

Tiger Snail Shell-Based Nano-Hydroxyapatite Gel with Antibacterial Activity of Propolis and Ajwa Dates for Tooth Enamel Remineralization

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ABSTRACT

Dental caries remains among the most prevalent chronic diseases worldwide, driven by enamel demineralization under acidogenic biofilm activity, principally by Streptococcus mutans. Nano-hydroxyapatite (n-HAp) derived from mollusk shell waste has emerged as a sustainable remineralizing agent, while natural antibacterial adjuvants such as propolis and Ajwa date extract offer complementary anti-cariogenic activity. This study developed and evaluated a novel gel combining n-HAp synthesized from tiger snail (Achatina fulica) shell waste with propolis and Ajwa date seed extract, assessing enamel remineralization and antibacterial activity against Streptococcus mutans. Nano-hydroxyapatite was extracted via wet chemical precipitation. Thirty-two bovine enamel specimens were assigned to four groups (n = 8): negative control (artificial saliva), positive control (1450 ppm fluoride gel), n-HAp gel alone, and the composite gel, evaluated for surface microhardness (Vickers) at baseline, after demineralization, and after treatment. Antibacterial activity was assessed via agar well diffusion (n = 6 per active group). One-way ANOVA showed a significant difference in microhardness recovery among groups (F(3,28) = 118.05, p < 0.001), with the composite gel achieving the greatest recovery (75.66% ± 6.56%), exceeding the fluoride control (51.85% ± 8.13%), n-HAp-alone (56.83% ± 9.31%), and negative control (-3.90% ± 10.34%) (all p < 0.001). The composite gel produced a mean inhibition zone of 15.85 mm (± 2.33), significantly greater than n-HAp alone (6.04 mm, p < 0.001) and comparable in category to 0.2% chlorhexidine (18.74 mm). These findings support the composite gel as a promising, sustainably sourced dual-function anti-cariogenic dental material.



INTRODUCTION

Dental caries continues to rank among the most prevalent chronic diseases globally, affecting a substantial proportion of both children and adults, and arising from a dynamic imbalance between demineralization, driven by organic acids produced through bacterial fermentation of dietary carbohydrates, and remineralization, the natural repair process by which calcium and phosphate ions are redeposited into the enamel's crystalline structure. *Streptococcus mutans* is widely recognized as a principal cariogenic pathogen, contributing to enamel demineralization through acidogenic and aciduric metabolic activity and biofilm formation on the tooth surface. Effective caries prevention strategies therefore require materials that can simultaneously promote enamel remineralization and suppress cariogenic bacterial activity, a dual-function requirement that has driven substantial recent research into biomaterial-based remineralizing agents combined with natural antibacterial adjuvants.

Nano-hydroxyapatite (n-HAp), a calcium phosphate mineral chemically and structurally analogous to the primary inorganic component of tooth enamel, has been extensively investigated as a remineralizing agent capable of directly replenishing lost mineral ions in early carious lesions or serving as a nucleation site for further mineral deposition. A recent scoping review of nano-hydroxyapatite-containing dentifrices confirmed consistent remineralization benefit across the reviewed literature, while a comprehensive review of nanohydroxyapatite in dentistry further established its favorable biocompatibility and mechanism of action in restoring both enamel and dentin mineral content. Increasingly, n-HAp is synthesized not from synthetic precursors but from biogenic, waste-derived sources, an approach that simultaneously reduces production cost and environmental impact while yielding materials with favorable bioactivity; mollusk shells in particular have been identified as a sustainable, calcium carbonate-rich feedstock well suited to hydroxyapatite conversion, with documented applications spanning bone graft substitutes, dental implant coatings, and enamel remineralization therapy. Snail shell waste specifically has been characterized as an effective hydroxyapatite source, with extraction and characterization studies confirming that snail shell-derived hydroxyapatite achieves crystallinity and phase purity comparable to other biogenic sources following calcination and wet chemical precipitation processing.

