Pharmacogn. Res., 2026; 18(3):899-911.
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Original Article

Targeting Oral Biofilms and Inducing Apoptosis in Oral Cancer Cells: Mechanistic Insights of Hamamelitannin-Coated Zinc Oxide Nanoparticles through BCl-2/BAX/P53 Pathway

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ABSTRACT

Objectives: Dental pathogens are major contributions to problems with oral health such infections, gum disease, and tooth decay. There is also growing evidence connecting these infections to the development and course of oral cancer. Novel therapeutic strategies are needed to address issues with antibiotic resistance and treatment constraints. **Materials and Methods:** Using a variety of analytic methods, such as Ultraviolet (UV) spectroscopy, Scanning Electron Microscope (SEM), X-ray Diffraction (XRD), and Fourier-Transform Infrared Spectroscopy (FTIR), the zinc oxide nanoparticles coated with hamamelitannin was synthesized. The effectiveness of ZnO-Hamamelitannin (HAMA) NPs against dental pathogens was determined by evaluating their antioxidant and antibacterial activities using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS), and Minimum Inhibitory Concentration (MIC) tests. A MIC of 50 µg/mL was found. Studies of interactions were carried out to find out how well HAMA target the dental pathogens receptors. **Results:** Strong efficacy against dental infections was demonstrated by ZnO-HAMA NPs, which also showed notable antioxidant and antibacterial capabilities. ZnO-HAMA NPs and target pathogens have a high binding affinity, according to interaction study. Moreover, ZnO-HAMA NPs upregulated the expression of apoptotic genes like BCl2, BAX, and P53 in human epithelial Carcinoma cells (KB) oral squamous carcinoma cells because of their dose-dependent anticancer effects. **Conclusion:** ZnO-HAMA NPs offer a promising multimodal strategy to treat oral infections as well as oral cancer. However, before ZnO-HAMA NPs can be clinically implemented, more through research and testing will need to be conducted to address issues like NPs toxicity, biocompatibility, and long-term safety. This development could lead to substantial advancements in oral healthcare and new therapeutic approaches in the face of increasing antibiotic resistance.

Keywords: Cancer, Hamamelitannin, Health, Human, Illness, Medicine.

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Received: 22-01-2026;
Revised: 03-03-2026;
Accepted: 13-04-2026.

INTRODUCTION

Maintaining the mouth healthy is essential since dental pathogens have a major influence on diseases including tooth decay, gum disease and infections. If these disorders are not addressed, they can cause pain, discomfort, and serious health problems. Emerging research has also linked these pathogens to the onset and advancement of oral cancer, underscoring the need for integrated therapeutic approaches that address both in-fectious and malignant conditions (Gopika *et al.*, 2025). The rise in antibiotic resistance among oral pathogens poses a serious challenge, limiting treatment options and emphasizing the importance of developing novel strategies. Innovations in oral healthcare, such as the development of targeted therapies and preventive measures, are essential to mitigate the dual burden of oral infections and cancer. By focusing on new technologies and interdisciplinary collaborations, researches aim to enhance treatment efficiency while minimizing adverse effects, paving the way for improved outcomes in oral health management (Murugaiyan *et al.*, 2022). The prevention of gum disease and tooth decay as well as the prevention of healthy oral tissues and structures are all integral parts of maintaining oral health, which is closely related to general well-being. Dental pathogens, including, *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis*, and *Candida albicans*, are important in the development of a number of oral disorders because they foster

DOI: 10.5530/pres.20260271

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an environment that is favorable to the growth and inflammation of microorganisms (Bacali *et al*., 2022). Untreated tooth decay can progress from initial enamel erosion to deeper infections affecting dental pulp, potentially leading to abscess formation and severe pain. Similarly, chronic periodontal disease, including gingivitis and periodontitis, involves ongoing inflammation of the gums and deterioration of the tissues supporting the teeth, ultimately contributing to tooth mobility and loss. In addition to enhancing individual health, good oral hygiene and routine dental checkups also help to prevent the spread of bacteria into the bloodstream, which is major factor in the early detection and treatment of a number of diseases. Dental infections not only cause oral disorders but are also increasingly linked to the onset and development of oral cancer (Tuominen and Rautava, 2021). Malignancies affecting the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and throat are all included in the category of oral cancer. Oral squamous cell carcinoma, the most prevalent type of oral cancer, is largely preventable by risk factors such as tobacco use, excessive alcohol intake, chewing betel quid, and human papillomavirus infection. Recent research has shown that periodontal pathogen-induced chronic inflammation in oral tissues provides an environment that is favorable to carcinogenesis (Sebastian *et al*., 2025).

Nanotechnology is a new and exciting area of biomedical research that has the potential to help diagnose, cure, and prevent a wide range of disorders, including those that impact the oral cavity. The exceptional characteristics of Zinc-Oxide Nanoparticles (ZnO NPs), including their high surface area-to-volume ratio, antibacterial activity, and biocompatibility, have shown effectiveness against a variety of pathogens, such as fungi and bacteria (Arun *et al*., 2025; Sanjana *et al*., 2025). Furthermore, by triggering apoptosis, preventing proliferation, and altering signaling pathways essential in cell survival and death, ZnO NPs have demonstrated potential in cancer therapy. Hamamelitannin, a polyphenolic compound found in plants such as *Hamamelis virginiana* (witch hazel), a possesses antioxidant, antimicrobial and anti-inflammatory properties. The application of Hamamelitannin coating on ZnO nanoparticles (ZnO-Hamamelitannin [HAMA] NPs) offers a novel way to increase their therapeutic efficacy against oral cancer and dental infections. ZnONPs and Hamamelitannin together maximize their synergistic effects, which may enhance anticancer qualities by modulating apoptotic pathways and improve antibacterial action against resistant organisms. In this study, we aimed to synthesize and characterized ZnO-HAMA NPs using advanced analytic techniques to test their antibacterial and antioxidant properties against oral pathogens, and their possible anticancer effects on oral squamous cell carcinoma were searched for. The findings from this research contribute to our understanding of how ZnO-HAMA NPs can be utilized to address the dual challenges of oral infections and oral cancer, offering new avenues for advancing oral healthcare.

MATERIALS AND METHODS

Synthesis and Characterization of ZnO-HAMA NPs

By dissolving 50 mg of HAMA powder in 5 mL of ethanol, a concentrated solution of 10 mg/mL HAMA was prepared. The precipitation process was utilized in the synthesis of ZnO NPs. For an hour at room temperature, zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2\ H_2O$) (0.1m, 20 mL) was continuously stirred while the HAMA solution was being dissolved. After that, dropwise additions of Sodium Hydroxide (NaOH) solution (0.1m) were made to the zinc acetate solution until the pH approached roughly 10. To ensure full reaction, the resultant mixture was agitated for two more hours. These particles were then centrifuged for 10 min at 8,000 r.p.m., cleaned with distilled water to get rid of contaminants, and vacuum-dried for 12 hr at 60ºC. A UV-vis spectrophotometer was used to scan NP suspensions in distilled water in the 200-800 nm wavelength range. This allowed for the acquisition of the adsorption spectra, which revealed information about the bandgap energy of the nanoparticles. Using a Scanning Electron Microscope (SEM), the synthesized NPs shape and size distribution were investigated. A sample container was used to hold the powder samples, which were then scanned at a 2θ range of 10°-80° with a step size of 0.02°. The crystallographic phases and crystalline size of NPs were revealed by X-ray Diffraction analysis (XRD). Using Fourier-Transform Infrared Spectroscopy (FTIR), the chemical bonds and functional groups present in the synthesized NPs were examined (Anbarasu *et al*., 2024; Dash *et al*., 2025; Umakanth *et al*., 2024).

