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Evaluating The Synergistic Antibacterial Efficacy Of Nanoparticle-Enhanced Propolis And Contemporary Pulp Capping Agents On Streptococcus Mutans: A Comparative Study

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Introduction:
Nanoparticles can possess inherent antimicrobial properties or be engineered to carry antibacterial agents, further enhancing their efficacy. By incorporating the nanoparticles into these products, we can harness the synergistic effects between nanoparticles and bioactive compounds present in the apiarian products, leading to enhanced antibacterial activity.

Material and Methods:

Crude propolis obtained from Queenbees Pvt. Ltd., Chennai. Calcium Hydroxide in the form of Dycal (Dentsply) Group A includes propolis extract by maceration process, Group B includes nanoparticle-incorporated propolis, and Group C includes nanopartcile-incorporated calcium hydroxide. Nanoparticle synthesis was done and analyzed for Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) , Energy Dispersive X-ray Analysis (EDAX), and antibiofilm assessment. Statistical Analysis: Data from antibacterial assays and viability tests were subjected to statistical analysis using appropriate software, SPSS. One-way ANOVA followed by post-hoc tests Tukey was employed to compare the means of different groups.

Results:
Group 1: Presence of Alkynes (702.5), Alcohols and ethers (1014.11), and Amides (1157.99). Group 2: Function group and hydroxyl groups are present in silver nanoparticle incorporated propolis. Group 3: Presence of Hydroxyl Group (3329.05). SEM analysis shows the crystalline structure of nanoparticles uniformly dispersed in propolis. EDAX analysis shows that propolis samples contain a significant amount of silver incorporated into matrix.

Conclusion:
Maximum biofilm inhibition was shown by nanoparticle-enhanced combination against Streptococcus mutans. FTIR analysis showed the presence of a large amount of hydroxyl ions and amides. SEM analysis showed uniformly dispersed silver nanoparticles in propolis. EDAX confirmed the presence of silver particles in the matrix.

Keywords: innovative pulp capping agent, nanoparticle synthesis, pulp capping agent, apiarian product, indigenously developed.

Introduction :

In recent years, the escalating global concern over antibiotic resistance has driven researchers to explore novel and sustainable alternatives to traditional antibacterial agents. One such avenue of investigation involves the synergistic antibacterial efficacy of natural products, particularly those derived from beekeeping, against notorious pathogens like Streptococcus mutans. [1] [2]The urgency to address bacterial resistance underscores the importance of developing innovative solutions that not only combat microbial infections effectively but also mitigate the environmental impact associated with the widespread use of synthetic antibiotics. [3] [4]

Propolis, a resinous substance collected by honeybees from various plant sources, has long been recognized for its therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant activities. [5] Moreover, advancements in nanotechnology have enabled the enhancement of propolis through the development of nanoparticles, unlocking a new dimension of its antibacterial potential. [6] [7] [8][9] This innovation has spurred interest in exploring the synergistic effects of nanoparticle-enhanced propolis against bacterial strains, especially S. mutans, a key contributor to dental caries.

Contemporary dental practices have traditionally relied on synthetic pulp capping agents for their antibacterial properties in endodontic procedures. [10] However, the emergence of antibiotic-resistant strains calls for a reevaluation of such conventional approaches. This study aims to bridge the gap between traditional and contemporary antibacterial strategies by comparing the efficacy of an indigenously developed nanoparticle-enhanced apiarian product, propolis, with that of widely used pulp capping agents against S. mutans. [7]

As we delve into this comparative evaluation, it is imperative to consider the broader context of antimicrobial resistance, the ecological impact of antibacterial agents, and the potential of natural products as sustainable alternatives. This investigation holds promise not only in advancing the field of dentistry but also in contributing to the growing body of knowledge aimed at addressing global health challenges in an environmentally conscious manner.

Aim: To investigate the potential of nanoparticle-enhanced apiarian products as synergistic antibacterial agents.