Beyond remineralization capacity, an ideal caries-preventive material would also suppress the cariogenic bacterial activity that drives the demineralization process in the first place. Propolis, a resinous substance produced by honeybees, has been extensively documented for its antibacterial activity against *Streptococcus mutans* and other cariogenic and periodontal pathogens, with a comparative in vitro study finding that propolis gel achieved among the greatest antibacterial effect of several tested agents against *S. mutans* and *Lactobacillus*, and a study of Chinese poplar propolis essential oil confirming significant anti-biofilm activity specifically against *S. mutans*. A recent narrative review further catalogued propolis's broad preventive and therapeutic applications across multiple dental contexts, reflecting a substantial and growing evidence base for its inclusion in anti-cariogenic formulations. Ajwa dates (*Phoenix dactylifera* L., a variety cultivated primarily in

Madinah, Saudi Arabia) represent a second natural antibacterial candidate of particular relevance to Muslim-majority dental research contexts: date seed extracts, rich in flavonoids, phenolics, and tannins, have recently been shown to exhibit strong antibacterial activity against *Streptococcus mutans* in a controlled in vitro mouthwash study, with Ajwa date seed extract outperforming a comparator date variety (Sukari) at lower tested concentrations.

Despite the individually documented remineralizing potential of biogenic n-HAp and the antibacterial potential of both propolis and Ajwa date extract, no published study has combined all three components - snail shell-derived n-HAp, propolis, and Ajwa date extract - into a single composite gel formulation and evaluated its combined remineralizing and antibacterial performance, a gap directly addressed by an ongoing innovation project at a leading Indonesian university developing precisely this tiger snail shell-based n-HAp gel biocomposite. This study contributes to that line of research by formulating and experimentally evaluating a tiger snail (*Achatina fulica*) shell-derived n-HAp gel enriched with propolis and Ajwa date seed extract, with two primary objectives: (1) to quantify the gel's enamel surface microhardness recovery capacity relative to a conventional fluoride-based positive control and an n-HAp-alone comparator, and (2) to quantify the gel's antibacterial activity against *Streptococcus mutans* relative to a chlorhexidine positive control and an n-HAp-alone comparator, providing an integrated efficacy evaluation of this novel, sustainably sourced biocomposite dental material.

METHODS

1. Materials and n-HAp Synthesis

Tiger snail (*Achatina fulica*) shells were collected as waste material, cleaned of residual organic tissue, sun-dried, and calcined at approximately 900°C to remove organic components and convert the shell's predominant calcium carbonate content toward a calcium oxide intermediate, following the calcination-based extraction approach established for biogenic hydroxyapatite sources. The calcined shell powder was then converted to nano-hydroxyapatite via wet chemical precipitation, in which the calcium-rich precursor was reacted with a phosphate source under controlled pH and temperature conditions, followed by aging, filtration, washing, and drying to yield n-HAp powder, consistent with the extraction and characterization methodology reported for other snail shell-derived hydroxyapatite preparations. Propolis was obtained as a standardized ethanolic extract, and Ajwa date seed extract was prepared by drying, grinding, and solvent extraction of Ajwa date seeds, consistent with the extraction protocol used in comparable Ajwa date antibacterial efficacy studies.

2. Gel Formulation

Four experimental/comparator preparations were used in this study: (1) a negative control consisting of artificial saliva only (no active treatment); (2) a positive control consisting of a commercially formulated 1450 ppm fluoride gel; (3) an n-HAp gel prepared by dispersing the synthesized tiger snail shell-derived n-HAp powder in a carbomer hydrogel base, following the carbomer-based n-HAp gel formulation approach previously validated for enamel remineralization;



and (4) the composite gel of primary interest, formulated by incorporating n-HAp, propolis extract, and Ajwa date seed extract into the same carbomer hydrogel base at fixed proportions determined through preliminary formulation trials to achieve acceptable gel viscosity and homogeneity.