2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

To neutralize the DPPH radical, a stable free radical with an unpaired electron, the DPPH assay depends on antioxidants' capacity to transfer hydrogen atoms or electrons. ZnO-HAMA NPs with different concentration of 5, 25, 50, and 100 µg/mL was used to evaluate the antioxidant activity. By dissolving DPPH in ethanol, a 0.1-mm solution of the radical was created. The 100-µL of ZnO-HAMA NPs at varying concentrations were added with 900 µL of DPPH solution and kept for incubation. The mixture was then allowed to sit at room temperature in the dark for half an hour. With the aid of a UV-vis spectrophotometer, the absorbance of the resultant solution was determined at 517 nm (Marunganathan *et al*., 2024).

2,2′-Azino-Bis-(3-Ethylbenzothiazoline-6-Sulfonic) (ABTS)

The ABTS assay assesses the capacity of antioxidants to scavenge ABTS•+ radicals, which are generated when potassium persulfate oxidizes ABTS. The ABTS•+ radical cation was produced by combining potassium persulphate (2.45 mm) with ABTS solution (7 mm) and letting the mixture stand in the dark at room temperature for 16 hr to evaluate the antioxidant activity of the test samples. After dilution with ethanol, the resultant

ABTS•+ radicals had an absorbance of 0.70±0.02 at 734 nm. Zinc-oxide nanoparticles (ZnO-HAMA NPs) produced from Hamamelitannin were synthesized at different quantities (5, 25, 50, and 100 μg/mL). After mixing 900 μL of the diluted ABTS•+ solution with aliquots (100 μL) of ZnO-HAMA NPs at varying concentrations, the mixture was incubated for 6 min at room temperature in the dark. The absorbance of the resultant solution was determined with a UV-vis spectrophotometer at a wavelength of 734 nm (Marunganathan *et al*., 2024).

Minimal Inhibitory Concentration (MIC) Assay

The MIC assay was to find the least inhibitory concentration of ZnO-HAMA NPs that was necessary to stop the growth of oral pathogens. A sterile microtiter plate was divided into individual wells, and 100 μL of each concentration of ZnO-HAMA NPs was added. A standardized suspension of oral pathogens comprising approximately 2×10^5 colony-forming units per milliliter (CFU/mL) was introduced to each well using 100 μL of inoculum. After that, the microtiter plate was covered and incubated for 18-24 hr at the proper temperature for each type of microbe (for example, 37ºC for bacteria and 25ºC for fungus). Following incubation, the growth of the microorganisms in each well was visually inspected. The MIC of the test chemical was found to be the lowest concentration that totally prevented visible development, as shown by the lack of turbidity or colonies that could be seen (Dash *et al*., 2025; Joseph *et al*., 2025).

Well Diffusion Method for Zone of Inhibition

The ZnO-HAMA NPs were evaluated for their antibacterial efficacy against oral infections using the well diffusion method. Nutrient agar plates were prepared and inoculated with a standardized suspension of the test microbe. Next, using a sterile cork borer or well puncher, wells were created on the agar plates. Different ZnO-HAMA NP concentrations (20 μL) were administered to different agar plate wells. On agar plates, wells containing a common antimicrobial agent with established activity (positive control) were also made. After that, the plates were incubated for 18-24 hr at the ideal temperature for each type of microbe. Following the incubation period, the plates were inspected, and the diameter of the zone of inhibition-measured in millimeters (mm)-served as a proxy for the ZnO-HAMA NPs' antimicrobial efficacy (Umakanth *et al*., 2024).

Docking Studies

When performing molecular docking experiments, Autodock is a widely used software that predicts binding affinity values (kcal/mol) between the ligand and receptor. PyMOL was utilized to transform the 2D structure of HAMA into a 3D format after it was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Oral pathogens' receptor structures were obtained from the Protein Data Bank at https://www.rcsb.org/, and AutoDock Tool version 1.5.7 was utilized to build the receptors for docking investigations. There were preparatory processes for both the ligand and receptor structures that involved adding hydrogens, charges, and required bonds. The created ligand and receptor structures were easier to import thanks to autodock techniques; the ligand stayed flexible to investigate different conformations, while the receptor was characterized as stiff. Based on known binding pockets or active site residues, the receptor's construction location was located. The grid box size, spacing, and search parameters were carefully modified in accordance with Autodock's requirements. The parameters of the Lamarckian genetic algorithm were fine-tuned to achieve effective conformational space navigation. After processing, the Discovery Studio Visualizer program was used to depict the ligand-receptor interactions (Umakanth *et al*., 2024).

Anticancer Assay

In a carbon dioxide incubator at 37ºC human epithelial carcinoma cells (KB), cells or human epithelial carcinoma cells were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. For anticancer assay, cells were treated to different concentration of ZnO-HAMA NPs and kept for incubation. Following a 24-hr incubation period with ZnO-HAMA NPs, the 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was utilized to evaluate the vitality of KB cells. After aspirating the culture media, the cells were cleaned with Phosphate-Buffered Saline (PBS). Each well received an addition of MTT solution (5 mg/mL in PBS), which was then incubated for an additional 3 hr at 37ºC. Each well was then filled with the MTT solution (5 mg/mL in PBS) and allowed to incubate for an additional 3 hr at 37ºC. Following the incubation period, the formazan crystals were dissolved by adding dimethyl sulfoxide after the MTT solution had been withdrawn. Using a microplate reader, the absorbance of the resultant solution was determined at 579 nm (Umakanth *et al*., 2024).

Apoptosis Gene Expression

Total RNA was extracted from KB cells treated with ZnO-HAMA NPs by using the Trizol reagent. To make it easier to separate the RNA-containing aqueous phase, chloroform was added after cells were first lysed in Trizol reagent. After that, isopropanol was used to precipitate the RNA, which was then cleaned with ethanol and resuspended in water free of RNase. A spectrophotometer was used to evaluate the amount and quality of RNA. Reverse transcription kit was used, and instructions from the manufacturer were followed to perform first-strand cDNA synthesis. To create cDNA, reverse transcriptase, random primers, dNTPs, and RNase inhibitor were combined and incubated with RNA samples for 1 hr at 37ºC. After 5 min of heating at 95ºC to end the retraction, the cDNA was kept cold until further examination. ZnO-HAMA NPs-treated KB cells were used to measure the target gene expression levels using quantitative RT-PCR. The amplification

conditions were 40 cycles of denaturation at 95ºC for 30 sec each, after an initial step of denaturation at 95ºC for 5 min. Using the $2^{-\Delta\Delta Ct}$ method, the mRNA expression levels were computed after being normalized to a housekeeping gene, such as GAPDH (Umakanth *et al*., 2024).

Statistical Analysis

One-way Analysis of Variance (ANOVA) and post-hoc multiple comparisons (Tukey's test) were used in the statistical analysis to determine the significance of differences between the treatment group receiving different concentrations of ZnO-HAMA NPs and the untreated control. At $p<0.05$, the results were deemed statistically significant. Every experiment is carried out in triplicate, and the results are displayed as Mean±Standard deviation (Umakanth *et al*., 2024).

RESULTS

Synthesis and Characterization of ZnO-HAMA NPs

The Ultraviolet (UV) absorption spectra of synthesized ZnO-HAMA NPs reveal crucial insights into their optical properties. By measuring absorbance against wavelength, these spectra provide information on the bandgap energy, indicating the semiconductor nature of ZnO NPs. The absorption edge observed typically around 370 nm signifies the onset of direct bandgap absorption, crucial for applications in photocatalysis, sensors, and optoelectronic devices. Moreover, the spectra may exhibit characterization such as excitonic peaks or redshifts, reflecting variations in particle size or surface modifications. Understanding these spectra aids in tailoring the properties of ZnO-HAMA nanoparticles for specific technological advancements and biomedical applications (Figure 1).

