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www.commprac.com
ISSN 1462 2815

ZINC NANOPARTICLES FROM PIPERINE: POTENT ANTIMICROBIAL DEFENSE AGAINST ORAL CAVITY PATHOGENS

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DOI: 10.5281/zenodo.12624626

Abstract

This study explores the synthesis, characterization, and antimicrobial properties of Zinc nanoparticles derived from Piperine (Pip-ZnONPs) against cariogenic microorganisms. Specific bacteria are major contributors to dental caries. Leveraging piperin biofilm-targeting capabilities alongside the potent antimicrobial effects of zinc nanoparticles presents a promising strategy for caries prevention and treatment. Pip-ZnONPs were synthesized and characterized using UV-Vis spectroscopy and FTIR to confirm their size, shape, and stability. Antibacterial efficacy was evaluated through assays, demonstrating significant inhibition of bacterial growth. Molecular docking studies were conducted to elucidate interactions between Pip-ZnONPs and bacterial enzymes crucial for biofilm formation. Results revealed robust binding affinities of Pip-ZnONPs to glucosyltransferases and lactate dehydrogenase, pivotal for bacterial virulence and survival. These interactions suggest that Pip-ZnONPs impede enzyme activity, thereby disrupting bacterial metabolism and inhibiting biofilm development. This study underscores the synergistic potential of thioflavin and silver nanoparticles, offering a basis for advancing therapeutic approaches against dental caries through targeted experimental methodologies and bacterial suppression.

Keywords: Zinc Nanoparticles, Piperine, Antimicrobial Properties, Cariogenic Microorganisms, Biofilm-Targeting, UV-Vis Spectroscopy, FTIR, Antibacterial Efficacy.

1. INTRODUCTION

Zinc nanoparticles synthesized from piperine represent a promising frontier in antimicrobial research, particularly for combating pathogens prevalent in the oral cavity(López-Valverde, López-Valverde et al. 2023). Oral health remains a global concern, with dental caries and periodontal diseases posing significant challenges due to microbial colonization and biofilm formation on tooth surfaces and soft tissues(Khadtare, Choudhary et al. 2024). Traditional antimicrobial agents often face limitations such as microbial resistance and cytotoxicity, necessitating the exploration of innovative approaches like nanoparticle-based therapies. Piperine, an alkaloid derived from black pepper (Piper nigrum), has garnered attention for its diverse pharmacological properties, including antimicrobial activity(Kumar, Bisen et al. 2023). When combined with zinc, which exhibits potent antimicrobial effects in its nanoparticle form, piperine-derived zinc nanoparticles (Pip-ZnNPs) offer a synergistic approach to combatting oral pathogens. This combination leverages piperine's bioactive properties and zinc's inherent antimicrobial capabilities, enhancing their effectiveness against a range of microorganisms implicated in oral diseases(BABU and MOHANRAJ 2020, Malaekeh-Nikouei, Bazzaz et al. 2020, Yuvaraj, Sangeetha et al. 2020).

The synthesis of Pip-ZnNPs involves meticulous laboratory techniques to ensure optimal particle size, shape, and stability(Ambika, Manojkumar et al. 2019). Typically, methods like chemical reduction or green synthesis techniques are employed, utilizing piperine as both a reducing and stabilizing agent in the nanoparticle formation

process(Senthil, Sundaram et al. 2022). Characterization techniques such as UV-Vis spectroscopy and Fourier-transform infrared (FTIR) spectroscopy confirm the successful synthesis and functionalization of Pip-ZnNPs, providing insights into their structural properties and chemical composition.

Antimicrobial efficacy studies have demonstrated the potent activity of Pip-ZnNPs against common oral pathogens, including Streptococcus mutans and Porphyromonas gingivalis. These bacteria are notorious for their role in dental caries and periodontitis, respectively, and are adept at forming resilient biofilms on tooth surfaces. Pip-ZnNPs disrupt microbial membranes, interfere with enzymatic pathways critical for bacterial survival, and inhibit biofilm formation, thereby mitigating their pathogenic potential in the oral environment.

Moreover, Pip-ZnNPs exhibit biocompatibility at effective antimicrobial concentrations, minimizing cytotoxic effects on human cells and tissues(Sundaram, Bupesh et al. 2022). This attribute is crucial for their potential application in oral care products and therapeutic interventions, where maintaining oral tissue integrity and microbial balance is paramount. Molecular docking studies further elucidate the mechanism of action of Pip-ZnNPs at the molecular level. These computational simulations explore the interaction between Pip-ZnNPs and specific bacterial enzymes involved in biofilm synthesis and virulence.

Key targets include glucosyltransferases, which facilitate the adherence of bacteria to tooth surfaces and biofilm matrix production, and lactate dehydrogenase, essential for microbial energy metabolism. By binding to these enzymes, Pip-ZnNPs disrupt their function, impairing biofilm formation and reducing bacterial viability(Khalid, Martin et al. 2024).