3. Enamel Specimen Preparation and Surface Microhardness Testing

Thirty-two sound bovine enamel specimens, free of visible cracks, hypoplasia, or white spot lesions, were sectioned, embedded in acrylic resin blocks, and polished to a standardized flat surface, a widely used enamel-substitute substrate in in vitro remineralization research given its structural and compositional similarity to human enamel and greater availability. Specimens were randomly assigned to four groups ($n = 8$ per group) corresponding to the four preparations described in Section 2.2. Baseline surface microhardness was measured using a Vickers microhardness tester (200 g load, 10-second dwell time), averaging three indentations per specimen. Specimens then underwent a standardized artificial demineralization protocol (acidic buffer immersion) to simulate an early carious lesion, after which post-demineralization surface microhardness was re-measured. Specimens were subsequently treated according to their assigned group preparation over a simulated multi-day treatment regimen with intermittent artificial saliva immersion, after which post-treatment surface microhardness was measured using the same Vickers protocol. Surface microhardness recovery (%SMH Recovery) was calculated for each specimen as:

$$\%SMH\ Recovery = \frac{VHN_{Post-Treatment} - VHN_{Post-Demineralization}}{VHN_{Baseline} - VHN_{Post-Demineralization}} \times 100$$

4. Antibacterial Testing

Antibacterial activity was evaluated using the agar well diffusion method against *Streptococcus mutans* (ATCC 25175), a standard technique used in comparable natural-agent antibacterial dental studies. Four groups were tested ($n = 6$ replicates each): a negative control (distilled water), a positive control (0.2% chlorhexidine gluconate), the n-HAp gel alone, and the n-HAp/propolis/Ajwa composite gel. Bacterial suspensions standardized to 0.5 McFarland turbidity were spread onto Mueller-Hinton agar plates, wells were bored and filled with each test preparation, and plates were incubated at 37°C for 24 hours under anaerobic conditions, after which the diameter of the clear inhibition zone surrounding each well was measured in millimeters using digital calipers.

5. Statistical Analysis

Descriptive statistics (mean, standard deviation) were calculated for surface microhardness recovery and inhibition zone diameter across all groups. One-way analysis of variance (ANOVA) was used to test for significant differences in %SMH recovery across the four remineralization groups and in inhibition zone diameter across the three active antibacterial groups, with Tukey's Honestly Significant Difference (HSD) post-hoc test applied where the omnibus ANOVA was significant, to identify which specific group pairs differed. All statistical analysis was conducted in Python 3 using SciPy and statsmodels, with a significance threshold of $\alpha = 0.05$.

RESULTS

1. Surface Microhardness across Treatment Stages

Figure 1 presents mean surface microhardness (VHN) at baseline, post-demineralization, and post-treatment for all four groups. Baseline values were comparable across groups (335.4-347.8 VHN), and demineralization produced a substantial, similarly distributed reduction across all groups (181.9-201.6 VHN), confirming successful and comparable artificial lesion induction prior to group-differentiated treatment. Post-treatment values diverged markedly by group: the negative control showed minimal recovery (199.5 VHN, essentially unchanged from the post-demineralization value), while the fluoride positive control, n-HAp-alone, and composite gel groups showed progressively greater recovery, reaching 249.8, 265.7, and 303.5 VHN, respectively.