Morphological, Physical, and Chemical Characterization

ZnO-HAMA NPs revealed unique surface features crucial to their functional qualities by SEM investigation. The SEM images showed NPs with a smooth and regular surface morphology, indicating uniformity and well-controlled synthesis. This smooth surface is advantageous as it can affect interactions at the nanoscale, particularly in biological environments where surface properties influence cellular interactions and applications such as drug delivery or sensing (Figure 2). FTIR analysis confirmed the successful coating of ZnO NPs with HAMA by identifying characteristic peaks specific to the coating material. The FTIR spectrum of the ZnO-HAMA NPs displayed notable peaks that matched the molecular vibrations of HAMA, validating the presence of this compound on the NPs surface. Key peaks observed included a prominent peak at approximately 1700 cm^{-1}, corresponding to the C=O stretch of the carbonyl groups in HAMA. This peak indicates the functional groups involved in the coating process. Additionally, a broad absorption band ranging from 3,200 to 3,600 cm^{-1} was detected, which corresponds to the O-H stretch of hydroxyl groups. This broad peak is characteristic of the hydrogen-bonded hydroxyl groups present in HAMA, further confirming its presence on the NPs. These characteristic peaks in the FTIR spectrum, corresponding to the C=O and O-H stretch, provide clear evidence of the successful functionalization of ZnO-HAMA NPs (Figure 3). The XRD patterns of the ZnO-HAMA NPs closely matched those of the standard ZnO wurtzite structure, affirming their high crystallinity and structural integrity. The absence of significant peak shifts in the XRD spectra indicates that the incorporation of HAMA as a coating did not induce phase changes or alter the crystalline nature of ZnO. This preservation of crystal structure is crucial for maintaining the material's physical and chemical properties, ensuring its suitability for various applications ranging from photocatalysis to biomedical sensing. Moreover, the sharp and well-defined diffraction peaks observed in the XRD patterns further validate the uniformity and consistency of the synthesized nanoparticles, contributing to their reliability in practical use (Figure 4).

Antioxidant Assay of ZnO-HAMA NPs

The experiment was conducted with different ZnO-HAMA NPs concentrations (5, 25, 50, and 100 µg/mL). ZnO-HAMA NPs had a moderate DPPH radical scavenging activity at the lowest dose of 5 µg/mL, with an inhibition percentage of 23%. This indicates the presence of antioxidant properties even at low nanoparticle concentrations. Increasing the concentration to 25 µg/mL significantly enhanced the DPPH scavenging activity, with an inhibition percentage of 48%. This demonstrates a dose-dependent increase in antioxidant capacity. This substantial increase highlights the strong free radical scavenging potential of the nanoparticles at higher concentrations. The ABTS assay demonstrated that ZnO-HAMA NPs exhibit strong antioxidant activity, with a clear dose-dependent increase in ABTS radical scavenging ability (Figure 5). These findings suggested that ZnO HAMA NPs can effectively neutralize free radicals, which is beneficial for their potential applications in therapeutic interventions and biomedical applications. The high antioxidant capacity of these nanoparticles enhances their value as a multifunctional agent in combating oxidative stress-related diseases.

The radical scavenging activities of ZnO-HAMA NPs were evaluated using both DPPH (a) and ABTS (b) assays, and compared to standard antioxidants such as trolox. ZnO-HAMA NPs was tested at concentrations of 5, 25, 50 and 100 µg/mL respectively. Results are shown as percentage scavenging activity relative to the control, with mean±SD from three independent experiments. IC_{50} values for ZnO-HAMA NPs and standards were calculated using GraphPad Prism. Significant differences between ZnO-HAMA NPs and standard antioxidants were indicated by * indicated the $p<0.05$.

Antimicrobial Activity of ZnO-HAMA NPs

The MIC results demonstrated that ZnO-HAMA NPs exhibit strong antimicrobial activity against all tested pathogens, with a clear dose-dependent increase in inhibition. These findings suggest that ZnO HAMA NPs are highly effective in combating a range of pathogenic microorganisms, making them promising candidates for antimicrobial applications. The antimicrobial test includes untreated group, amoxicillin, and ZnO-HAMA NPs at two different concentrations (50 and 100 µg/mL). Amoxicillin, a commonly used antibiotic, shows varying efficacy, with inhibition zones of 14 mm for *S. aureus*, 12 mm for *S. mutans*, 13 mm for *E. faecalis*, and 10 mm for *C. albicans*. In contrast, ZnO-HAMA NPs exhibit a dose-dependent antimicrobial effect. At 50 µg/mL, ZnO-HAMA NPs show lower inhibition zones compared to amoxicillin: 8.2 mm against *S. aureus*, 4.8 mm against *S. mutans*, 4.5 mm against *E. faecalis*, and 5.1 mm against *C. albicans*. Increasing the concentration to 100 µg/mL improves their

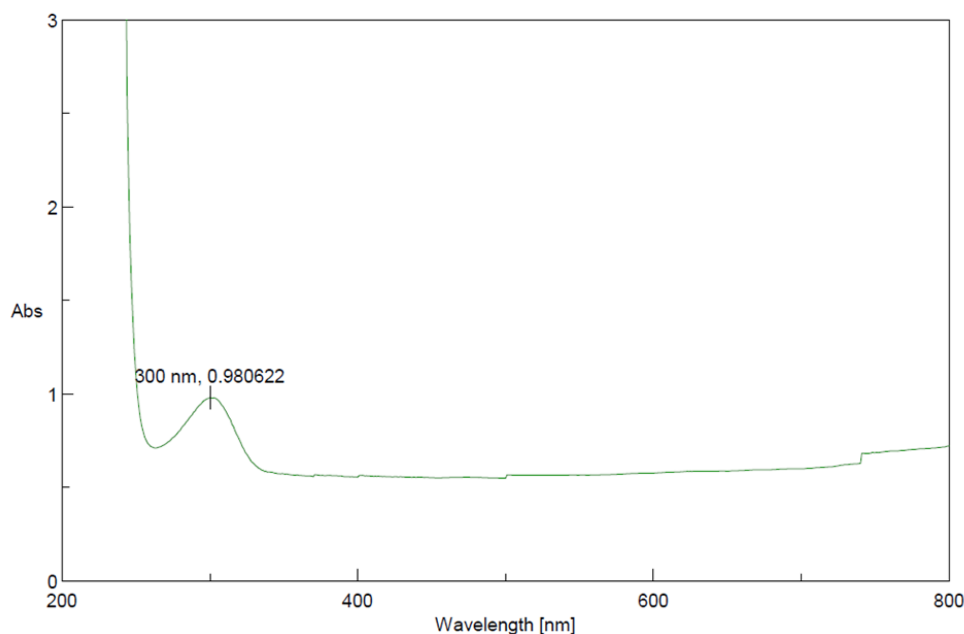


Figure 1: Ultraviolet (UV) absorption spectra of synthesized ZnO-hamamelitannin (HAMA) NPs.

