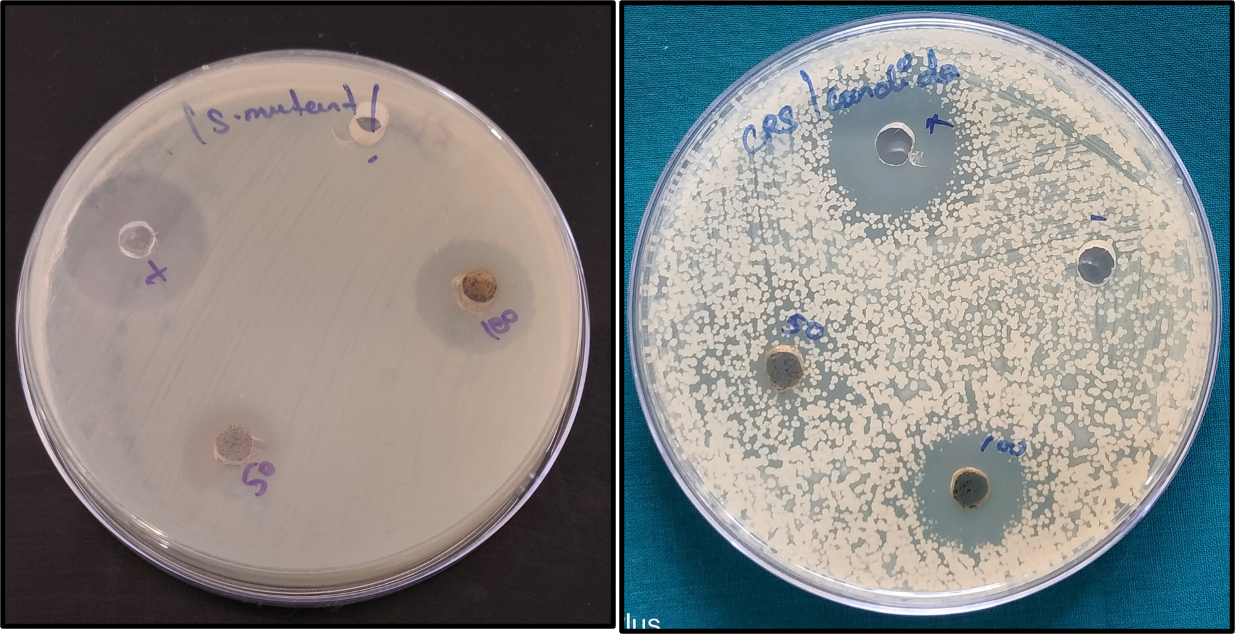
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Fig:1 Antibacterial activity of ZnO NPs against S. mutans and C. albicans

The zone of inhibition of *Tridax procumbans* extract synthesised ZnO nanoparticles against *S. mutans* and *C. albicans* carried out by well diffusion method shows increased rate of inhibition with the increased rate of concentration of extract from 50ug/ml to 100ug/ml. The standard used in the above experiment is chloramphenicol.

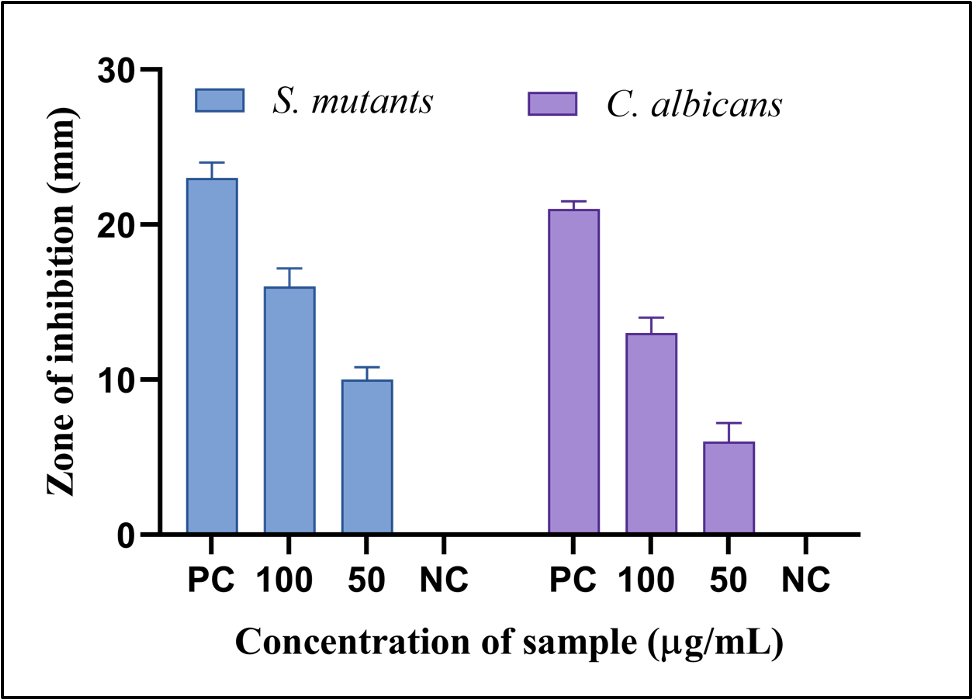
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Fig:2 Zone of inhibition on S.mutans and C. albicans

