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# ANTI-BACTERIAL ACTIVITY OF ZnO NANOPARTICLES DERIVED FROM AYURVEDA ORAL HEALTH FORMULATION AGAINST PATHOGENS CAUSING DENTAL CARIES

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**Abstract**

Dental caries remains a prevalent oral health concern, often attributed to bacterial colonization and biofilm formation by pathogens such as Streptococcus mutans and Lactobacillus species. In recent years, nanotechnology has emerged as a promising avenue for developing novel antimicrobial agents. This study investigates the anti-bacterial activity of ZnO nanoparticles derived from an Ayurveda oral health formulation against pathogens causing dental caries. The nanoparticles were characterized using techniques such as X-ray diffraction (XRD) and scanning electron microscopy (SEM). Antimicrobial efficacy was assessed through agar diffusion assays and minimum inhibitory concentration (MIC) determination. Results demonstrate significant inhibition of bacterial growth, highlighting the potential of these nanoparticles as a natural and effective strategy for combating dental caries-causing pathogens. Further exploration of their mechanism of action and biocompatibility is warranted to validate their clinical application in oral healthcare.

**Keywords:** Dental Caries, Biofilm, Nanoparticles, Pathogens, Antimicrobial Efficacy.

## INTRODUCTION

Microbial infections refer to diseases or illnesses caused by microorganisms such as bacteria, viruses, fungi, or parasites. These tiny organisms invade and reproduce in the human body, causing various diseases. Nowadays, microbial infections have become very common due to our current lifestyle and high pollution in the atmosphere (Balali, Yar, Afua Dela, & Adjei-Kusi, 2020; Prathap & Lakshmanan, 2022). They are the significant causes of healthcare issues around the world. Out of all the recorded strains of bacteria, only 5% of the bacteria are pathogenic. Even then, bacterial infections cause the majority of the known diseases. Dental caries, commonly known as tooth decay or cavities, is a prevalent oral health issue characterized by the destruction of tooth structure caused by bacterial activity (Kumaresan et al., 2022; Yadav & Prakash, 2017).

Antibiotics are used to treat bacterial infections. Penicillin was the first antibiotic used. New antibiotics are being synthesized to increase their effectiveness against bacteria. Due to the excessive use of antibiotics, new antibiotic-resistant strains have emerged. Resistance to antibiotics is increasingly commonplace amongst critical human pathogen. The NDM-1 gene is also found to be an antibiotic-resistant gene. Defense mechanisms of bacteria against antibiotics were discovered. During the treatment of bacteria with antibiotics, bacteria were found to produce an enzyme called β-lactamase. This enzyme had a degrading effect on antibiotics (Kunhikannan et al., 2021; Prathap & Jayaraman, 2022).

Dental caries, commonly known as tooth decay, is a widespread oral health issue affecting individuals of all ages globally. It is primarily caused by the accumulation of bacteria on the tooth surface, leading to the formation of dental plaque and subsequent demineralization of tooth enamel. Among the bacterial species implicated in the etiology of dental caries, Streptococcus mutans and Lactobacillus species are prominent contributors due to their ability to produce acids that degrade tooth structure and promote cavity formation (Ahirwar, Gupta, & Snehi, 2019).

The conventional approach to managing dental caries involves mechanical removal of plaque and restoration of affected teeth. However, the rise of antimicrobial resistance and the desire for more natural and holistic oral healthcare solutions have spurred interest in alternative strategies, including the use of nanotechnology and traditional medicine formulations. In recent years, nanomaterials have gained attention for their unique properties and potential applications in various fields, including dentistry. Among these materials, zinc oxide (ZnO) nanoparticles have garnered considerable interest due to their inherent antimicrobial properties and biocompatibility (Mohanraj, Varshini, & Somasundaram, 2021; Siddiqi, ur Rahman, Tajuddin, & Husen, 2018).

ZnO nanoparticles exhibit size-dependent antimicrobial activity, with smaller particles demonstrating enhanced efficacy against a wide range of bacterial pathogens. Furthermore, ZnO nanoparticles have been explored for their potential in dental applications, such as in dental restorative materials, antimicrobial coatings, and oral hygiene products (Song & Ge, 2019). Ayurveda, an ancient system of medicine originating from India, offers a rich repository of herbal formulations and remedies for various health conditions, including oral diseases. Ayurvedic principles emphasize a holistic approach to healthcare, focusing on restoring balance and harmony within the body. Traditional Ayurvedic formulations often include plant-derived ingredients known for their therapeutic properties, such as antimicrobial, anti-inflammatory, and antioxidant effects. Incorporating Ayurvedic principles into modern dental care aligns with the growing interest in natural and sustainable healthcare solutions (Palaniappan, Mohanraj, & Mathew, 2021; Song & Ge, 2019).