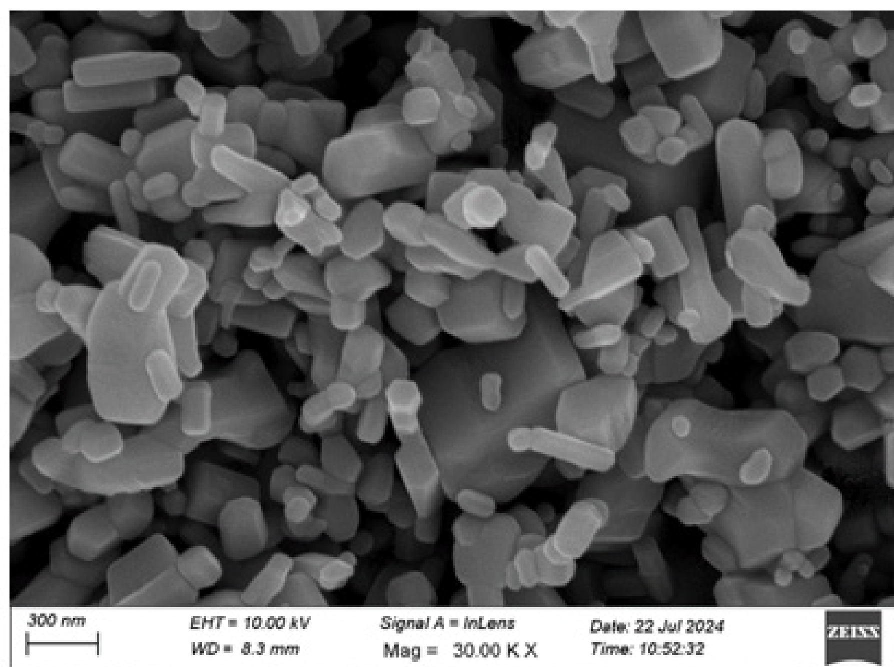


Figure 2: Scanning Electron Microscope (SEM) images revealed crystal-like structure in the ZnO-Hamamelitannin (HAMA) NPs.

Table 1: Molecular Docking analysis of hamamelitannin (HAMA) with different oral pathogens targets proteins.

Sl. No.	Protein	PDB id	Amino acids in hydrogen bonding	Amino acids in other type of bonding except hydrogen bond
1	*S. aureus*: surface protein G (SasG)	5DBL	TYR349, ASP307, GLY346	LYS309
2	*S. mutans*: Antigen I/II carboxy-terminus (3QE5)	3QE5	TYR190, PRO188	MET209, TYR85
3	*E. faecalis*: Esp.	6ORI	GLU234, TYR235, GLU252, VAL237	LYS248, GLU236
4	*C. albicans*: adhesin Als3	4LE8	VAL189	TYR495

Abbreviations: Esp.: Enterococcal Surface Protein; **PDB:** Protein Data Bank.

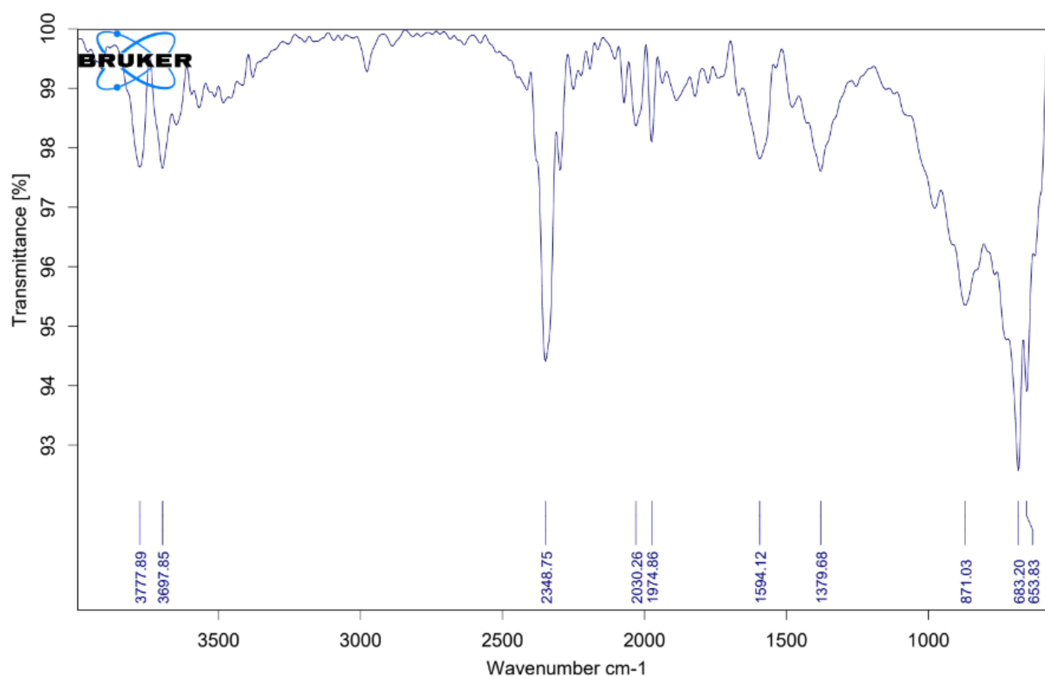


Figure 3: Fourier-Transform Infrared Spectroscopy (FTIR) characterization of ZnO-Hamamelitannin (HAMA) NPs.

antimicrobial activity, though still less effective than amoxicillin for most microorganisms. At this higher concentration, the inhibition zones are 12 mm for *S. aureus*, 10.5 mm for *S. mutans*, 8.6 mm for *E. faecalis*, and 10.2 mm for *C. albicans* (Figures 6 and 7). The results suggest that while ZnO-HAMA NPs exhibit antimicrobial properties, they are less effective than amoxicillin at the concentrations tested.

Molecular Docking Analysis of HAMA with Target Proteins

Molecular docking studies were performed to investigate the binding interactions of HAMA with four target proteins (PDB IDs: 5DBL, 3QE5, 6ORI, and 4LE8) using AutoDock software. The docking analysis aimed to elucidate the potential molecular mechanisms underlying the biological activities of HAMA by evaluating its affinity and interaction modes with these proteins. HAMA exhibited a strong binding affinity for the *S. aureus* surface protein G (SasG) of *S. aureus*, a known receptor involved in biofilm formation. The analysis revealed that HAMA forms three hydrogen bonds with key amino acid residues within the active site, including TYR349, ASP307 and GLY346. Additionally, hydrophobic interactions with Val83 and Ile125 further stabilized the complex (Table 1). The Antigen I/II carboxy-terminus (3QE5) of *S. mutans* associated with bacteria adhere to surfaces showed a significant interaction with HAMA. HAMA was found to form hydrogen bonds with residues Tyr190, Pro188, which are critical for the protein's activity. Enterococcal surface protein (6ORI) of *E. faecalis*, involved in biofilm formation. The docking analysis identified hydrogen bonds with Glu 234, Tyr235, Glu252 and Val237, which are crucial for the protein's function. Additionally, van der Waals interactions with surrounding residues such as Lys248 and Glu 236 contributed to the binding stability. HAMA also showed a strong interaction with the adhesin Als3 (4LE8) of *C. albicans*, a receptor implicated in apoptotic pathways.