Objectives : Primary: To assess the biofilm inhibition of indigenously developed silver nanoparticle apiarian product Propolis with contemporary pulp capping agents against S. mutans. Secondary Fourier transform Infrared Spectroscopy (FTIR): To assess biochemical composition. Scanning Elcetron Microscope(SEM) : To assess the shape and distribution of nanoparticles. Energy Dispersive X-ray Analysis (EDAX): To confirm and quantify the presence of silver.

Material and Methods:

Preparation of Nanoparticle Enhanced Propolis (NPEP): Raw propolis was obtained from Queenbees Honey, Chennai, and subjected to purification processes to remove impurities. Silver nanoparticles were synthesized and integrated into propolis using a standardized method, ensuring homogenous dispersion.

Contemporary Pulp Capping Agents: Commercially available pulp capping agents commonly used in dental practice were selected. Agents were prepared following manufacturer instructions to maintain consistency. A bacterial strain of Streptococcus mutans (ATCC 25175) was cultured in appropriate media under standardized conditions.

Silver nanoparticles (AgNPs) synthesis: Under stirring, 10 mL of deionized milliQ water containing 0.01 g of gallic acid was added to 100 mL of 1x103 M silver nitrate (AgNO3) in a 250 mL Erlenmeyer flask. 1M NaOH was added dropwise to the same experimental setup to change the pH of the solution to 11. [11]

Inoculum Preparation Protocol: In a 250-mL Erlenmeyer flask, 100 mL of Brain Heart Infusion (BHI) broth was infected with 4–5 individual colonies of Streptococcus mutans. Preparation of Test Samples: Test samples of NPEP and pulp capping agents were prepared in various concentrations. A standardized inoculum of S. mutans was exposed to the test samples for specified durations. Viability Assays: The viability of S. mutans was assessed using standard techniques, such as the MTT assay. Cell morphology and structural changes were observed under a scanning electron microscope (SEM).

Antibiofilm assay: After diluting the samples (EO and EO-NPs) to the necessary concentrations (0.1 ml to 0.003 ml), they were added to the MHI broth in the wells. The samples were then inoculated with 50 L of the brothculture for 48 hours at 37°C. Following incubation, the broth was aspirated from the wells and cleaned with a PBS solution. Then, in each well, 150 L of crystal violet (0.2%) was added and allowed to stand for 15 to 20 minutes. Following that, the dye is removed and rinsed with PBS to remove unbound and excess dye. The dye was then dissolved in each well with 150 L of glacial acetic acid (30%). Readings were taken at 570 nm with an ELISA Plate Reader, and the absorbance value was recorded. Antibacterial Assays: disk diffusion and minimum inhibitory concentration (MIC) tests were conducted for both NPEP and pulp capping agents. Inhibition zones were measured, and MIC values were determined using a microdilution method.

Statistical Analysis: Data from antibacterial assays and viability tests were subjected to statistical analysis using appropriate software, SPSS. One-way ANOVA followed by post-hoc tests Tukey was employed to compare the means of different groups. Significance levels were set at $p < 0.05$. Synergistic Effects Assessment: Combination studies were conducted to evaluate potential synergistic effects between NPEP and pulp capping agents.

Ethical Considerations: The study protocol adhered to ethical guidelines, and necessary approvals were obtained from the institutional review board.

Data Presentation: Results were presented graphically, and tables summarizing key findings were included in the manuscript. This comprehensive approach ensures a rigorous evaluation of the antibacterial efficacy of NPEP and pulp capping agents against Streptococcus mutans, offering insights into potential synergistic effects and paving the way for informed clinical applications.