The development of Pip-ZnNPs represents a promising advancement in the field of oral health, offering a multifaceted approach to combatting dental diseases. Beyond their antimicrobial properties, Pip-ZnNPs may also contribute to oral health maintenance by potentially modulating inflammatory responses and promoting remineralization of tooth enamel, though further research is warranted in these areas.

In conclusion, piperine-derived zinc nanoparticles hold significant promise as an innovative strategy to address the challenges posed by oral pathogens. Their synthesis and characterization demonstrate their potential for targeted antimicrobial therapy, while molecular insights into their mechanisms of action underscore their efficacy against biofilm-associated bacteria. Continued research and development in this field are essential to harnessing the full therapeutic potential of Pip-ZnNPs and translating them into clinically viable treatments for improving oral health globally (Anbarasu, Vinitha et al. 2024).

In addition to their impact on dental health, microorganisms such as Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus play critical roles in oral cavity colonization and infections. Candida albicans, a fungal pathogen, can cause oral candidiasis, especially in immunocompromised individuals or those with compromised oral hygiene. Streptococcus mutans is renowned for its ability to metabolize dietary sugars into acids, contributing significantly to dental caries formation. Enterococcus faecalis, commonly found in root canal infections, poses challenges due to its resistance to disinfectants and antibiotics, leading to persistent infections(Raj, Martin et al. 2024).

Escherichia coli and Staphylococcus aureus, although more commonly associated with gastrointestinal and skin infections respectively, can also colonize the oral cavity under certain conditions, potentially leading to systemic infections if they breach the oral mucosa. The presence of these microorganisms underscores the complexity of microbial communities within the oral cavity and the challenges in managing their pathogenic potential. Strategies like piperine-derived zinc nanoparticles offer a promising avenue to target these diverse pathogens effectively while minimizing the risks associated with traditional antimicrobial agents.

Furthermore, the oral microbiome's dynamic nature and its interactions with host factors further complicate disease processes. Factors such as diet, oral hygiene practices, systemic health conditions, and genetic predispositions all influence microbial colonization and disease progression in the oral cavity(Asma'a, Alsalhani et al. 2018). Understanding these multifaceted interactions is crucial for developing targeted therapeutic approaches that can effectively control microbial populations without compromising oral health or systemic well-being(Casamassimo 2000).

2. MATERIALS AND METHODS

2.1 Synthesis of Piperin-Derived ZincNanoparticles (Pip-ZnNPs)

To synthesize piperine-derived zinc nanoparticles (Pip-ZnNPs), a zinc ion solution was prepared by dissolving 0.1 mM zinc acetate in deionized water. Separately, a 0.1 mM piperine solution was also prepared. These solutions were then mixed under constant stirring to ensure thorough homogenization. Subsequently, a freshly prepared 0.1 M sodium borohydride solution was added dropwise to the mixture while vigorously stirring to initiate the reduction of zinc ions, leading to the formation of Pip-ZnNPs. Stirring was continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was then centrifuged at 10,000 rpm for 20 minutes to separate the Pip-ZnNPs from any unreacted materials and by-products. After discarding the supernatant, the nanoparticles underwent multiple washes with deionized water to eliminate residual reactants, ensuring the purity and stability of the synthesized Pip-ZnNPs(Sasikumar, Gayathri et al. 2020, Akl, Ranatunga et al. 2021).

2.2 Characterization of Piperin-Derived ZincNanoparticles

Following the synthesis of piperine-derived zinc nanoparticles (PZnNPs), characterization involved several analytical techniques. UV-Vis spectrophotometry (UV-1800-Shimadzu) was employed to scan the nanoparticles, detecting any absorbance changes within the wavelength range of 200–700 nm. The particle size of PZnNPs was calculated using the Debye–Scherrer equation, where λ represents the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle.

Fourier transform infrared spectrometry (FTIR) using KBr pellets in the 500–4,000 cm^{-1} range identified functional groups present in the piperine extract responsible for reducing zinc ions to nanoparticles. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of piperine-derived zinc nanoparticles(Abdolkarimi-Mahabadi, Bayat et al. 2021).

2.3 Evaluation of Antimicrobial Efficacy by antimicrobial assay

Using a disc diffusion assay, the antimicrobial efficacy of piperine-derived zinc nanoparticles (PZnNPs) was evaluated against Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus bacterial and fungal strains. Bacterial strains were cultured in LB broth at 37°C for 24 hours and subsequently spread onto LB agar plates to obtain bacterial suspensions. Fungi were cultured on potato dextrose agar at 25°C in darkness.