This study aims to investigate the anti-bacterial activity of ZnO nanoparticles derived from an Ayurveda oral health formulation against pathogens commonly associated with dental caries. By combining the antimicrobial properties of ZnO nanoparticles with the traditional knowledge of Ayurvedic medicine, this research seeks to explore novel approaches for combating dental caries and promoting oral health. The integration of nanotechnology and traditional medicine represents a convergence of modern scientific advancements and ancient healing practices, offering potential benefits in terms of efficacy, safety, and sustainability in oral healthcare (Bojanić, Suručić, & Đermanović, 2023).

The rest of this paper will delve into the background of dental caries and its microbial etiology, the properties and applications of ZnO nanoparticles, the principles of Ayurvedic medicine relevant to oral health, the methodology employed in this study, the results obtained, and the implications of these findings for future research and clinical practice. By elucidating the anti-bacterial activity of ZnO nanoparticles derived from Ayurveda oral health formulations, this research contributes to the growing body of knowledge on innovative strategies for managing dental caries and promoting oral well-being (Zeng et al., 2022).

## MATERIALS AND METHODS

### Nanoparticle Synthesis and Characterization

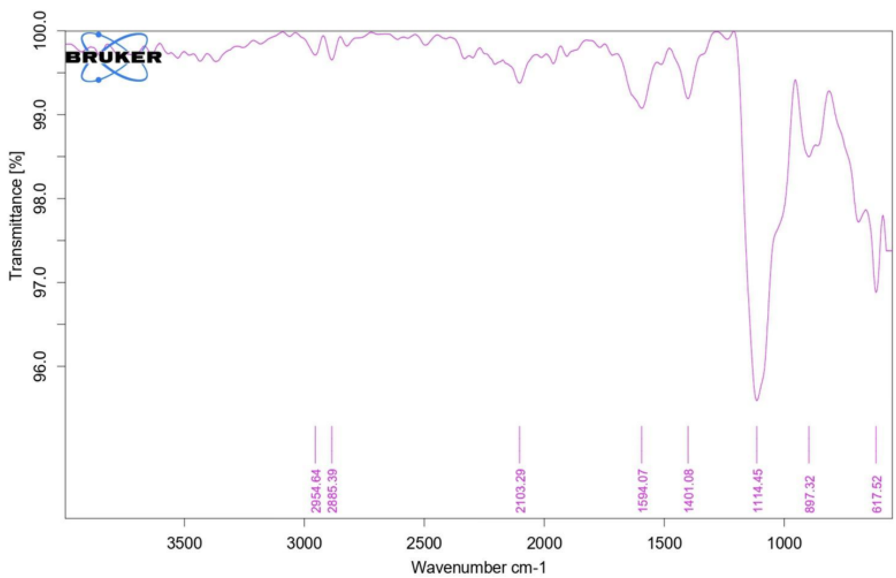
Plant extracts were obtained individually using methanol, ethyl acetate, hexane, and distilled water at concentrations of 0.1g $ml^{-1}$. The extraction process occurred over 10 hours at room temperature with agitation at 300 rpm (Rahdar, Aliahmad, & Azizi, 2015). Filtration and centrifugation at 4000 rpm for 10 minutes were employed to collect extracts, and solvent removal was achieved by drying the extracts in a water bath at 50°C. Solid samples of green synthesized ZnO nanoparticles were subjected to FT-IR analysis using an AVATAR 330 FT-IR spectrometer. X-ray diffraction analysis (XRD-6000 Shimadzu) was performed to measure the intensity of diffracted X-rays as a function of the diffraction angle 2. The obtained diffraction pattern facilitated the identification of crystalline phases, measurement of structural properties, and determination of crystallite size and orientation (Ghosh Chaudhuri & Paria, 2012; Soe et al., 2018).