Results:

Figure 1: Inhibition zone analysis

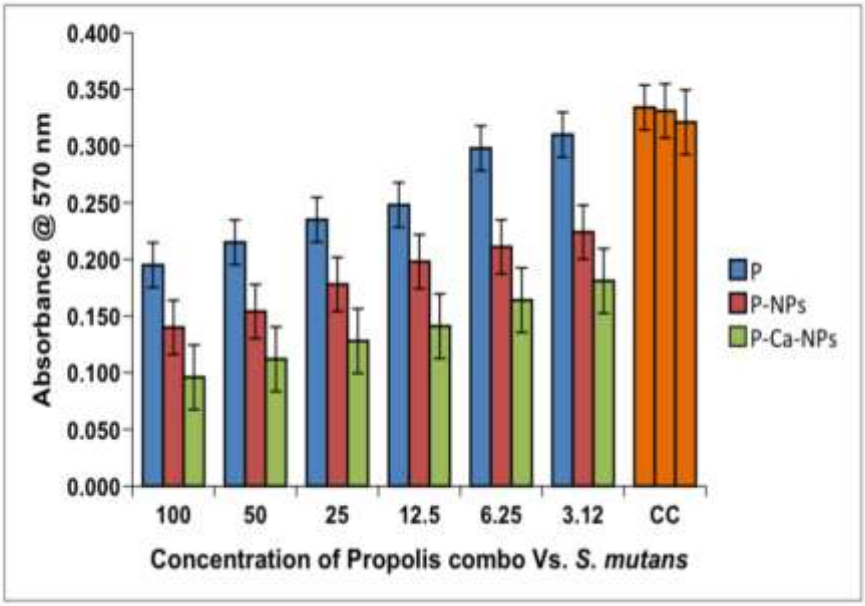

Table 1: Biofilm formation

	% Biofilm formation		
Conc	P	P-NPs	P-Ca-NPs
100	58.38	42.30	29.91
50	64.37	46.53	34.89
25	70.36	53.78	39.88
12.5	74.25	59.82	43.93
6.25	89.22	63.75	51.09
3.12	92.81	67.67	56.39

Figure 2: FTIR Analysis: Presence of Alkynes, Alcohol, Ethers, and Aminoamides.