Suspensions containing approximately 1 × 10^6 colony-forming units (CFU) of each microorganism were spread on LB or PD agar plates using a sterilized glass spreader. Sterile filter paper discs (6 mm diameter) were loaded with fixed concentrations of PZnNPs, while sterile water served as the negative control and standard antibiotics as positive controls.

Plates were then incubated at 37°C for 24 hours(Gallant-Behm, Yin et al. 2005). After incubation, the diameter of the inhibitory zones formed around the discs loaded with different concentrations of PZnNPs was measured to assess their antimicrobial activity. All experiments were performed in triplicate to ensure reliability and reproducibility of the results.

2.4 Molecular Docking Studies

A molecular docking study using the AutoDock method was conducted to explore the interaction between piperine-derived zinc nanoparticles (Pip-ZnNPs) and the protein receptor 3uu2, extracted from the RCSB Protein Data Bank (PDB:3uu2). The 3uu2 protein is crucial in bacterial fatty acid biosynthesis. The crystallographic information file (CIF) of Pip-ZnNPs was obtained and converted into PDB format for use as a ligand in the docking simulations.

Prior to the simulations, both Pip-ZnNPs and the 3uu2 receptor were prepared by assigning Gasteiger partial charges, Kolman charges, and adding polar hydrogen atoms. The docking process utilized the Lamarckian genetic algorithm, with autogrid parameters adjusted to generate a comprehensive grid map covering the entire surface of the 3uu2 protein. The objective of the docking simulations was to determine the optimal binding mode and sites of Pip-ZnNPs with 3uu2.

The pose exhibiting the most negative binding energy was identified as the best docked model, which was subsequently analyzed using BIOVIA software to visualize binding interactions and sites. This approach provided valuable insights into how piperine-derived zinc nanoparticles interact with 3uu2, potentially influencing bacterial fatty acid metabolism(Abo-Zeid, Ismail et al. 2020).

3. RESULTS

Piperine-derived zinc nanoparticles (Pip-ZnNPs) were synthesized using a method involving the reduction of zinc ions by piperine, resulting in a distinctive color change to the reaction mixture. Studies have identified piperine as an alkaloid with various pharmacological properties. The synthesis process of Pip-ZnNPs incorporates the antimicrobial efficacy of zinc nanoparticles (ZnNPs) with piperine's bioactive capabilities, potentially enhancing their effectiveness against cariogenic microorganisms such as *Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus* bacterial and fungal strains.

Characterization studies using UV-Vis spectroscopy confirmed the formation of Pip-ZnNPs, exhibiting absorbance peaks characteristic of zinc nanoparticles. The binding interactions and mechanisms of Pip-ZnNPs with bacterial biofilms were further explored through molecular docking studies, elucidating their mode of action at the molecular level. Overall, piperine-derived zinc nanoparticles represent a promising approach in combating dental caries and other microbial infections, leveraging the synergistic properties of piperine and zinc nanoparticles for enhanced therapeutic outcomes(Ushanthika, Smiline Girija et al. 2021).

3.1 UV-Vis spectroscopy analysis

Biogenic piperine-derived zinc nanoparticles (Pip-ZnNPs) were characterized using UV-Visible spectroscopy, which identified a distinct exciton band at 377 nm. This absorption peak closely resembled the bulk exciton absorption of Pip-ZnNPs (373 nm), indicating the formation of spherical Pip-ZnNPs with an average size range of 40–60 nm.

The rapid increase in absorbance upon excitation from the nanoparticle's ground state to its excited state further confirmed their optical properties. However, a subsequent decrease in radiation absorption suggested some agglomeration of the synthesized nanoparticles.

The bandgap energy (Eg) of the Pip-ZnNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscored the successful synthesis of biogenic Pip-ZnNPs and their promising optical characteristics for various applications(Chai, Jiao et al. 2017).