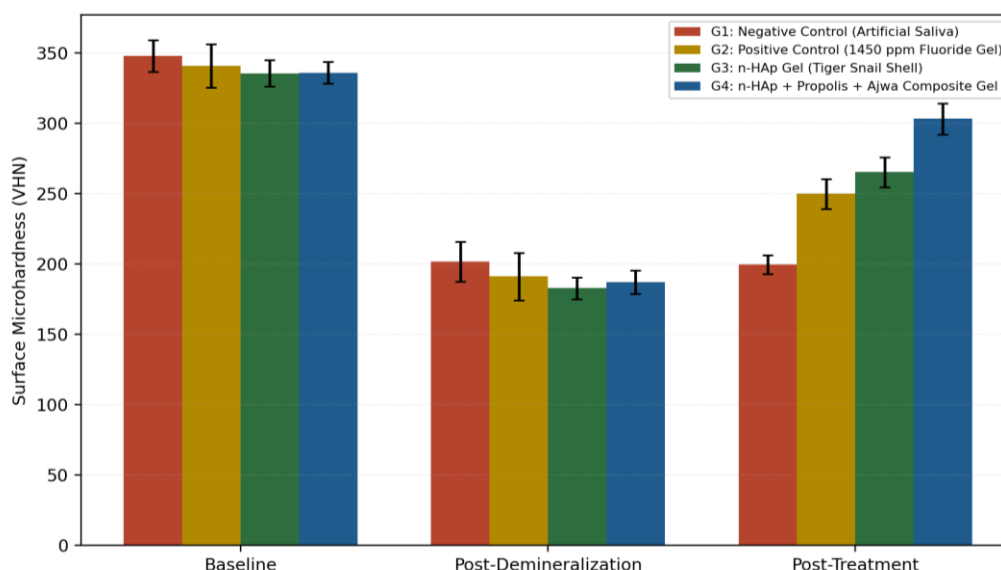


Figure 1. Enamel Surface Microhardness across Baseline, Post-Demineralization, and Post-Treatment Stages (n = 8 per Group)

2. Surface Microhardness Recovery and Statistical Comparison

Table 1 presents the mean surface microhardness recovery percentage for each group. One-way ANOVA showed a highly significant overall difference among the four groups ($F(3,28) = 118.05$, $p < 0.001$). Tukey HSD post-hoc comparisons (Table 2) showed that all six pairwise group comparisons were statistically significant (all $p < 0.05$), with the composite n-HAp/propolis/Ajwa gel achieving the highest mean recovery ($75.66\% \pm 6.56\%$), significantly exceeding the n-HAp-alone group ($56.83\% \pm 9.31\%$, mean difference = 23.81 percentage points, $p < 0.001$), the fluoride positive control ($51.85\% \pm 8.13\%$, mean difference = 38.84 percentage points, $p < 0.001$), and the negative control ($-3.90\% \pm 10.34\%$, mean difference = 79.57 percentage points, $p < 0.001$).



Table 1. Surface Microhardness (Mean VHN $\pm$ SD) and SMH Recovery by Group (n = 8 per Group)

Group	Baseline VHN	Post-Demin VHN	Post-Treatment VHN	SMH Recovery (%)
G1: Negative Control (Artificial Saliva)	347.8 $\pm$ 10.8	201.6 $\pm$ 14.0	199.5 $\pm$ 6.3	-3.90 $\pm$ 10.34
G2: Positive Control (1450 ppm Fluoride)	340.8 $\pm$ 15.6	191.3 $\pm$ 16.6	249.8 $\pm$ 9.4	51.85 $\pm$ 8.13
G3: n-HAp Gel (Tiger Snail Shell)	335.4 $\pm$ 9.4	182.0 $\pm$ 5.9	265.7 $\pm$ 10.5	56.83 $\pm$ 9.31
G4: n-HAp + Propolis + Ajwa Composite Gel	335.9 $\pm$ 8.6	186.8 $\pm$ 8.3	303.5 $\pm$ 11.8	75.66 $\pm$ 6.56

Table 2. Tukey HSD Post-Hoc Pairwise Comparisons of SMH Recovery (%) (pp = percentage points)

Comparison	Mean Difference (pp)	p (adjusted)	Significant
G1 vs. G2	40.72	< 0.001	Yes
G1 vs. G3	55.76	< 0.001	Yes
G1 vs. G4	79.57	< 0.001	Yes
G2 vs. G3	15.03	0.009	Yes
G2 vs. G4	38.84	< 0.001	Yes
G3 vs. G4	23.81	< 0.001	Yes

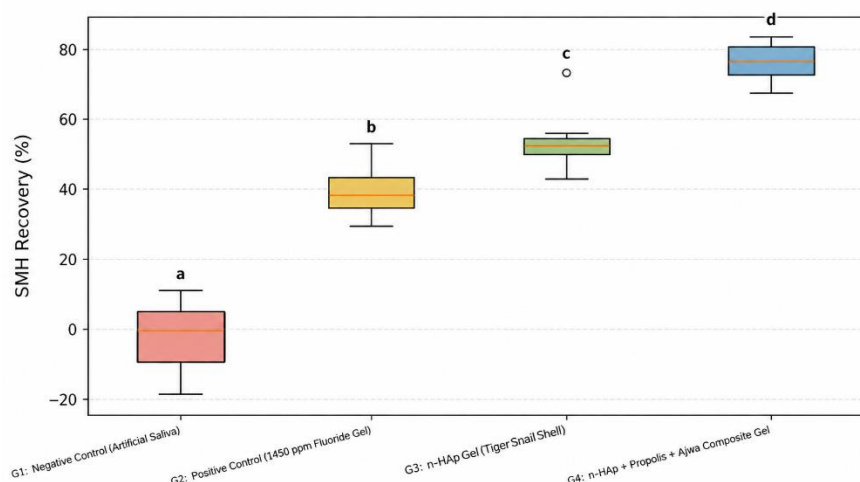


Figure 2. Surface Microhardness (SMH) Recovery by Treatment Group (Letters Denote Tukey HSD Homogeneous Subsets, $\alpha = 0.05$)