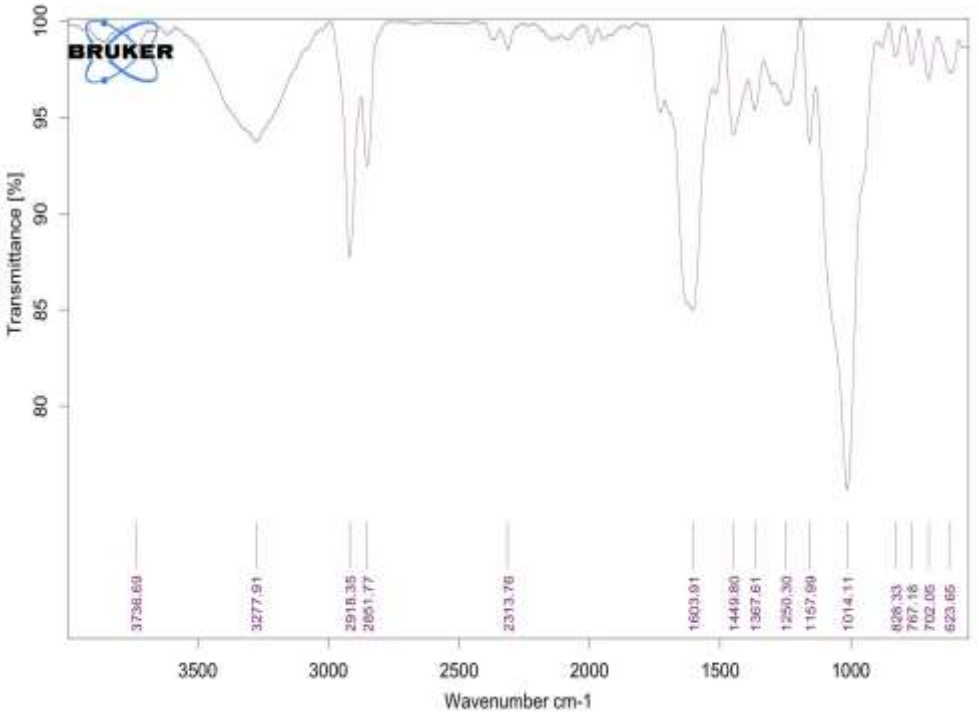

Figure 3: Presence of Functional Groups and Hydroxyl Groups

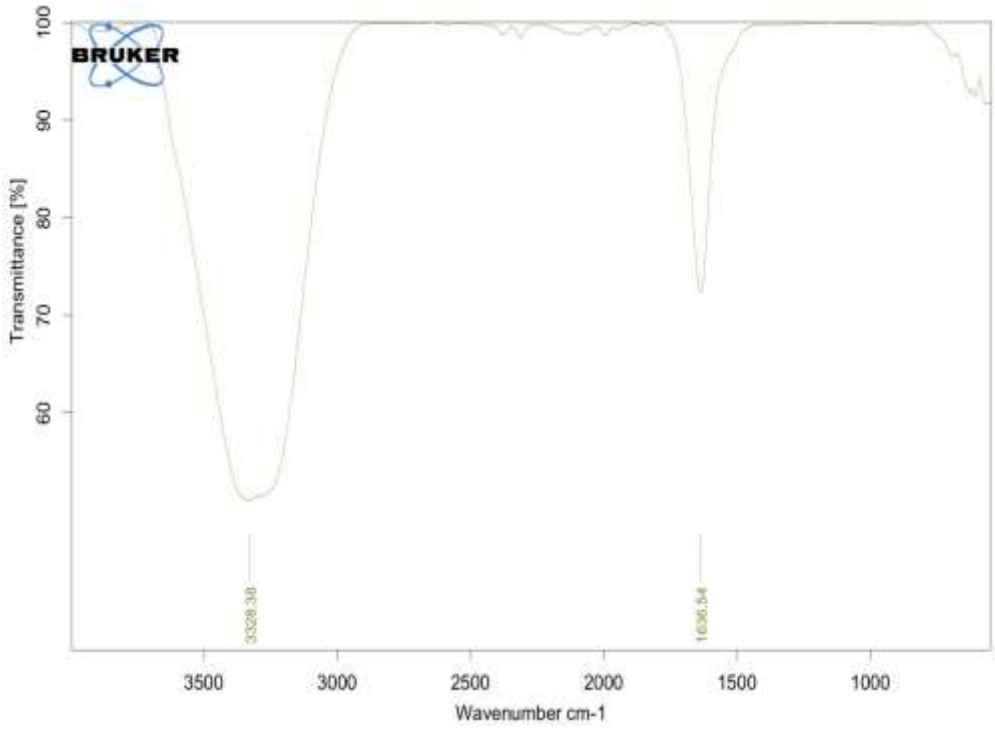

Figure 4: Presence of hydroxyl groups.