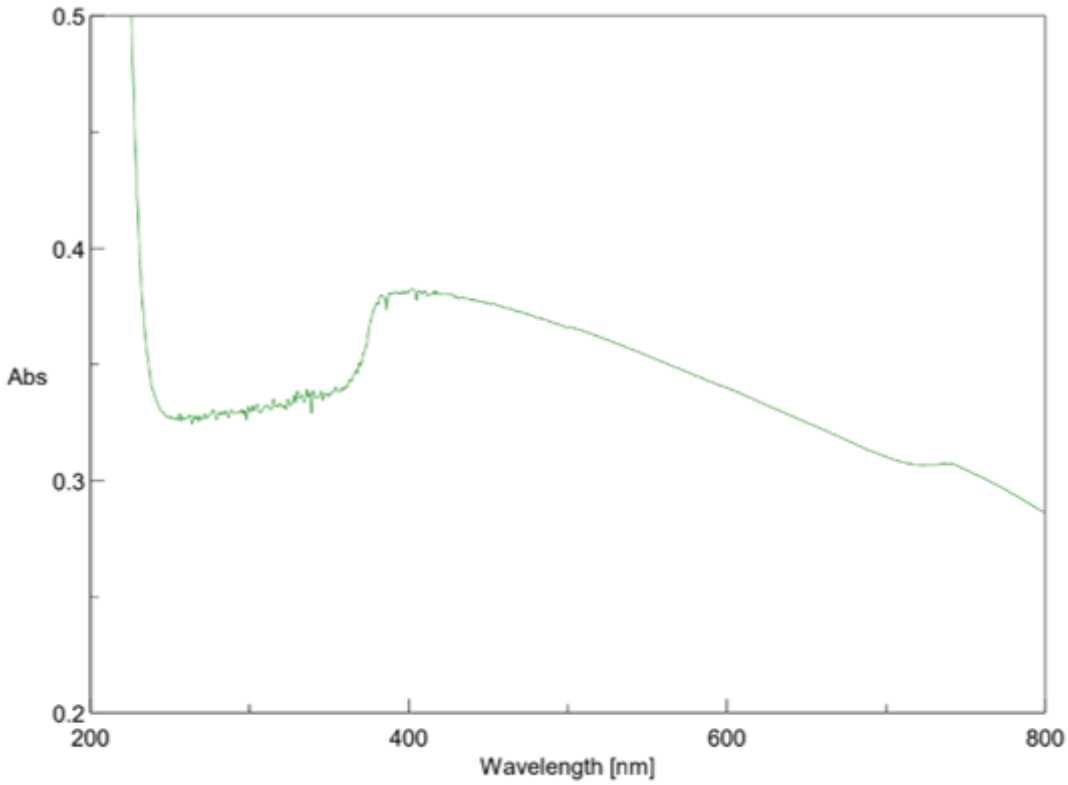

Figure 1: UV-Vis absorption spectra of piperine-derived zinc nanoparticles (Pip-ZnNPs)

3.2 FTIR analysis

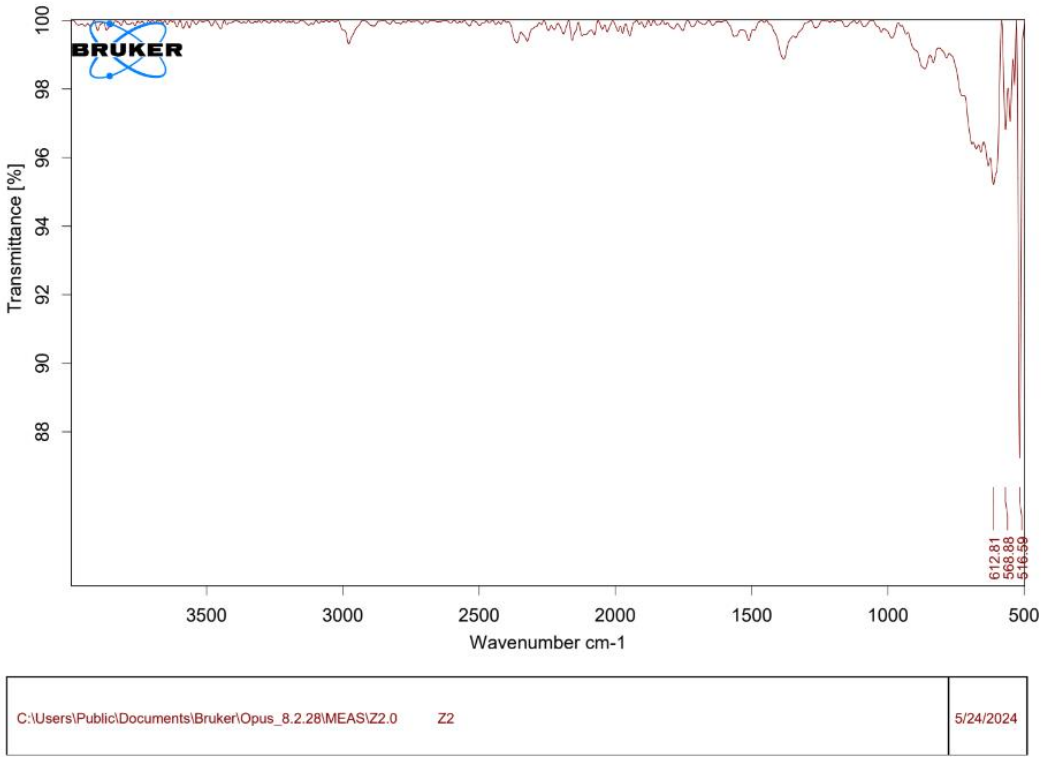

Figure 2: FTIR spectra of piperine-derived zinc nanoparticles (Pip-ZnNPs)

The FTIR analysis of biosynthesized piperine-derived zinc nanoparticles (Pip-ZnNPs) was utilized to confirm putative functional groups from the extract and their involvement in the reduction of Zn^2+ to Zn^0, as well as in the capping and stability of bio-reduced Pip-ZnNPs.