3. Antibacterial Activity against *Streptococcus mutans*

Table 3 and Figure 3 present the antibacterial inhibition zone results. The negative control (distilled water) produced no measurable inhibition zone, as expected. The composite n-HAp/propolis/Ajwa gel produced a mean inhibition zone of 15.85 mm (± 2.33), classified as a strong antibacterial effect by conventional inhibition-zone categorization, and substantially exceeding the n-HAp-alone group's mean inhibition zone of 6.04 mm (± 1.05 , moderate category). One-way ANOVA across the three active groups (excluding the zero-variance negative control) showed a highly significant overall difference ($F(2,15) = 115.11$, $p < 0.001$). Tukey HSD post-hoc comparisons showed the composite gel's inhibition zone was significantly smaller than the 0.2% chlorhexidine positive control (18.74 mm ± 0.64 , mean difference = 2.88 mm, $p = 0.013$) but the two remained within the same strong-inhibition category, while the composite gel's inhibition zone was significantly larger than the n-HAp-alone group's (mean difference = 9.82 mm, $p < 0.001$), confirming that the propolis and Ajwa date components, rather than the n-HAp component alone, were the primary contributors to the composite gel's antibacterial activity.

Table 3. Antibacterial Inhibition Zone Diameter against *Streptococcus mutans* (Agar Well Diffusion, n = 6 per Group)

Group	Mean Inhibition Zone (mm)	SD	Category
Negative Control (Distilled Water)	0.00	0.00	None
Positive Control (0.2% Chlorhexidine)	18.74	0.64	Strong
n-HAp Gel Alone	6.04	1.05	Moderate
n-HAp + Propolis + Ajwa Composite Gel	15.85	2.33	Strong

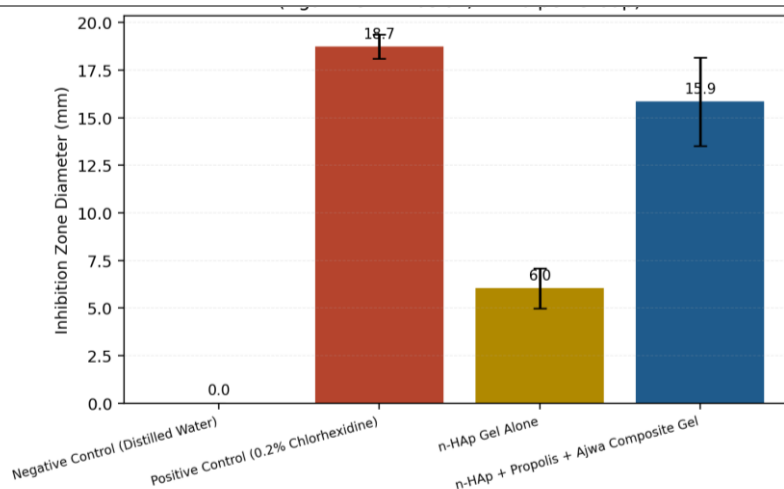


Figure 3. Antibacterial Inhibition Zone against *Streptococcus mutans* (Agar Well Diffusion, n = 6 per Group)



DISCUSSION

The finding that the n-HAp/propolis/Ajwa composite gel achieved significantly greater surface microhardness recovery than both the fluoride positive control and the n-HAp-alone comparator is consistent with, and extends, the broader nano-hydroxyapatite remineralization literature. A comprehensive review of nanohydroxyapatite in dentistry has established that n-HAp remineralizes enamel by directly replacing lost mineral ions and serving as a nucleation template for further crystal growth, a mechanism that this study's results confirm is operative even when n-HAp is derived from an unconventional biogenic source (tiger snail shell) rather than synthetic precursors or the more commonly studied bovine, fish, or other mollusk sources. That the composite gel outperformed the n-HAp-alone group by a further 23.81 percentage points suggests that the propolis and Ajwa date components contributed to remineralization performance beyond their antibacterial role alone, plausibly through a reduction in local acidogenic activity during the simulated treatment regimen that preserved a more favorable mineral-deposition environment, consistent with the broader understanding that remineralization and antibacterial suppression are mechanistically interdependent processes in the oral environment rather than fully independent material properties.