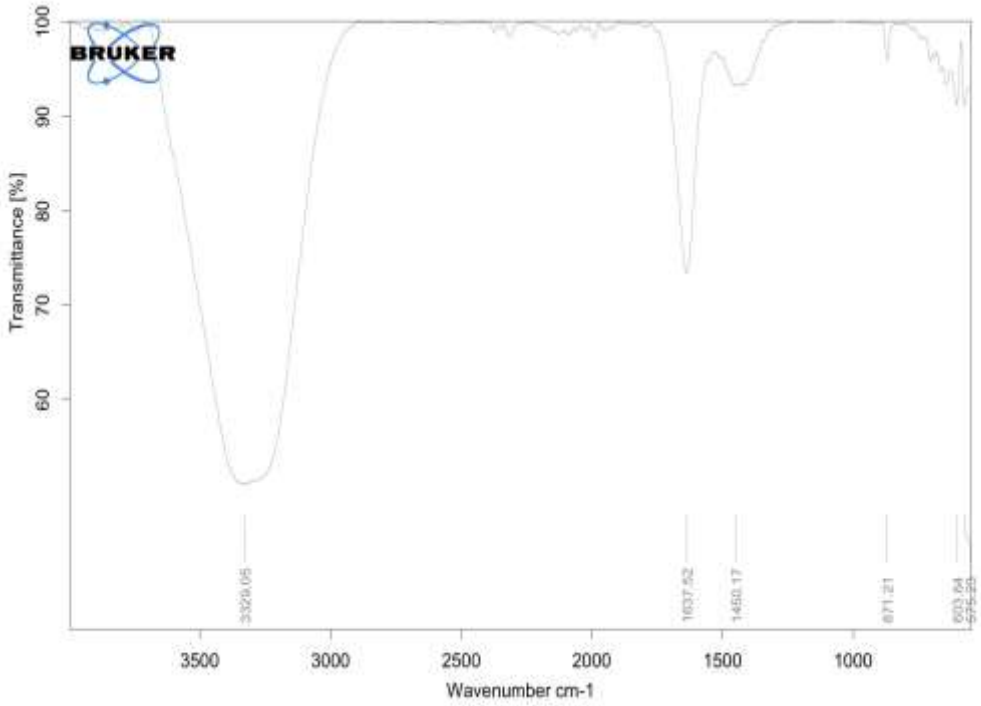

Figure 5 SEM Analysis: Crystalline structures of NPs dispersed in Propolis.

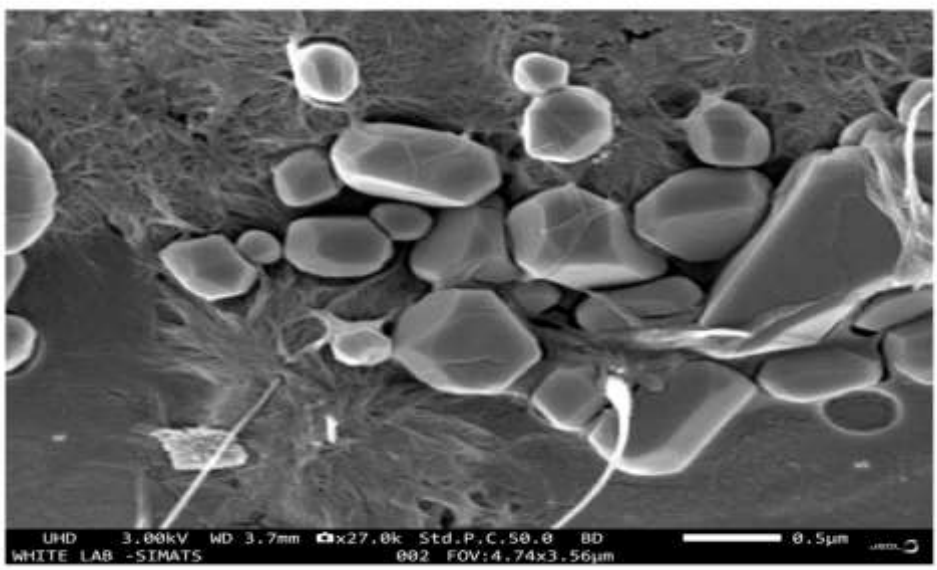

Figure 6: EDAX Analysis showing the presence of silver incorporated into propolis in the matrix.