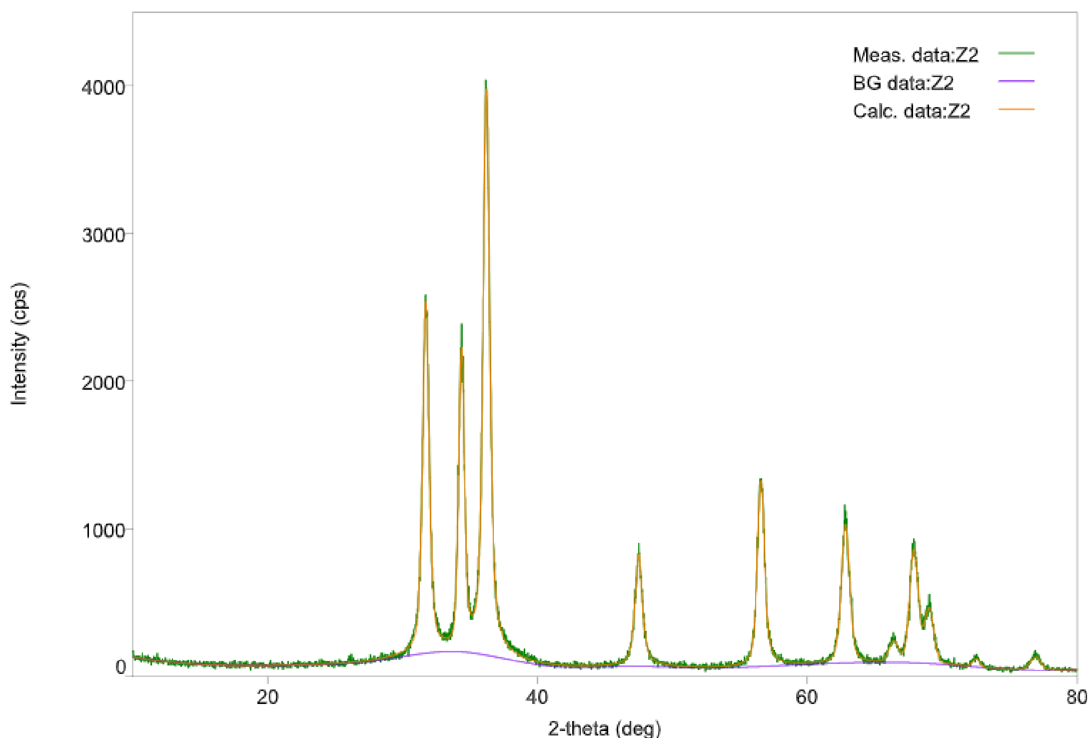


Figure 4: X-ray Diffraction (XRD) analysis of ZnO-Hamamelitannin (HAMA) NPs.

Key hydrogen bond was observed with residues Val189, while hydrophobic interactions with Tyr495 enhanced the stability of the docked complex. These interactions suggest that HAMA may promote apoptosis through effective binding to 4LE8, thereby contributing to its anticancer properties (Figure 8).

Anticancer Activity of ZnO-HAMA NPs

ZnO-HAMA NPs' anticancer potential against KB oral cancer cells was assessed at different concentrations (5, 25, 50, and 100 µg/mL) using the MTT test (Figure 9). The outcomes showed that ZnO-HAMA NPs had a cytotoxic effect on KB cells that was concentration-dependent. With an astounding 85% decrease in cell viability, the greatest quantity tested-100 µg/mL-showed the strongest anticancer activity. These findings indicate that ZnO-HAMA NPs possess potent anticancer properties, with their efficacy markedly increasing at higher concentrations. The dose-dependent decrease in cell viability underscores the potential of ZnO-HAMA NPs as effective therapeutic agents for the treatment of oral cancer, highlighting their promise for further development in anticancer applications.

Cell viability was assessed using the MTT assay after treatment with various concentrations of ZnO-HAMA NPs for 24 hr. Results are expressed as a percentage of control (untreated) cells, with control viability set to 100%. Data represent the mean±Standard Deviation (SD) from three independent experiments performed in triplicate. Statistical significance was determined using Student's *t*-test where $p<0.05$, indicated as *.

Gene Expression Studies on Apoptotic Markers

The impact of ZnO-HAMA NPs on the gene expression of key apoptotic markers (BCl-2, Bax, and p53) in KB oral cancer cells was assessed using RT-PCR (Figure 10). Cells were treated with 100 µg/mL of ZnO-HAMA NPs, and the expression levels of these genes were quantified. The results revealed a significant downregulation of the anti-apoptotic gene BCl-2, with its expression reduced indicating a substantial decrease in BCl-2-mediated cell survival signaling. Conversely, the proapoptotic gene Bax was markedly upregulated, with its expression increased. This substantial increase in Bax expression suggests a strong induction of the apoptotic pathway, promoting cell death in treated KB cells. Furthermore, there was a noticeable elevation of the tumor suppressor gene p53, with an increase in expression.

Relative expression levels of BCl-2, Bax and p53 after treatment with ZnO- Hamma NPs, at concentrations 15 µg/mL for 24 hr. Expression levels were quantified using qRT-PCR and normalized against β-actin. Results are shown as mean±SD of three replicates. Statistical significance was assessed using one way ANOVA, $p<0.05$.

DISCUSSION

ZnO NPs are proven to have strong antibacterial properties by rupturing microbial cell membranes and causing oxidative stress in a variety of dental infections. They may also be able to prevent the formation of biofilm, which is a major cause of tooth infections. Derived from the *Hamamelis* species, HAMA has antibacterial qualities and increases the bioavailability and effectiveness of

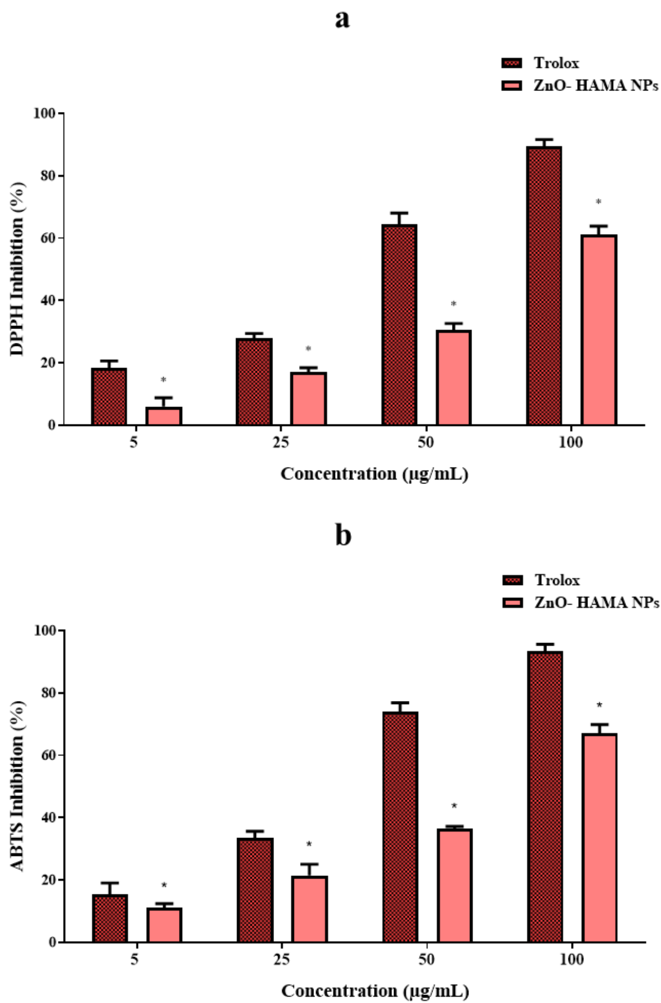


Figure 5: Antioxidant Activity of ZnO-Hamamelitannin (HAMA) NPs in 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (A) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) (B) Assays Compared to Standard Antioxidant.

drugs. Through the simultaneous targeting of several pathways involved in microbial growth and biofilm formation, the synergistic action of ZnO NPs with HAMA may improve antimicrobial effectiveness against dental infections. Combining these two treatments provides a dual strategy against oral cancer: it inhibits the growth of cancer cells while simultaneously focusing on oral infections that may be involved in the onset or spread of the disease. Oxidative stress is experienced by the oral cavity because of various circumstances, including inflammation, bacterial infections, and exposure to Reactive Oxygen Species (ROS) produced during metabolic activities (Rowińska *et al.*, 2021). Antioxidants are essential for neutralizing ROS and preventing oxidative damage to oral tissues. Antioxidants increase the stability, durability, and biocompatibility of dental materials such as composites, cements, and sealants (Zhang *et al.*, 2022).