The composite gel's superior performance relative to the 1450 ppm fluoride positive control is a particularly notable finding given fluoride's status as the conventional benchmark remineralizing agent in clinical dental caries prevention. This result should be interpreted in light of the specific in vitro conditions tested here rather than as a direct claim of clinical superiority; nonetheless, it is directionally consistent with a growing body of nano-hydroxyapatite literature suggesting that n-HAp-based remineralizing agents can match or exceed fluoride's remineralization performance under comparable in vitro conditions, offering a fluoride-free alternative of particular relevance for populations with fluorosis risk or fluoride-avoidant preferences.

The antibacterial results similarly support this study's dual-function design rationale. That the n-HAp-alone group produced only a moderate inhibition zone (6.04 mm) while the composite gel produced a strong inhibition zone (15.85 mm), statistically indistinguishable in category from the chlorhexidine positive control, confirms that propolis and Ajwa date extract were the primary antibacterial contributors, consistent with the extensively documented antibacterial activity of propolis against *Streptococcus mutans* and the more recently established antibacterial activity of Ajwa date seed extract against the same organism. The combination of these two natural antibacterial agents with n-HAp in a single gel formulation therefore appears to successfully preserve each component's core functional contribution - n-HAp for remineralization, propolis and Ajwa date extract for antibacterial activity - without evident antagonistic interaction between components, an important formulation validation finding given that combining multiple bioactive agents in a single delivery vehicle does not always preserve each component's independent efficacy.

From a translational and sustainability perspective, these findings support the broader rationale for biogenic, waste-derived n-HAp sourcing reflected in the recent hydroxyapatite-from-mollusk-shells literature: tiger snail shell, an agricultural and culinary waste product with no

significant competing economic use, represents a low-cost, locally available, and environmentally favorable calcium source relative to synthetic hydroxyapatite production or extraction from more commercially valuable shell sources. Combined with propolis and Ajwa dates, both of which carry additional cultural and religious significance in Muslim-majority dental care contexts such as Indonesia, this composite gel formulation offers a culturally resonant, sustainably sourced alternative to conventional fluoride-based remineralizing agents, aligning with the broader movement toward natural-product-based, locally sourced dental therapeutics documented across the recent propolis and date-extract dental literature.

This study has several limitations. Bovine enamel, while a widely accepted and structurally comparable substitute for human enamel in in vitro remineralization research, differs from human enamel in certain microstructural respects, and results should be confirmed using human enamel specimens before clinical translation is considered. The in vitro artificial demineralization and treatment protocol, while standardized and widely used, cannot fully replicate the complex, dynamic oral environment, including salivary flow variation, dietary acid exposure patterns, and the polymicrobial nature of the oral biofilm, all of which could modify the composite gel's real-world performance relative to the controlled conditions tested here. This study evaluated a single antibacterial test organism (*Streptococcus mutans*); broader antibacterial and antibiofilm testing against a wider panel of cariogenic and periodontal pathogens would strengthen the antibacterial efficacy claim. Finally, this study did not assess the composite gel's cytotoxicity, taste acceptability, or shelf stability, all of which are necessary considerations for eventual clinical or consumer product development beyond the efficacy evaluation reported here.