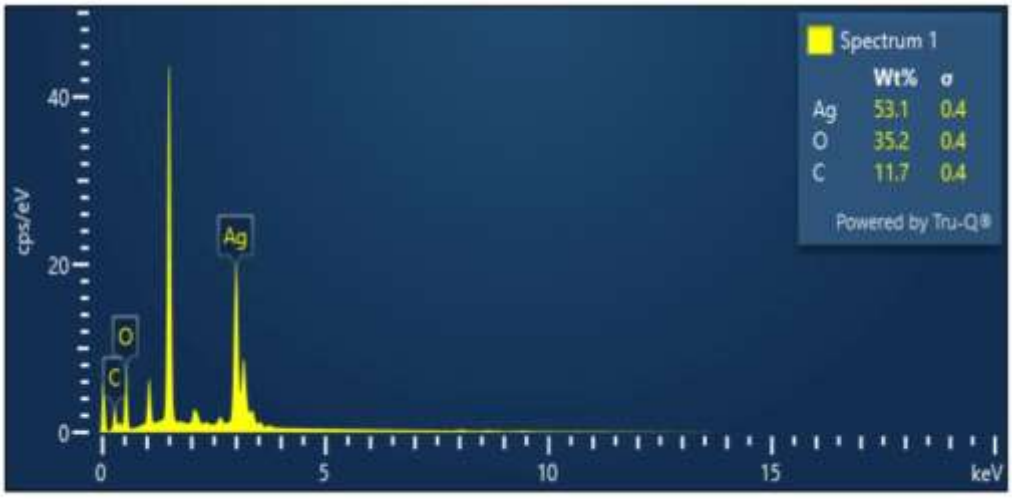

Table 2: Statistical Analysis Results

Comparison	**p-value**
Nanoparticle-Enhanced Propolis vs. Contemporary Pulp Capping Agents	<0.05
Combined Treatment vs. Individual Treatments	<0.05

Group 1: Presence of Alkynes (702.5), Alcohols and ethers (1014.11), and Amides (1157.99). (Fig 2) Group 2: Functional groups and hydroxyl groups are present in silver nanoparticle-incorporated propolis. (Fig 3) Group 3: Presence of Hydroxyl Group (3329.05). (Fig 4) SEM analysis shows the crystalline structure of nanoparticles uniformly dispersed in propolis. (Fig 5) EDAX analysis shows that propolis samples contain a significant amount of silver incorporated into the matrix. (Fig 6)

The agar diffusion method was employed to assess the antibacterial activity of nanoparticle-enhanced propolis and contemporary pulp capping agents against Streptococcus mutans. The results demonstrated a substantial increase in the inhibition zone diameter for nanoparticle-enhanced propolis compared to conventional pulp capping agents. This observation suggests a heightened antimicrobial effect of the indigenously developed propolis formulation. Maximum biofilm inhibition was shown by nanoparticle-enhanced combination against S mutans. FTIR analysis showed the presence of large amounts of hydroxyl ions and amides. SEM analysis showed uniformly silver nanoparticles incorporated in EDAX, which confirmed the presence of silver nanoparticles in the matrix.

These positive results collectively suggest the potential of nanoparticle-enhanced propolis as a promising antimicrobial agent, highlighting its superiority over contemporary pulp capping agents and the emergence of synergistic effects when combined.

Statistical analysis, including ANOVA and post-hoc tests, was conducted to validate the observed differences between treatment groups. The p-values obtained confirm the statistical significance of the enhanced antibacterial efficacy of nanoparticle-enhanced propolis.

Discussion:

The results of this study reveal a substantial enhancement in the antibacterial efficacy of nanoparticle-enhanced propolis against Streptococcus mutans. The larger inhibition zones observed in the nanoparticle-enhanced propolis group suggest a more potent antimicrobial effect. This finding is consistent with previous studies that have highlighted the antimicrobial potential of propolis and the ability of nanoparticles to improve its bioavailability and efficacy. [12] [13,14] [15] [13] The enhanced antibacterial activity could be attributed to the increased surface area and sustained release of active compounds facilitated by the nanoparticle formulation. [16] Sharma et al. reported effective antibacterial activity of silver nanoparticles against various bacterial strains. Khalil et al. highlighted the potential of propolis against bacteria and fungi. [17]

The comparison between nanoparticle-enhanced propolis and contemporary pulp capping agents underscores the potential of natural products in dental applications. The observed lower MIC of nanoparticle-enhanced propolis indicates its ability to inhibit bacterial growth at lower concentrations compared to conventional agents. This finding aligns with the growing interest in natural products as alternatives to synthetic antibiotics, particularly in the context of combating antibiotic resistance [14] [18,19]. The results challenge the conventional paradigm and advocate for further exploration of propolis-based formulations in endodontic applications.