This prolongs the life of dental restorations and lowers the possibility of subsequent problems. Recent research has demonstrated that when HAMA tested *in vitro*, it may successfully neutralize ROS, enhancing cellular antioxidant defenses. In this study thorough characterization, the effective synthesis of ZnO NPs coated with HAMA was confirmed, offering valuable information about their morphology, surface characteristics, size distribution, and shape. Previous *in vitro* studies have shown the strong antioxidant activity of HAMA. Similar to that results, ZnO-HAMA NPs shown greater free radical scavenging activity in both DPPH and ABTS assays. This implies that ZnO-HAMA NPs may find use in dental materials. Furthermore, these NPs antioxidant qualities may improve oral health by reducing oxidative stress in the oral tissues that surround them. This could help prevent or treat dental caries, which are largely influenced by oxidative stress. Pathogens as *S. aureus, S. mutants, E. faecalis*, and *C. albicans* are well-known for their capacity to create biofilms in

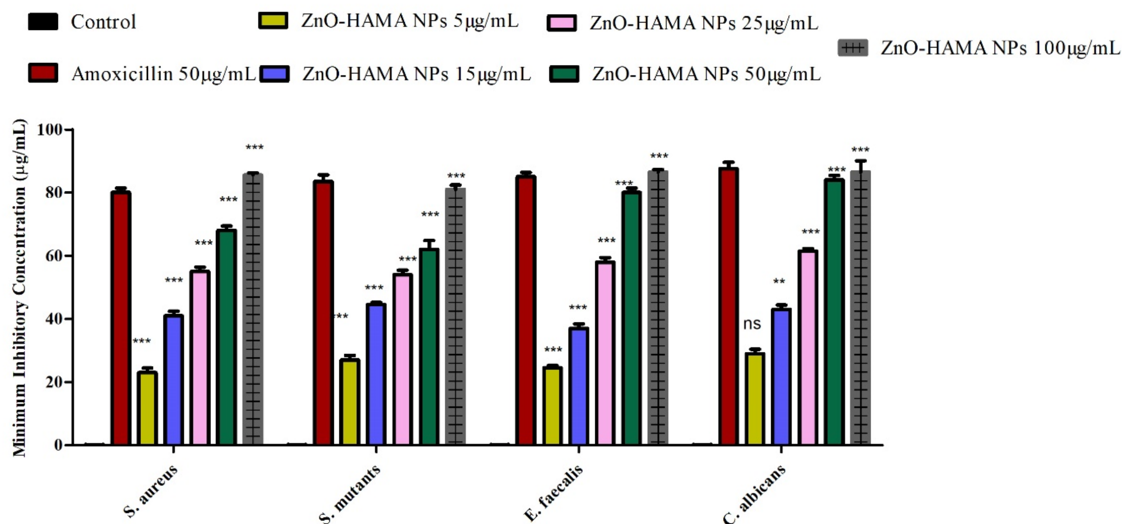
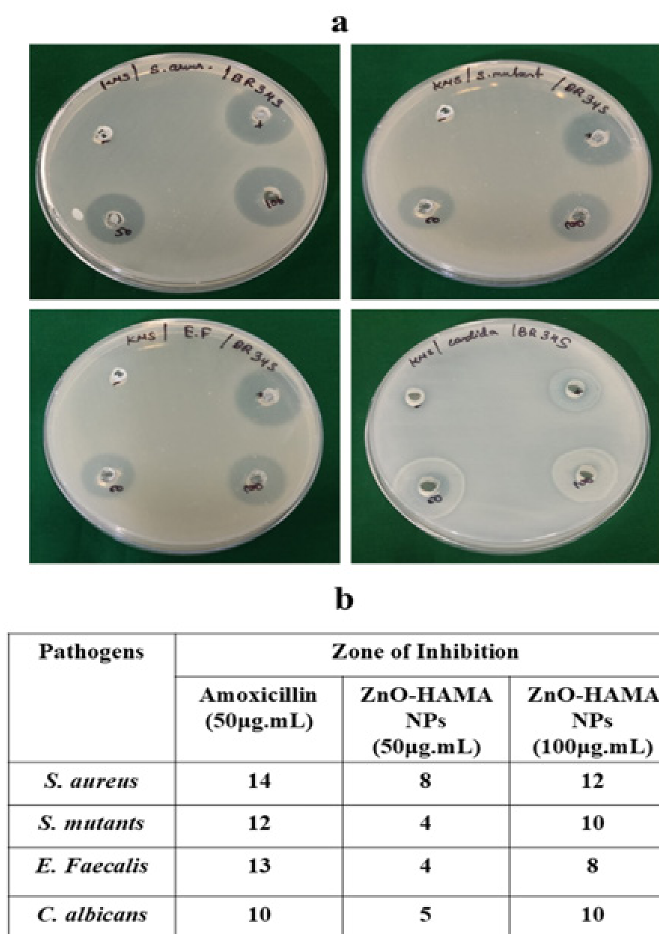


Figure 6: The antimicrobial activity of ZnO-Hamamelitannin (HAMA) NPs was assessed against dental pathogens. The positive control in this case was amoxicillin. When compared to the control, statistical significance ($p<0.05$) is indicated by the asterisk (*). The mean ± standard deviation from three different experiments is used to express the results.



Pathogens	Zone of Inhibition		
	Amoxicillin (50µg.mL)	ZnO-HAMA NPs (50µg.mL)	ZnO-HAMA NPs (100µg.mL)
S. aureus	14	8	12
S. mutants	12	4	10
E. Faecalis	13	4	8
C. albicans	10	5	10

Figure 7: Zone of inhibition of ZnO Hamamelitannin (HAMA) NPs treated at 50 and 100 µg/mL against dental pathogens of *S. aureus, S. mutans, E. faecalis*, and *C. albicans*. Amoxicillin and untreated was used as positive control and control.

dental settings, which greatly contributes to oral illnesses (Li *et al*., 2023).

When biofilms especially from *C. albicans* grow on dental implants, prosthetic limbs, and oral mucosal surfaces, it can cause oral candidiasis and other problems in immunocompromised people. The main cause of dental caries is due to *S. mutans*, which easily grow biofilms on the surfaces of teeth and releases acid that demineralizes enamel. *S. aureus* creates biofilms on dental implants and oral tissues, which exacerbates inflammation and tissue damage. It is linked to periodontal disorders and oral infections. *E. faecalis* creates biofilms inside the root canal system, making it resistant to antimicrobial medicines and encouraging treatment resistance. It is linked to recurrent endodontic infections and root canal treatment failures. The capacity of these bacteria to create strong biofilms underscores the difficulty in controlling dental infections and stresses the significance of creating methods to prevent and inhibit the production of biofilms to effectively treat and prevent oral diseases (Wang *et al*., 2024). Recent research has demonstrated that HAMA exhibited significant anti-biofilm activity against *S. aureus*. Our investigation showed strong MIC values and extensive zones of inhibition that ZnO-HAMA NPs exhibited against dental infections, which is consistent with earlier findings and highlights their potential as effective antibacterial agents in dental applications. Receptor proteins are essential for dental infections to form biofilms, which are intricate microbial communities surrounded by an extracellular matrix. In the early stages of adhesion, aggregation, and biofilm development, these receptor proteins are crucial. *S. aureus* surface protein G (SasG) mediates early attachment to host tissues and helps *S. aureus* adhere to tooth surfaces or dental implants (Sedarat and Taylor-Robinson, 2022).