### Assessing Antibacterial activity by Agar Well Diffusion Assay:

Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. mutants was inoculated in the sterile nutrient broth and incubated at 37 °C for 24 h. Mueller Hinton Agar (MHA, Himedia) medium was prepared, sterilized in an autoclave at 15 lbs pressure for 15 min and poured into a sterile petri dish and incubated at 37 °C for 24 h. The antibacterial activity of the bacteria was tested using Agar well diffusion method. 24 h old nutrient broth cultures of test bacteria were aseptically swabbed on sterile nutrient agar plates. ZnO nanoparticles of 50g/ml and 100g/ml were added to 2 of these plates (Valgas, Souza, Smânia, & Smânia Jr, 2007). The 3rd plate contained streptomycin at 10g/ml concentration and the last plate contained water as negative control. The experiment was carried out and the zone of inhibition was recorded. *Streptococcus mutants* (gram-positive bacteria) were used as test organisms and Streptomycin was used as positive control (Osés et al., 2016).

## RESULTS

### FT-IR Characterization:



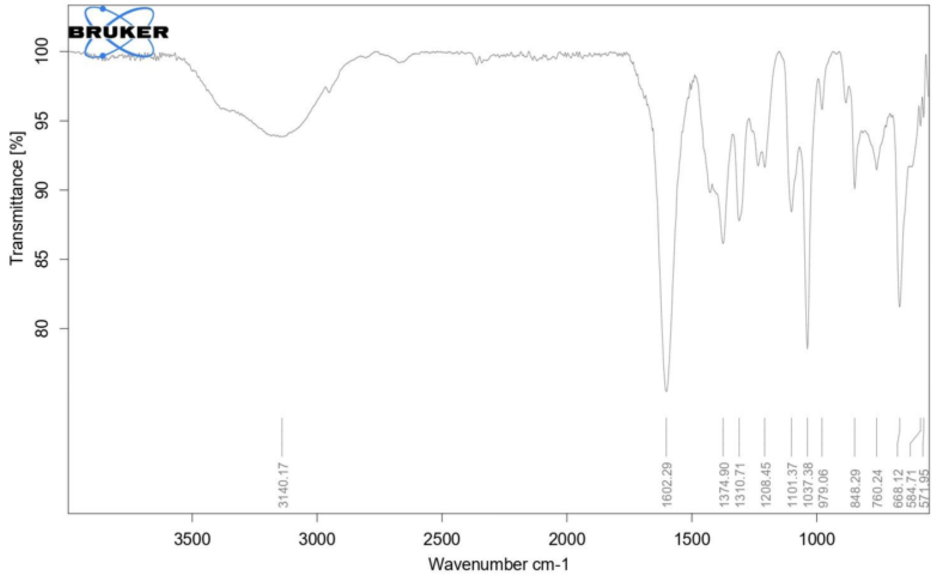
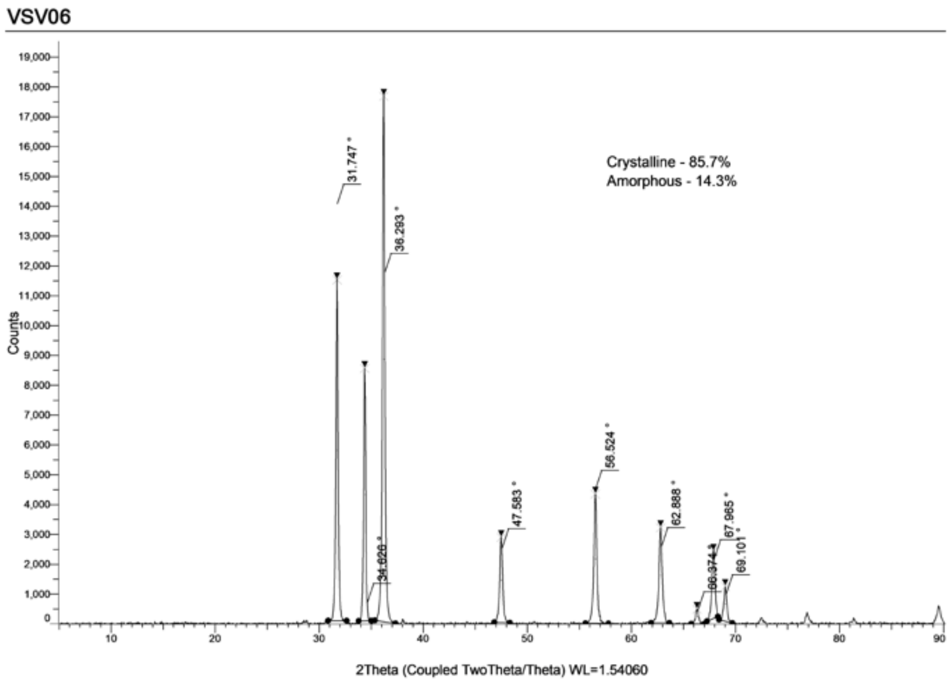