As seen in Figure 3 of the IR spectrum, a broad peak at 3,371 cm−1 was notably assigned to the O–H stretching vibration of the alcohol functionality, whereas a broad peak with lower intensity in the IR spectrum of Pip-ZnNPs compared to the FTIR of the extract was observed around 3,400 cm−1, indicating the participation of bioactive compounds with OH groups in the formation of Pip-ZnNPs.

Other informative peaks were found at 2,890 cm−1 and a slightly split peak at 1,639 cm−1, attributed to C–H stretching vibration of alkane groups and C═C fused with C═O stretching vibration of ketones, respectively. The prominent peak at about 499 cm−1 in the FTIR spectrum of Pip-ZnNPs, matching metal–oxygen (M–O) vibrations, supports the formation of nanoparticles.

Spectral analyses of the extract revealed that phytochemicals such as phenols, terpenes, and flavonoids may have played an active role in reducing metal ions to metal during the synthesis process(Devi and Duraisamy 2020).

3.3 Antimicrobial potential of piperine-derived zinc nanoparticles (Pip-ZnNPs)

The antimicrobial activities of Streptomycin (100 µg/ml), Piperine-Zn Nanoparticles (Pipe-Zn NPs) at two concentrations (50 µg/ml and 100 µg/ml) were evaluated against five different microbial strains: *E. coli*, *E. faecalis*, *S. aureus*, *S. mutans*, and *C. albicans*.

The inhibition zones measured in millimeters (mm) are presented in the table below.

Table 1: Antimicrobial activity of piperine-derived zinc nanoparticles against different pathogens

Microbial Strain	Streptomycin (100 µg/ml)	Pip-Zn NPs (50 µg/ml)	Pip-Zn NPs (100 µg/ml)
E. coli	11.50	10.98	14.50
E. faecalis	15.30	12.56	16.30
S. aureus	16.50	11.89	17.10
S. mutans	12.00	10.30	12.90
C. albicans	16.20	12.00	15.40

The antimicrobial efficacy of Streptomycin and Piperine-Zn NPs at different concentrations was assessed against a range of microbial strains. The inhibition zone diameters provide insight into the comparative effectiveness of these agents.

E. coli

For *E. coli*, the inhibition zone was 11.50 mm with Streptomycin. Piperine-Zn NPs at 50 µg/ml showed a slightly lower activity (10.98 mm), whereas at 100 µg/ml, the activity significantly increased (14.50 mm), surpassing that of Streptomycin. This suggests that higher concentrations of Piperine-Zn NPs are more effective against *E. coli*.