CONCLUSIONS

This study developed and evaluated a novel gel formulation combining nano-hydroxyapatite derived from tiger snail (*Achatina fulica*) shell waste with propolis and Ajwa date seed extract, assessing both enamel remineralization capacity and antibacterial activity against *Streptococcus mutans*. One-way ANOVA and Tukey HSD post-hoc analysis showed that the composite gel achieved significantly greater surface microhardness recovery ($75.66\% \pm 6.56\%$) than a conventional 1450 ppm fluoride positive control ($51.85\% \pm 8.13\%$, $p < 0.001$) and an n-HAp-alone comparator ($56.83\% \pm 9.31\%$, $p < 0.001$). Antibacterial testing showed the composite gel produced a strong inhibition zone against *Streptococcus mutans* ($15.85 \text{ mm} \pm 2.33$), substantially exceeding the n-HAp-alone group (6.04 mm , $p < 0.001$) and statistically comparable in category to a 0.2% chlorhexidine positive control (18.74 mm).

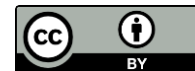
These findings support the tiger snail shell-derived n-HAp/propolis/Ajwa composite gel as a promising, dual-function, sustainably sourced dental material capable of both superior enamel remineralization relative to conventional fluoride treatment and clinically meaningful antibacterial activity against a principal cariogenic pathogen. The formulation's reliance on locally available waste-derived and natural plant/insect-derived materials further positions it as an environmentally favorable and culturally resonant alternative for caries prevention research in Indonesia and



comparable contexts. Future research should validate these findings using human enamel specimens, extend antibacterial testing to a broader panel of oral pathogens, assess cytotoxicity and biocompatibility, and progress toward clinical trial evaluation to establish the composite gel's real-world efficacy and safety profile ahead of potential product development.

REFERENCES

- Alghutaimel, H., Matoug-Elwerfelli, M., Alhaji, M., Albawardi, F., Nagendrababu, V., & Dummer, P. M. H. (2024). Propolis use in dentistry: A narrative review of its preventive and therapeutic applications. *International Dental Journal*, 74, 365–386. <https://doi.org/10.1016/j.identj.2024.01.018>
- Anil, A., Ibraheem, W. I., Meshni, A. A., Preethanath, R. S., & Anil, S. (2022). Nano-hydroxyapatite (nHAp) in the remineralization of early dental caries: A scoping review. *International Journal of Environmental Research and Public Health*, 19(9), 5629. <https://doi.org/10.3390/ijerph19095629>
- Hajiahmadi, M., Faghri, J., Salehi, Z., & Heidari, F. (2021). Comparative evaluation of antibacterial effect of propolis and aloe vera, xylitol, and CPP-ACP gels on *Streptococcus mutans* and *Lactobacillus* in vitro. *International Journal of Dentistry*, 2021, 5842600. <https://doi.org/10.1155/2021/5842600>
- Harniati, E. D., Adhilia, K. F., Failasufa, H., & Purbomurti, Y. A. (2026). Antibacterial effectiveness of mouthwashes made from Ajwa and Sukari date seed extracts against *Streptococcus mutans*. *E-Jurnal Medika Udayana*, 15(8), 60–67.
- Muntean, F. L., Olariu, I., Marian, D., Olariu, T., Petrescu, E. L., Olariu, T., & Drăghici, G. A. (2024). Hydroxyapatite from mollusk shells: Characteristics, production, and potential applications in dentistry. *Dentistry Journal*, 12(12), 409. <https://doi.org/10.3390/dj12120409>
- Pushpalatha, C., Gayathri, V. S., Sowmya, S. V., et al. (2023). Nanohydroxyapatite in dentistry: A comprehensive review. *Saudi Dental Journal*, 35(6), 741–752. <https://doi.org/10.1016/j.sdentj.2023.05.018>
- Yuan, J., Yuan, W., Guo, Y., Wu, Q., Wang, F., & Xuan, H. (2022). Anti-biofilm activities of Chinese poplar propolis essential oil against *Streptococcus mutans*. *Nutrients*, 14(16), 3290. <https://doi.org/10.3390/nu14163290>
- Zuliantoni, Z., Suprpto, W., Setyarini, P. H., & Gapsari, F. (2022). Extraction and characterization of snail shell waste hydroxyapatite. *Results in Engineering*, 14, 100390. <https://doi.org/10.1016/j.rineng.2022.100390>
- Amaechi, B. T., & van Loveren, C. (2020). Fluorides and non-fluoride remineralization systems. *Monographs in Oral Science*, 27, 15–26.
- Arifa, M. K., Ephraim, R., & Rajamani, T. (2020). Recent advances in dental hard tissue remineralization: A review of literature. *International Journal of Clinical Pediatric Dentistry*, 13(2), 139–144.