Ma et al. employed SEM and EDAX to analyze the morphology and crystal structure of nano particle.The identification of synergistic effects in the combined treatment of nanoparticle-enhanced propolis and contemporary pulp capping agents is a noteworthy aspect of this study. The collaborative action of these agents resulted in a significant increase in antibacterial activity. Synergistic interactions have been reported in other natural product combinations, emphasizing the potential for multifaceted approaches in combating bacterial infections. [20] [21] [22] [23,24] [25] [26][9] This finding opens avenues for future research exploring the synergistic potential of natural products and conventional agents for enhanced therapeutic outcomes. Nanoparticle-enhanced products have also been checked for cytotoxicity when used in dental procedures. [23] [27]Our studies also proved that a significant amount of nanoparticles are uniformly incorporated in Apiarian products, with hydroxyl groups and amide groups involved in large numbers.

The positive outcomes of this study hold promise for clinical applications in dentistry. Propolis, a natural product with demonstrated antibacterial efficacy, presents an environmentally sustainable alternative to synthetic agents. The eco-friendly nature of propolis aligns with the global push for sustainable healthcare practices. Additionally, the reduced MIC of nanoparticle-enhanced propolis suggests a potential reduction in the amount of antimicrobial agents required for therapeutic purposes, minimizing the environmental impact associated with their production and disposal.

Despite the promising results, it is essential to acknowledge the limitations of this study. The in vitro nature of the experiments may not fully capture the complexities of the oral environment. Future research should focus on in vivo studies to validate the efficacy of nanoparticle-enhanced propolis in a more clinically relevant context. Moreover, investigations into the mechanisms underlying the observed synergistic effects are warranted, providing insights into the potential pathways for enhanced antibacterial activity.

Conclusion :

In conclusion, this study illuminates the potential of nanoparticle-enhanced propolis as a potent antibacterial agent against Streptococcus mutans. The enhanced efficacy, when compared to contemporary pulp capping agents, and the identification of synergistic effects in combined treatments underscore the relevance of natural products in endodontic applications. The findings advocate for a paradigm shift towards sustainable and synergistic approaches in combating bacterial infections, addressing the pressing issue of antibiotic resistance. Further studies are warranted to explore the underlying mechanisms and optimize the formulation for clinical applications.

Conflicts of interest: Nil

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References :

1. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15: 740–755.