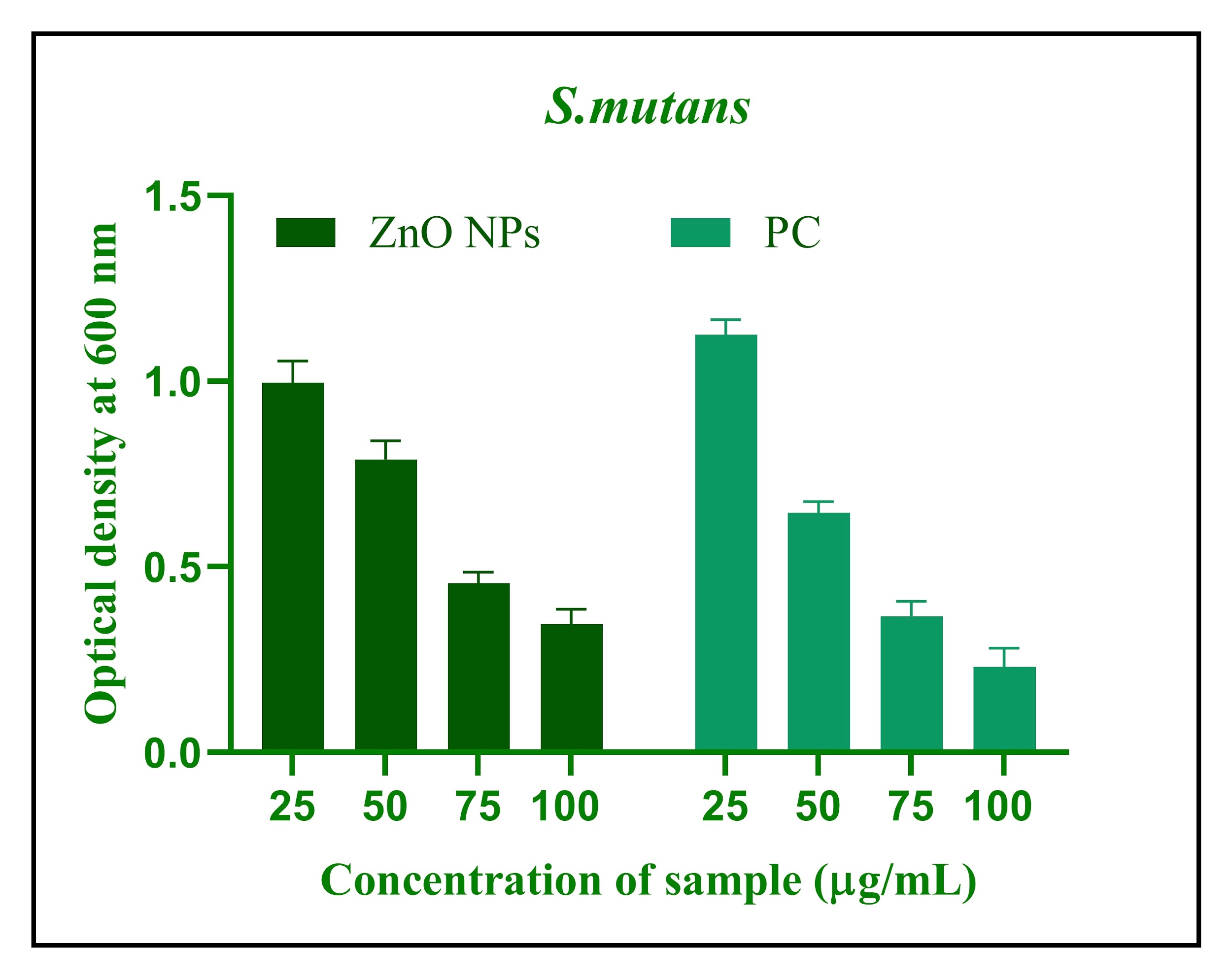
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Fig:3 Crystal violet assay to assess the antibiofilm activity of ZnO NPs against S. mutans