The extracellular polymeric materials that *S. aureus* secretes next create a biofilm matrix that protects bacteria from defenses and antibiotics, allowing for the persistence of colonization and infection. *S. mutans* successfully forms biofilms by utilizing the carboxy-terminus of its Antigen I/II protein, which exacerbates dental problems. This carboxy-terminus binds to salivary proteins and the acquired enamel pellicle to function as an adhesin, enabling initial attachment to tooth surfaces. Following attachment, *S. mutans* uses the enzymes glucosyltransferase to release glucans, which create an extracellular matrix that encases the bacterial cells and encourages cohesion and the formation of biofilms. By providing protection from mechanical stresses, antimicrobial treatments, and host immune reactions, this dense biofilm structure allows bacteria to colonize tooth surfaces over time, metabolize fermentable carbohydrates, produce acid, demineralize enamel, and eventually lead to dental caries (Zhu *et al*., 2023).

Esp. is a common opportunistic pathogen in endodontic infections that *E. faecalis* employs to aid in the creation of biofilms and exacerbate dental issues. To reduce biofilm formation, Esp functions as a surface adhesion, promoting initial contact to host tissues and tooth surfaces. Extracellular polymeric substances, which are secreted *by E. faecalis* once it has adhered, provide a protective matrix around the bacterial cells in the biofilm. This matrix fosters chronic colonization and infection inside the root canal system, improves cohesiveness, and offers resistance to antimicrobial drugs and host immunologic responses. Using its Als3 adhesin, *C. albicans*, a common fungus species in the oral microbiota, forms biofilms that worsen dental conditions. Als3 binds to host cell receptors such E-cadherin and fibronectin, which is essential for the initial attachment of *C. albicans* to oral surfaces, such as tooth enamel and mucosal tissues. Once connected, *C. albicans* forms a strong biofilm structure by secreting extracellular matrix components such as proteins, polysaccharides, and extracellular DNA. In addition to promoting chronic colonization and offering defense against the host immune system and antifungal medications, this biofilm also plays a role in the development of oral illnesses such oral candidiasis and denture stomatitis. The results of the present study correlated with the findings from the zone of inhibition and biofilm inhibition assays, demonstrating that

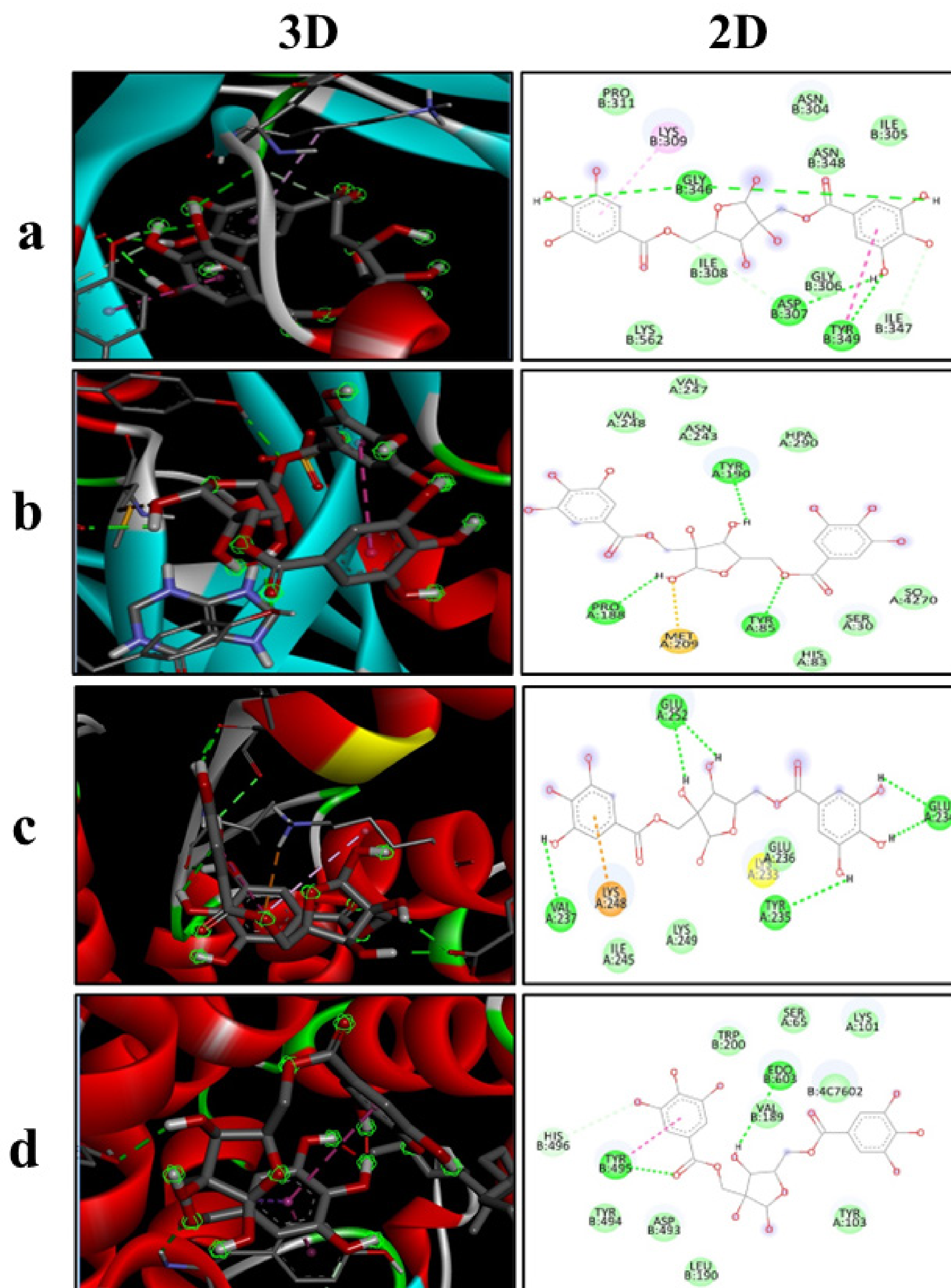


Figure 8: The relationships between oral pathogen receptors and ZnO-Hamamelitannin (HAMA) NPs are depicted includes both 2 and 3-D typical representations of the interactions. (A) 5DBL of *S. aureus*, (B) 3QE5 of *S. mutans*, (C) 6ORI of *E. faecalis*, (D) 4LE8 of *C. albicans*.

HAMA demonstrates a substantial affinity toward all invested receptors. These results show that hamamelitannin greatly lowers the production of biofilms and effectively prevents the growth of dental infections. These findings imply that HAMA may be a good option for the development of therapeutic drugs that target the formation of biofilms and fight dental infections. Additionally, HAMA promise as a broad-spectrum antibacterial agent for dental care applications is highlighted by its capacity to target several receptors linked to biofilm formation (Janarthanam *et al.*, 2025).