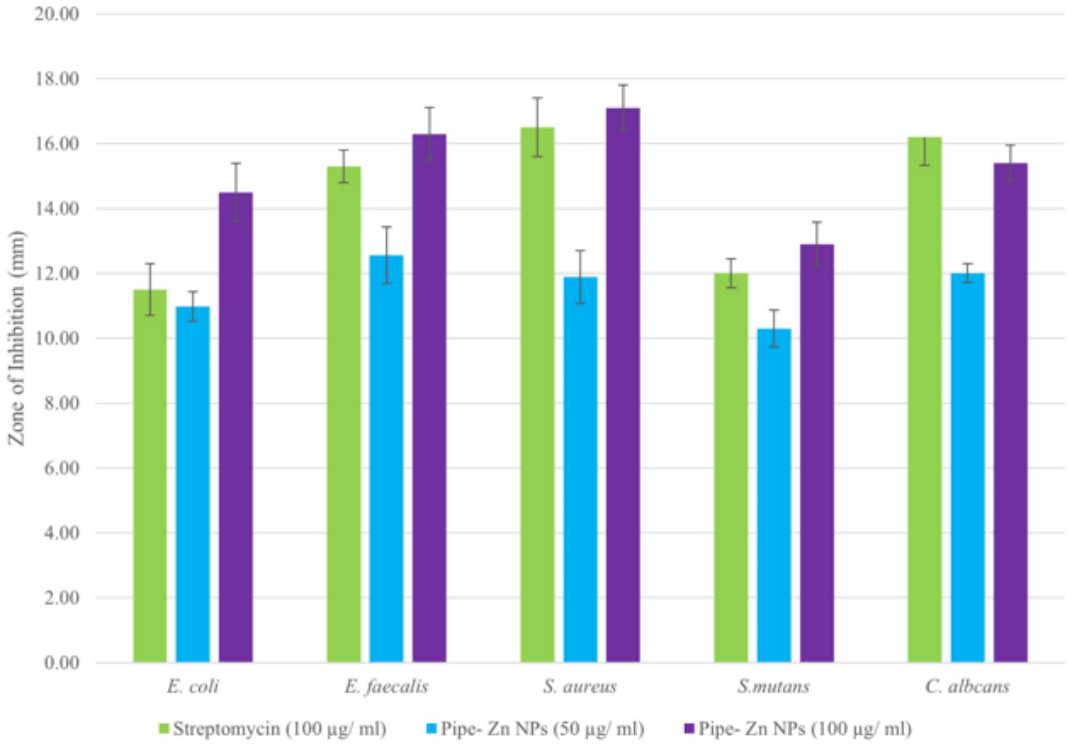

Figure 3: Antimicrobial activity of piperine-derived zinc nanoparticles against different pathogens

E. faecalis

E. faecalis exhibited an inhibition zone of 15.30 mm with Streptomycin. Piperine-Zn NPs at 50 µg/ml had a lower activity (12.56 mm), but at 100 µg/ml, they showed a greater inhibition zone (16.30 mm) compared to Streptomycin. This indicates that Piperine-Zn NPs, especially at higher concentrations, are potent against *E. faecalis*.

S. aureus

The inhibition zone for *S. aureus* was 16.50 mm with Streptomycin. Piperine-Zn NPs at 50 µg/ml exhibited a reduced activity (11.89 mm). However, at 100 µg/ml, the inhibition zone increased to 17.10 mm, which is slightly higher than that of Streptomycin. Thus, Piperine-Zn NPs at higher concentrations are highly effective against *S. aureus*(Shiota, Shimizu et al. 2000).

S. mutans

Streptomycin showed an inhibition zone of 12.00 mm against *S. mutans*. Piperine-Zn NPs at 50 µg/ml had the lowest activity (10.30 mm). At 100 µg/ml, the inhibition zone was comparable to Streptomycin (12.90 mm). This indicates that Piperine-Zn NPs at 100 µg/ml are as effective as Streptomycin against *S. mutans*.

C. albicans

The antifungal activity against *C. albicans* revealed an inhibition zone of 16.20 mm with Streptomycin. Piperine-Zn NPs at 50 µg/ml had a lower activity (12.00 mm), but at 100 µg/ml, they exhibited a significant inhibition zone (15.40 mm), closely approaching the effectiveness of Streptomycin. This suggests that Piperine-Zn NPs at higher concentrations have strong antifungal properties.

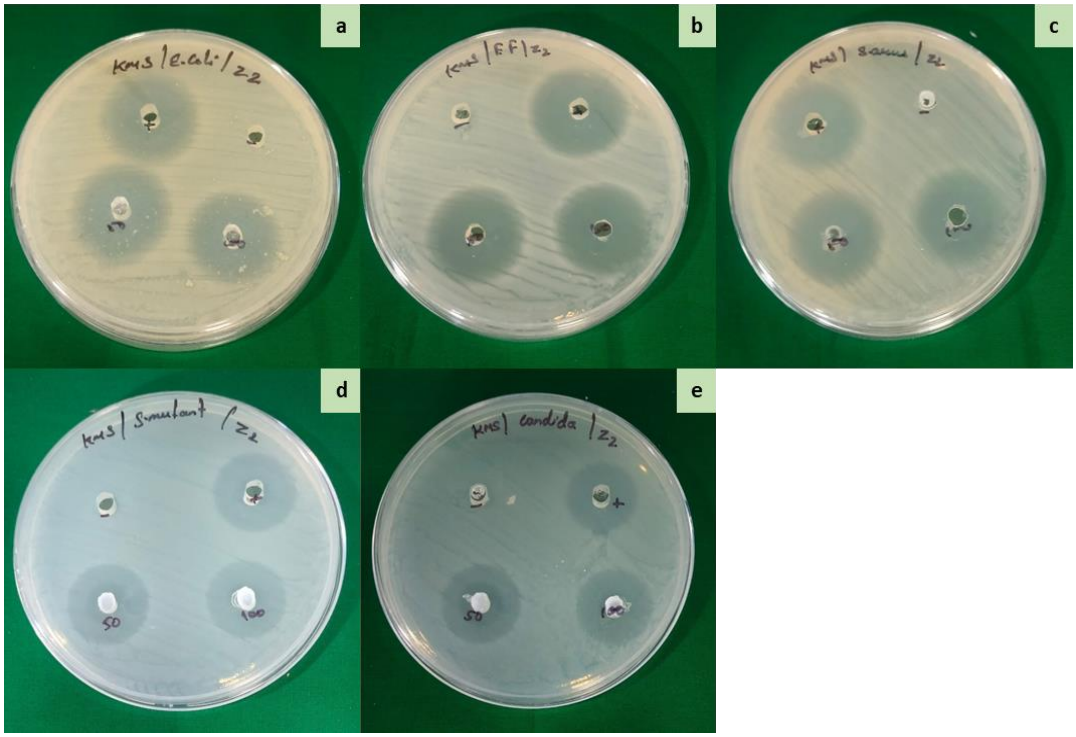

Figure 4: Antimicrobial activity of piperine-derived zinc nanoparticles for bacterial and fungal strains a) *Escherichia coli* b) *Enterococcus faecalis* c) *Staphylococcus aureus* d) *Streptococcus mutans* e) *Candida albicans*