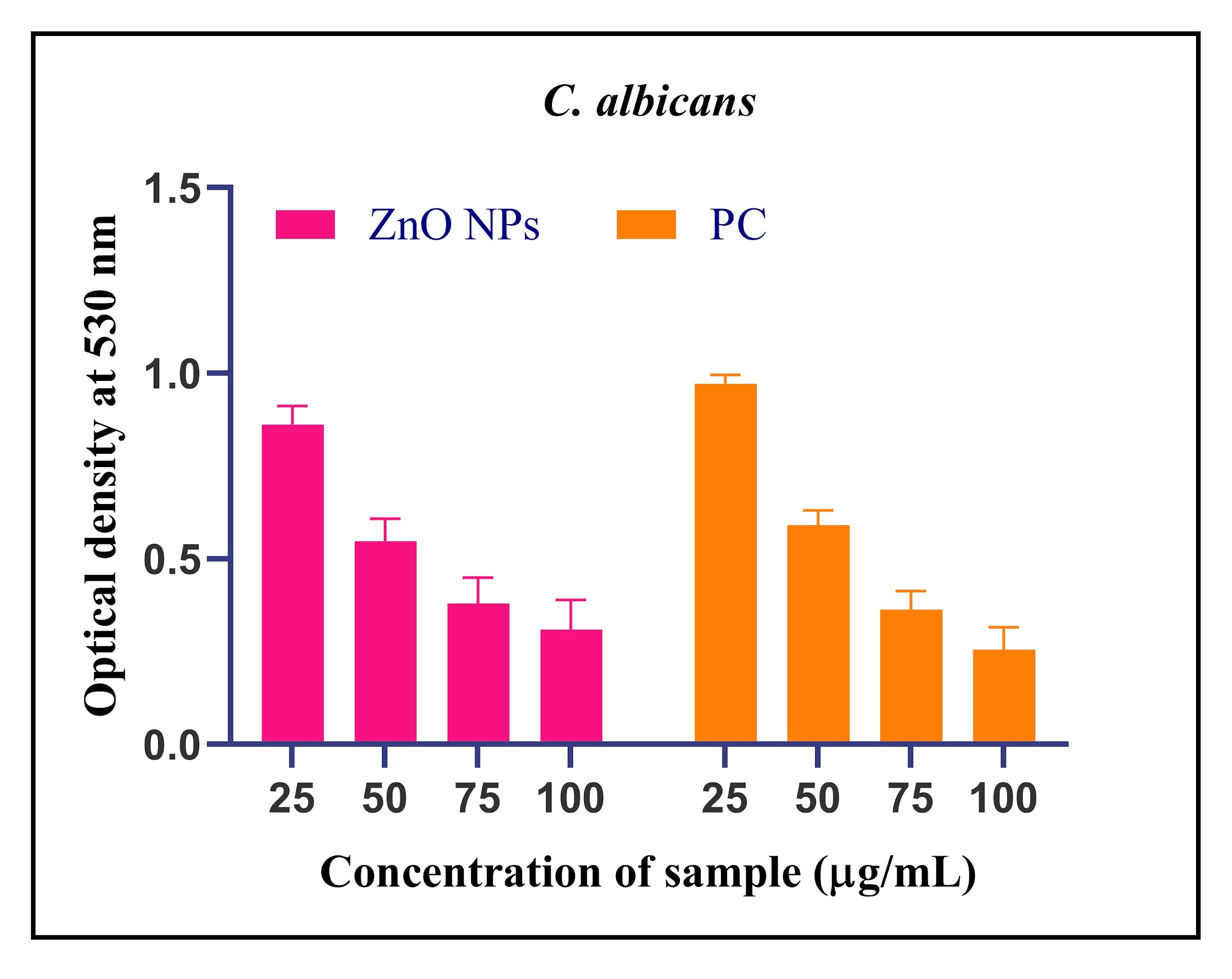
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Fig:4 Crystal violet assay to assess the antibiofilm activity of ZnO NPs against C. albicans