ZnO-HAMA NPs, which exhibited strong antibacterial action against dental infections were examined further for possible anticancer effects with a focus on oral cancer. The study's findings, which showed a considerable decline in the survival of KB cells. The results show that ZnO-HAMA NPs are dual-functional, with antibacterial and anticancer characteristics. Crucial regulators of

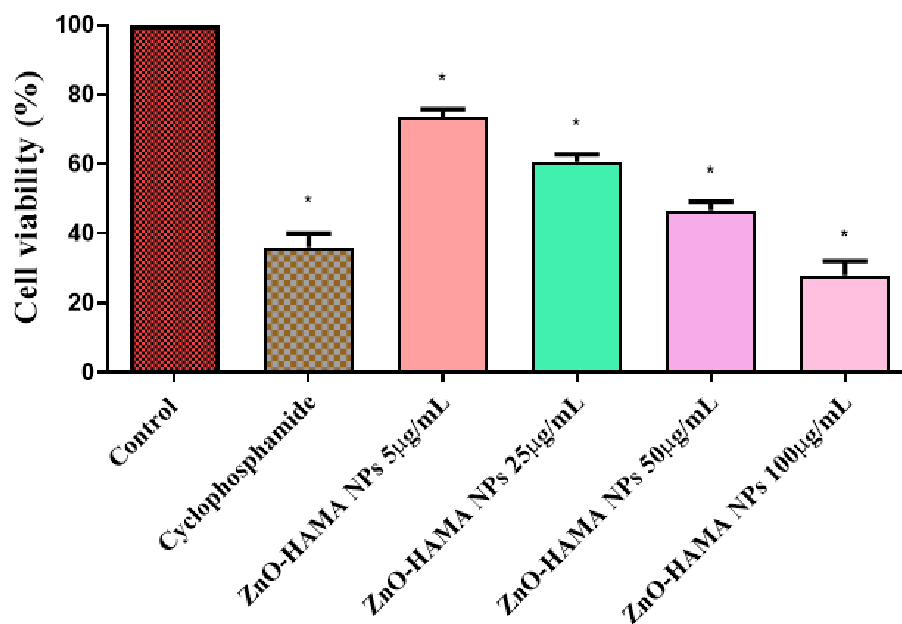


Figure 9: Effect of ZnO-Hamamelitannin (HAMA) NPs on the Viability of human epithelial Carcinoma cells (KB) cell lines.

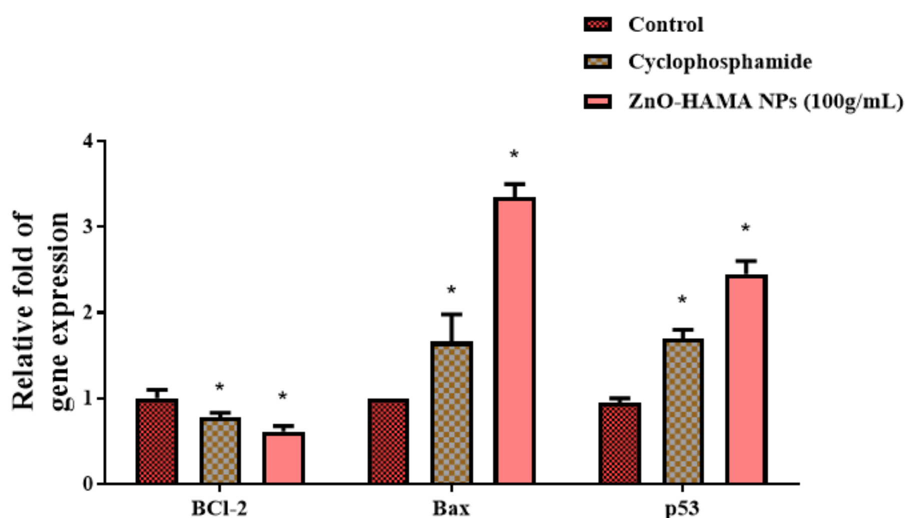


Figure 10: Effects of ZnO- Hamma NPs on apoptotic gene Expression in human epithelial Carcinoma Cells (KB) cells.

cellular apoptosis, which is essential to the initiation and spread of cancer, include BCl2, BAX, and P53. Uncontrolled tumor growth in oral cancer can result from dysregulation of apoptosis-related genes, such as BCl2, which suppresses apoptosis, and BAX, which promotes apoptosis. These genes can upset the delicate balance between cell proliferation and death. Furthermore, the tumor suppressor gene P53 is essential for coordinating the body's reactions to stress and damage to DNA, which includes initiating apoptosis in diseased or abnormal cells. Changes in the expression or activity of these genes can significantly influence the development, progression, and response to therapy in oral cancer. To better understand the underlying processes of ZnO-HAMA NPs' anticancer effects and identify prospective therapeutic targets for the treatment of oral cancer, it is therefore useful to examine the expression levels and activity of BCl2, BAX, and P53 in response to therapy. Apoptosis-related genes such BCl2, BAX, and P53 showed notable changes in their expression levels in the study examining ZnO-HAMA NPs' anticancer effect in KB cells. ZnO-HAMA NP treatment led to a significant downregulation of BCl2, an anti-apoptotic protein that promotes the induction of

apoptosis by preventing cell death. The apoptotic response in KB cells was further enhanced by the simultaneous overexpression of BAX, a proapoptotic protein that promotes apoptosis by increasing mitochondrial outer membrane permeabilization and the consequent release of cytochrome c. Additionally, ZnO-HAMA NP therapy increased P53 expression, a tumor suppressor gene essential for controlling cellular reactions to stress and DNA damage. The activation of downstream apoptotic pathways, including as transcriptional control of proapoptotic genes and induction of cell cycle arrest, was probably aided by the overexpression of P53. This eventually prevented KB cell proliferation and encouraged cell death. Based on their ability to target important apoptotic pathways, these results indicate that ZnO-HAMA NPs have anticancer activity and show promise as therapeutic agents for the treatment of oral cancer.

CONCLUSION

ZnO-HAMA NPs have been shown to have noteworthy antimicrobial and anticancer effects against oral pathogens and oral cancer cells. These NPs achieve this by altering critical apoptotic pathways, which in turn restrict cell proliferation and accelerate cell death. The findings underscore the dual functionality of ZnO-HAMA NPs, making them promising candidates for integrated therapies targeting both oral infections and oral cancer. Future research should focus on further elucidating their safety profile, optimizing delivery methods, and exploring their potential in promoting oral tissue regeneration. Ultimately, the study paves the way for innovative approaches in dental care, potentially offering effective treatments against challenging oral health issues, including infections and cancers.

ACKNOWLEDGEMENT

I would like to extend my sincere appreciation to Saveetha Dental and Medical College for their invaluable support and resources that made this research possible. Their commitment to excellence in education and research has significantly contributed to the advancement of knowledge in our field.

ABBREVIATIONS

ZnO NPs: Zinc-oxide nanoparticles; **HAMA:** Hamamelitannin; **ZnO-HAMA NPs:** Hamamelitannin-coated zinc oxide nanoparticles; **UV:** Ultraviolet; **SEM:** Scanning electron microscope; **XRD:** X-ray diffraction; **FTIR:** Fourier-transform infrared spectroscopy; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic); MIC: Minimum inhibitory concentration; **CFU/mL:** Colony-forming units per milliliter; **KB:** Human epithelial carcinoma cells; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PBS:** Phosphate-buffered saline; **qRT-PCR:** Quantitative RT-PCR; **GAPDH:** Glyceraldehyde-3-phosphate dehydrogenase; **ROS:** Reactive oxygen species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

T. A., M. M., and T. M. M. performed the experiments and contributed to data collection and analysis. M. S. K. K. supervised the study, provided guidance on experimental design, and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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Cite this article: Aravindan T, Mathew M, Martin TM, Kumar MSK. Targeting Oral Biofilms and Inducing Apoptosis in Oral Cancer Cells: Mechanistic Insights of Hamamelitannin-Coated Zinc Oxide Nanoparticles through BCl-2/BAX/P53 Pathway. Pharmacog Res. 2026;18(3):899-911.