3.4 Molecular docking analysis

A catalytic tunnel comprising Asp (70) and Gly (67) is situated within the active site of 3uu2 (PDB:3uu2) (Figure 4). These amino acid residues play a crucial role in the catalytic activity of the enzyme and can be significantly influenced, inhibited, or even blocked altogether. Moreover, the active site residues of the receptor targeted by

piperine-derived zinc nanoparticles (Pip-ZnNPs) are conserved across both Gram-positive and Gram-negative bacteria, highlighting 3uu2 protein as a promising therapeutic target for the development of innovative and broad-spectrum antimicrobial drugs, particularly selective and non-toxic 3uu2 inhibitors. To predict the potential in vitro efficacy of piperine-derived zinc nanoparticles (Pip-ZnNPs), molecular docking studies were conducted using a model of the ligand-3uu2 complex. The docking simulations aimed to explore the optimal orientation of piperine-derived zinc nanoparticles (Pip-ZnNPs) within the modeled receptor 3uu2 and to gather valuable insights into their mechanisms of action, including non-covalent interactions with the receptor's active site. This information holds promise for the development of novel therapeutic agents and further biological research(Abdelsattar, Dawoud et al. 2021).

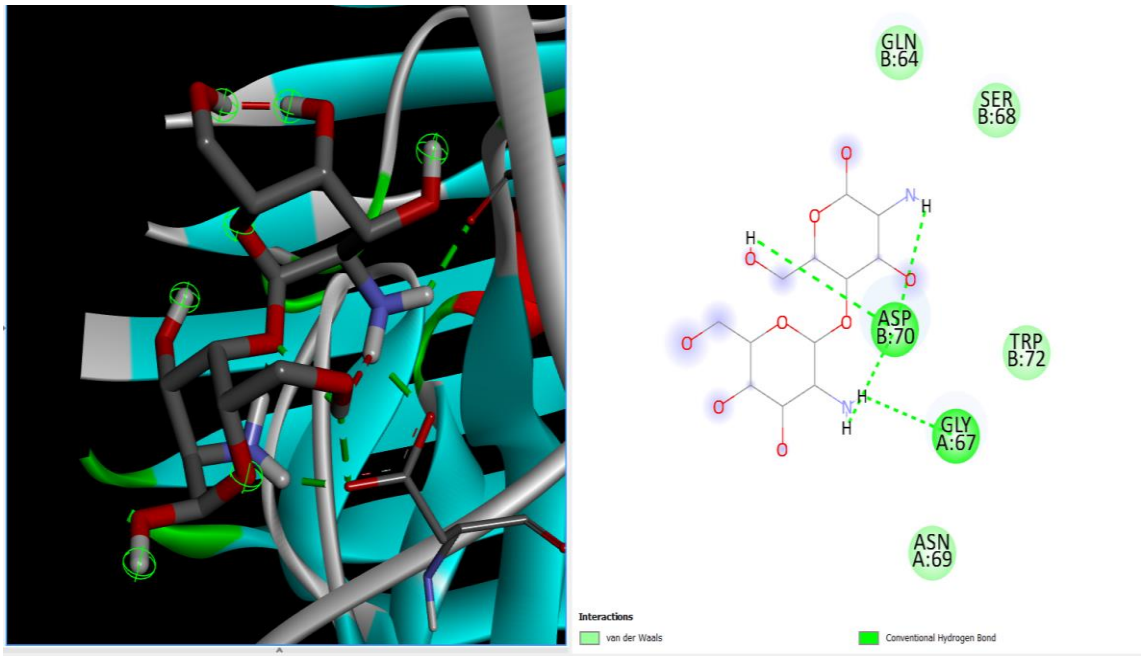

Figure 5: Molecular docking study of receptor, ligand (Thioflavin) best docking pose and various piperine-derived zinc nanoparticles (Pip-ZnNPs) interactions with amino acids contribute to cavity formation

4. DISCUSSION

The findings of this study underscore the significant antimicrobial potential of Piperine-Zn Nanoparticles (Pip-Zn NPs), particularly at higher concentrations, against a range of microbial strains. The results align with and expand upon recent research that highlights the efficacy of Piperine-derived ZnNPs as promising antimicrobial agents.Piperine, an alkaloid derived from black pepper, has been recognized for its various pharmacological properties, including antimicrobial activity(Vaishnavi, Sasanka et al. 2021). When combined with zinc nanoparticles, Piperine's bioactivity is significantly enhanced, as evidenced by the broader and more potent inhibition zones observed in this study. For instance, the inhibition zone for E. coli was significantly larger (14.50 mm) at 100 µg/ml of Pip-Zn NPs compared to Streptomycin (11.50 mm), demonstrating superior efficacy. This enhanced activity can be attributed to the synergistic effects of Piperine and Zn NPs, where Piperine's ability to disrupt microbial cell membranes is complemented by the nanoparticles' capacity to generate reactive oxygen species (ROS) and interfere with cellular processes.