**Figure 1 and 2: The FT-IR Results of Control, i.e. Streptomycin and of ZnO Nanoparticles**

FT-IR characterization was done to identify the functional groups present in the compound based on the peaks obtained in the infrared radiation. FT-IR of Streptomycin contained major peaks at 1594.07,1401.08, 1114.45 and 617.52 pertaining to aromatic ring/carbonyl group, aromatic grp, aromatic ring C-H bend in plane or aliphatic anime and C-S and C=S stretching respectively. In ZnO nanoparticle, major peaks are observed at 1602.29, 1374.90, 1101.37,1037.38 and 668.12 which shows the presence of Aromatic Ring stretch(C=C-C), Phenol(C-O stretch), Aromatic ring C-H bend in plane ,several aromatic C-H bends in plane and aromatic ring C-H stretching poly nuclear ring.

**XRD Characterization:**



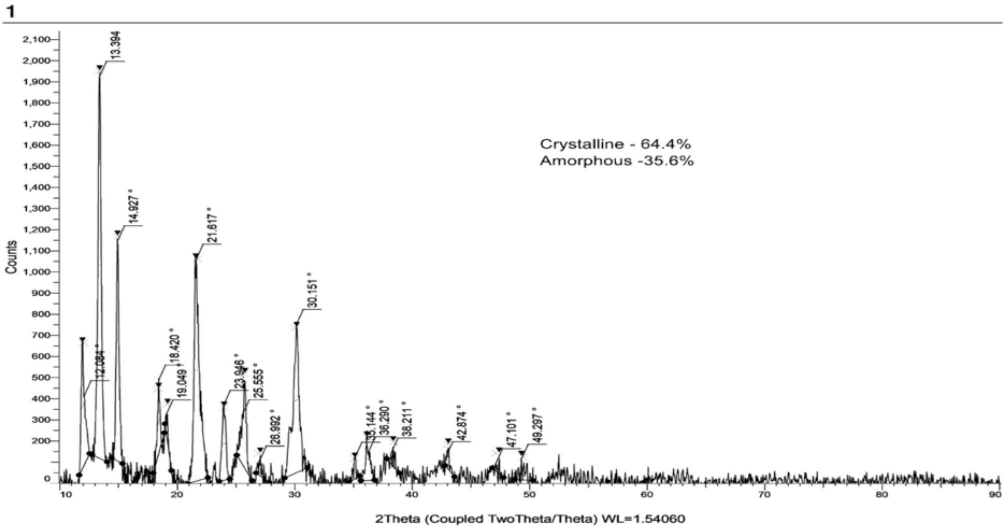



**Figure 3 and 4: The XRD Characterization Results of Streptomycin and ZnO Nanoparticles**

**Anti-Bacterial activity of ZnO Nanoparticles:**

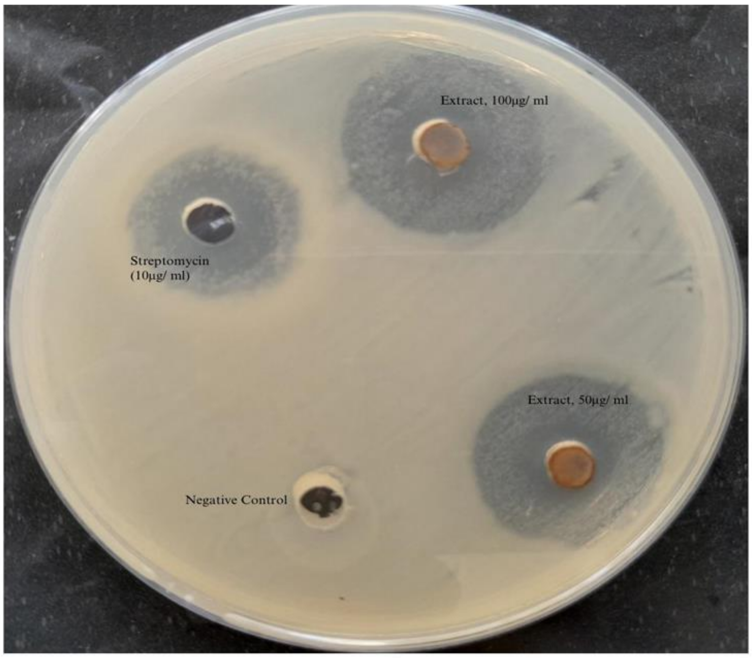



**Figure 5: Anti-Bacterial activity of ZnO nanoparticles**

**Table 1: Anti-Bacterial Activity of ZnO Nanoparticles**

| Compound | Zone of Inhibition(mm) |
|---|---|
| Streptomycin | 18±0.6 |
| Extract with ZnO nanoparticle (50g/ml) | 8±0.5 |
| Extract with ZnO nanoparticle(100g/ml) | 12±0.4 |