It was observed that Zinc Oxide nanoparticles synthesised from *Tridax procumbans* extract showed promising results by showing antibacterial activity (Chehelgerdi et al., 2023). The results demonstrate that ZnO nanoparticles synthesised using Tridax procumbens exhibit significant antibiofilm and antibacterial activities against dental caries causing pathogens.

# Discussion

Eco-friendly synthesis approach using plant extracts reduces the environmental impact associated with conventional nanoparticle synthesis methods. The unique properties of ZnO NPs make them promising candidates for dental caries treatment[(Chokkattu et al., 2022; Merchant et al., 2022; Papetti et al., 2018)](https://paperpile.com/c/tIYIr5/6NE0+BdKP+afUX). Eco-Friendly Synthesis: The eco-friendly synthesis of ZnONPs using Tridax procumbens extract is a significant achievement. This approach not only reduces the environmental impact associated with conventional nanoparticle synthesis methods but also utilises a natural, sustainable source for nanoparticle production. The thorough characterization of the synthesis ZnO NPs is crucial. The size, structure, and morphology of the nanoparticles play a pivotal role in their antibacterial and antibiofilm activities[(Marya et al., 2022; Mu et al., 2023; Ramamurthy et al., 2022)](https://paperpile.com/c/tIYIr5/Oo1q+8JEh+AJMA). It's essential to discuss how these properties align with the desired outcomes and their potential impact on application. The observed antibiofilm activity of ZnO NPs against dental caries-causing pathogens underscores their potential as a preventive strategy[(Adel et al., 2023; Giddens, 2012)](https://paperpile.com/c/tIYIr5/LMpk+mKMn). Discuss the mechanisms through which ZnO NPs inhibit biofilm formation and disrupt pre-formed biofilms. Explore any variations in activity based on nanoparticle concentration and exposure time. The antibacterial efficacy of ZnO NPs against

specific dental pathogens are noteworthy. Discuss the potential mechanisms of action, such as the generation of reactive oxygen species (ROS), that contribute to bacterial inhibition. [(Solanki et al., 2023; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/tIYIr5/yf45+ZoDj)Additionally, address any differences in sensitivity between different bacterial strains. Compare the antibacterial and antibiofilm activities of ZnO NPs with existing methods and materials used in dental caries prevention and treatment.[(Sreevarun et al., 2023; Wadhwani et al., 2022)](https://paperpile.com/c/tIYIr5/6Vq2+0m40) Highlight the advantages, such as broad-spectrum activity and eco-friendliness, that ZnO NPs may offer over traditional approaches. While ZnO NPs show promise, it's critical to address safety concerns. Discuss potential cytotoxicity and any adverse effects on oral tissues. Emphasise the importance of conducting further studies, including in vitro and in vivo assessments, to ensure the biocompatibility of ZnO NPs[(R. K. Jain & Verma, 2022; Thomas & Baiju, 2023)](https://paperpile.com/c/tIYIr5/5HJ4+9mqT). Outline future research directions based on the study's findings. Consider areas such as optimising synthesis methods for enhanced nanoparticle properties, conducting animal or clinical trials, and exploring the incorporation of ZnO NPs into dental materials like toothpaste or coatings.

# Conclusion

This study highlights an eco-friendly approach for the synthesis of ZnO nanoparticles using Tridax procumbens. The synthesised nanoparticles exhibited significant antibiofilm and antibacterial activities against dental caries-causing pathogens. These findings suggest the potential application of ZnO nanoparticles in dentistry as an alternative strategy for managing dental caries. Further studies are warranted to explore the mechanism of action and evaluate the biocompatibility and long-term effects of these nanoparticles in vivo. Future studies may focus on Optimising the synthesis process to enhance nanoparticle yield and properties, Evaluating the cytotoxicity and biocompatibility of ZnO NPs towards oral tissues, Conducting in vivo studies to validate the antibiofilm and antibacterial efficacy of ZnO NPs, Developing ZnO NP-based dental materials for preventive and therapeutic purposes.

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