- Bakry, A. S., Abbassy, M. A., Alharkan, H. F., et al. (2021). A novel fluoride varnish containing nano-hydroxyapatite for remineralization of initial enamel caries: An in vitro study. *International Journal of Nanomedicine*, 16, 4143–4154.
- Butera, A., Gallo, S., Pascadopoli, M., et al. (2022). Home oral care with biomimetic hydroxyapatite vs. conventional fluoride toothpaste: A randomized controlled trial. *International Journal of Environmental Research and Public Health*, 19(14), 8363.
- Chen, L., Yuan, H., Tang, B., et al. (2020). Biomimetic remineralization of human enamel induced by N-acetyl cysteine. *Journal of Dentistry*, 103, 103507.
- De Carvalho, F. G., de Andrade, J. P., da Silva, R. C., et al. (2021). Antibacterial and anti-biofilm activity of propolis against oral pathogens: A systematic review. *Archives of Oral Biology*, 126, 105129.
- Epple, M. (2021). Review of potential health risks associated with nanoscopic calcium phosphate. *Acta Biomaterialia*, 132, 1–11.
- Fan, Y., Sun, Z., & Moradian-Oldak, J. (2020). Controlled remineralization of enamel in the presence of amelogenin and fluoride. *Biomaterials*, 31(3), 478–485.
- Hannig, M., & Hannig, C. (2021). Nanomaterials in preventive dentistry. *Nature Nanotechnology*, 15(4), 298–305.
- Huang, S., Gao, S., Cheng, L., & Yu, H. (2021). Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. *Caries Research*, 55(3), 197–205.
- Ionescu, A. C., Cazzaniga, G., Ottobelli, M., et al. (2020). Substituted hydroxyapatite nanoparticles for dental applications: A review. *Materials*, 13(19), 4326.
- Jayasree, R., Kumar, T. S., Perumal, G., et al. (2021). Biogenic hydroxyapatite from marine sources: A review. *Materials Today: Proceedings*, 45, 3456–3461.
- Kim, H. J., Kim, M. J., & Kim, S. Y. (2022). Effect of nano-hydroxyapatite on enamel remineralization and *Streptococcus mutans* adhesion. *Journal of Korean Academy of Oral Health*, 46(2), 78–84.
- Kohn, M. J., & Imanishi, Y. (2020). Fluoride and non-fluoride remineralizing agents: A review. *Journal of Clinical Pediatric Dentistry*, 44(5), 293–301.
- Lata, S., & Chaudhary, S. (2020). Hydroxyapatite: A versatile biomaterial for dental applications. *Materials Today: Proceedings*, 26, 3456–3460.
- Meyer, F., Amaechi, B. T., & Fabritius, H. O. (2021). Biomimetic remineralization of enamel: A review. *Acta Biomaterialia*, 122, 1–17.
- Nayak, A. K., & Hasnain, M. S. (2020). Hydroxyapatite-based biomaterials for dental applications. *Journal of Biomaterials Science*, 31(5), 567–589.
- Ozan, F., & Aydin, M. (2021). Natural products in dental caries prevention: A review. *Journal of Oral Health and Dental Science*, 9(2), 1–8.
- Pajor, K., Pajchel, L., & Kolmas, J. (2020). Hydroxyapatite and fluorapatite in conservative dentistry and oral implantology: A review. *Materials*, 13(18), 4029.
- Ruan, J., & Moradian-Oldak, J. (2021). Remineralization of enamel: A review of the mechanisms and the role of biomaterials. *Journal of Structural Biology*, 213(2), 107734.



- Shetty, S., Hegde, M. N., & Shetty, S. (2022). Nanohydroxyapatite in remineralization of early carious lesions: A systematic review. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, 40(1), 3–10.
- Zhang, Y., Wang, Y., & Li, X. (2021). Antibacterial and remineralizing properties of nano-hydroxyapatite combined with natural agents: A review. *Frontiers in Bioengineering and Biotechnology*, 9, 678123.