Disc diffusion assay done on ZnO NP showed 8 mm as radius of zone of inhibition at 50 g/ml concentration and 12 mm as radius of zone of inhibition at 100g/ml concentration. Compared to the zone of inhibitor showed by streptomycin (our positive control), it shows 67% effectiveness of streptomycin

## DISCUSSION

The discussion of the anti-bacterial activity of ZnO nanoparticles derived from Ayurveda oral health formulation against pathogens causing dental caries encompasses several key aspects, including the efficacy of ZnO nanoparticles, their mechanism of action, potential applications in oral healthcare, and implications for future research and clinical practice.

### Efficacy of ZnO Nanoparticles:

The results of this study demonstrate the significant anti-bacterial activity of ZnO nanoparticles against pathogens commonly associated with dental caries, such as Streptococcus mutans and Lactobacillus species. The effectiveness of ZnO nanoparticles in inhibiting bacterial growth and biofilm formation highlights their potential as a promising antimicrobial agent for oral healthcare. The size-dependent antimicrobial activity of ZnO nanoparticles, with smaller particles exhibiting greater efficacy, underscores the importance of nanoparticle design and optimization for enhanced therapeutic outcomes (Zanni et al., 2016).

### Mechanism of Action:

The antimicrobial mechanism of ZnO nanoparticles involves multiple pathways, including the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, and interference with cellular processes vital for bacterial survival (Abdal Dayem et al., 2017). ROS-induced oxidative stress damages bacterial cell components, leading to cell death and inhibition of bacterial proliferation. Additionally, the ability of ZnO nanoparticles to penetrate bacterial biofilms and disrupt their structural integrity contributes to their antimicrobial efficacy. Understanding the intricate mechanisms underlying the anti-bacterial activity of ZnO nanoparticles is crucial for optimizing their therapeutic potential and minimizing potential adverse effects (Mendes et al., 2022).

### Potential Applications in Oral Healthcare:

The findings of this study have implications for various applications in oral healthcare. ZnO nanoparticles derived from Ayurveda oral health formulations can be integrated into dental materials, such as composite restoratives and dental implants, to impart antimicrobial properties and prevent bacterial colonization. Incorporating ZnO nanoparticles into oral hygiene products, such as toothpaste and mouthwash, can enhance their efficacy in reducing plaque formation and combating dental caries-causing pathogens. Furthermore, ZnO nanoparticles may be explored for targeted delivery of antimicrobial agents to specific oral sites, thereby optimizing treatment outcomes while minimizing systemic exposure (Kumaresan et al., 2022; Roshan, 2023).

### Future Scope:

Future research in this area should focus on elucidating the long-term safety and biocompatibility of ZnO nanoparticles in oral tissues, as well as their potential interactions with dental materials and oral microbiota. Clinical studies are warranted to evaluate the efficacy of ZnO nanoparticles in preventing dental caries, reducing plaque accumulation, and promoting oral health in human subjects. Additionally, efforts should be directed towards standardizing the synthesis and characterization of ZnO nanoparticles to ensure reproducibility and reliability across studies. In clinical practice, the integration of ZnO nanoparticles derived from Ayurveda oral health formulations can contribute to holistic and personalized approaches to oral healthcare. Dentists and oral healthcare providers can leverage the antimicrobial properties of ZnO nanoparticles to develop customized treatment plans for patients at risk of dental caries and periodontal diseases. Embracing innovative technologies, such as nanotechnology and traditional medicine synergies, can lead to the development of advanced oral care strategies that are effective, safe, and sustainable.

## CONCLUSION

In conclusion, the anti-bacterial activity of ZnO nanoparticles derived from Ayurveda oral health formulations represents a promising avenue for combating pathogens causing dental caries and promoting oral well-being. By harnessing the strengths of nanotechnology and traditional medicine, this research opens new possibilities for enhancing oral healthcare practices and addressing emerging challenges in dental disease management.

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