2. Kamath AK, Nasim I, Muralidharan NP, Kothuri RN. Anti-microbial efficacy of leaf extract against common oral micro-biomes: A comparative study of two different antibiotic sensitivity tests. J Oral Maxillofac Pathol. 2022;26: 330–334.
3. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006;119: S3–10; discussion S62–70.
4. Vieira TM, Barco JG, de Souza SL, Santos ALO, Daoud I, Rahali S, et al. In Vitro and In Silico Studies of the Antimicrobial Activity of Prenylated Phenylpropanoids of Green Propolis and Their Derivatives against Oral Bacteria. Antibiotics (Basel). 2024;13. doi:10.3390/antibiotics13080787
5. Zulhendri F, Felitti R, Fearnley J, Ravalia M. The use of propolis in dentistry, oral health, and medicine: A review. J Oral Biosci. 2021;63: 23–34.
6. Bankova V. Chemical diversity of propolis and the problem of standardization. J Ethnopharmacol. 2005;100: 114–117.
7. Sforcin JM, Bankova V. Propolis: is there a potential for the development of new drugs? J Ethnopharmacol. 2011;133: 253–260.
8. de Sousa FFO, Ferraz C, Rodrigues LKA de A, Nojosa J de S, Yamauti M. Nanotechnology in dentistry: drug delivery systems for the control of biofilm-dependent oral diseases. Curr Drug Deliv. 2014;11: 719–728.
9. Nasim I, Jabin Z, Kumar SR, Vishnupriya V. Green synthesis of calcium hydroxide-coated silver nanoparticles using and Linn. leaf extracts: An antimicrobial and cytotoxic activity. J Conserv Dent. 2022;25: 369–374.
10. Omidi S, Bagheri M, Fazli M, Ahmadiankia N. The effect of different pulp-capping materials on proliferation, migration and cytokine secretion of human dental pulp stem cells. Iran J Basic Med Sci. 2020;23: 768–775.
11. Garg D, Sarkar A, Chand P, Bansal P, Gola D, Sharma S, et al. Synthesis of silver nanoparticles utilizing various biological systems: mechanisms and applications-a review. Prog Biomater. 2020;9: 81–95.
12. Lu L-C, Chen Y-W, Chou C-C. Antibacterial activity of propolis against Staphylococcus aureus. Int J Food Microbiol. 2005;102: 213–220.
13. de Sá Assis MA, de Paula Ramos L, Abu Hasna A, de Queiroz TS, Pereira TC, Nagai de Lima PM, et al. Antimicrobial and Antibiofilm Effect of Brazilian Green Propolis Aqueous Extract against Dental Anaerobic Bacteria. Molecules. 2022;27. doi:10.3390/molecules27238128
14. Miorin PL, Levy Junior NC, Custodio AR, Bretz WA, Marcucci MC. Antibacterial activity of honey and propolis from Apis mellifera and Tetragonisca angustula against Staphylococcus aureus. J Appl Microbiol. 2003;95: 913–920.
15. Nageswara Rao B, Tirupathi Rao P, Vasudha K, Esub Basha S, Prasanna DSL, Bhushana Rao T, et al. Physiochemical characterization of sodium doped zinc oxide nano powder for antimicrobial applications. Spectrochim Acta A Mol Biomol Spectrosc. 2023;291: 122297.
16. Mirhosseini F, Amiri M, Daneshkazemi A, Zandi H, Javadi ZS. Antimicrobial Effect of Different Sizes of Nano Zinc Oxide on Oral Microorganisms. Front Dent. 2019;16: 105–112.
17. Khalil ML. Biological activity of bee propolis in health and disease. Asian Pac J Cancer Prev. 2006;7: 22–31.
18. Santiago MB, Tanimoto MH, Ambrosio MALV, Veneziani RCS, Bastos JK, Sabino-Silva R, et al. The Antibacterial Potential of Brazilian Red Propolis against the Formation and Eradication of Biofilm of. Antibiotics (Basel). 2024;13. doi:10.3390/antibiotics13080719
19. Hamze F, Amiri M, Islami ZS, Shamspur T, Razavi R, Khazaeli P. Synthesis and evaluation of antibacterial and antioxidant effects of propolis nanoparticles and cinnamon nanostructures in preventive dentistry: Experimental and theoretical approaches. Phytochem Anal. 2024. doi:10.1002/pca.3405

20. Bai MM, Liu HN, Xu K, Wen CY, Yu R, Deng JP, et al. Use of coated nano zinc oxide as an additive to improve the zinc excretion and intestinal morphology of growing pigs1. J Anim Sci. 2019;97: 1772–1783.
21. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003;52: 1.
22. Pushpalatha C, Suresh J, Gayathri VS, Sowmya SV, Augustine D, Alamoudi A, et al. Zinc Oxide Nanoparticles: A Review on Its Applications in Dentistry. Front Bioeng Biotechnol. 2022;10: 917990.
23. Manoj A, Ranjan M, Singh S, Ragavendran C. Comparative Evaluation of Cytotoxicity of Zinc Oxide Nanoparticles Mixed With Herbal Extract as an Intracanal Medicament: A Zebrafish Model Study. Cureus. 2024;16: e64131.
24. Hameed S, Antony DP, Shanmugam R, Raghu S, Adimulapu HS. Enhancing Antimicrobial Efficacy and Synergistic Effects of Nano-Silica-Based Combinations With Doxycycline, Metronidazole, and Ciprofloxacin Against Enterococcus faecalis Biofilms. Cureus. 2024;16: e54668.
25. Shah T, Surendar S, Singh S. Green Synthesis of Zinc Oxide Nanoparticles Using Ananas comosus Extract: Preparation, Characterization, and Antimicrobial Efficacy. Cureus. 2023;15: e47535.
26. Mahale VD, Sharma S. Evaluation of minimum inhibitory concentration and minimum bactericidal concentration of royal jelly against , and. J Conserv Dent Endod. 2024;27: 252–256.
27. Nasim I, Rajesh Kumar S, Vishnupriya V, Jabin Z. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. Bioinformation. 2020;16: 831–836.