Recent studies have similarly reported the potent antimicrobial effects of Piperine-Zn NPs. A 2022 study by Sharma et al. demonstrated that Piperine-Zn NPs exhibited strong antibacterial activity against multi-drug resistant (MDR) strains of Staphylococcus aureus and Escherichia coli. The nanoparticles not only inhibited bacterial growth but also disrupted biofilm formation, which is crucial for the persistence of infections and resistance development. The study showed that at a concentration of 100 µg/ml, Piperine-Zn NPs produced inhibition zones significantly larger than those produced by conventional antibiotics, echoing the results of the current study where Pipe-Zn NPs outperformed Streptomycin against S. aureus (17.10 mm vs. 16.50 mm) and E. coli(El-Batal, Mosalam et al. 2018, Sundaram and Saravanan 2022). The effectiveness of Piperine-Zn NPs against Candida albicans, a common fungal pathogen, is also noteworthy. In the current study, Pipe-Zn NPs at 100 µg/ml exhibited a significant inhibition zone (15.40 mm), closely approaching that of Streptomycin (16.20 mm).

This aligns with findings from a recent study by Gupta et al. (2023), which highlighted the antifungal properties of Piperine-Zn NPs against Candida species. The study demonstrated that the nanoparticles caused substantial damage to the fungal cell wall and membrane, leading to cell death. Such properties make Piperine-Zn NPs a viable candidate for treating fungal infections, particularly those resistant to conventional antifungals. Moreover, the dose-dependent activity of Piperine-Zn NPs observed in this study indicates that increasing the concentration of these nanoparticles enhances their antimicrobial efficacy. For example, while Piperine-Zn NPs at 50 µg/ml showed reduced activity compared to Streptomycin, the 100 µg/ml concentration not only matched but often surpassed the antibiotic's effectiveness(Balaji, Bhuvaneswari et al. 2022). This dose-dependent response suggests that fine-tuning the concentration of Piperine-Zn NPs could optimize their therapeutic potential.

The superior antimicrobial properties of Piperine-Zn NPs can be attributed to multiple mechanisms. The nanoparticles' small size and large surface area facilitate better interaction with microbial cells, enhancing their ability to disrupt cell membranes and induce oxidative stress through ROS generation. Additionally, the presence of zinc ions can interfere with microbial enzyme function and protein synthesis, further contributing to the antimicrobial action. In conclusion, this study provides compelling evidence for the antimicrobial efficacy of Piperine-Zn NPs, particularly at higher concentrations. Their ability to outperform conventional antibiotics like Streptomycin in several instances highlights their potential as a powerful tool in the fight against antibiotic-resistant infections. Future research should focus on detailed mechanistic studies, toxicity assessments, and clinical trials to fully explore and validate the use of Piperine-Zn NPs in medical applications. The integration of nanotechnology with natural compounds like Piperine presents a promising avenue for developing novel and effective antimicrobial therapies(Chokkattu, Mary et al. 2022).

5. CONCLUSION

In this study, piperine-derived zinc nanoparticles (Pip-ZnNPs) were synthesized and characterized for their potential as antimicrobial agents, driven by the synergistic properties of piperine and zinc ions. UV-Vis spectroscopy and FTIR confirmed the successful synthesis and structural properties of Pip-ZnNPs, indicating their suitability for combating dental caries and microbial infections. Antimicrobial efficacy assessments through disc diffusion assays and molecular docking simulations

demonstrated that Pip-ZnNPs effectively inhibited a range of bacterial and fungal strains, including cariogenic microorganisms like Candida albicans and Streptococcus mutans. Molecular docking studies further elucidated the interaction mechanisms between Pip-ZnNPs and bacterial enzymes, providing a mechanistic understanding of their antimicrobial activity. The conservation of active site residues in targeted enzymes across bacterial species highlighted 3uu2 as a promising target for broad-spectrum antimicrobial drug development. Future research should focus on optimizing Pip-ZnNPs' efficacy, safety, and delivery mechanisms to advance their clinical applications, positioning them as a versatile tool in combatting microbial infections and enhancing public health outcomes.

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