Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 24 May 2026
Revised 26 June 2026
Accepted 10 July 2026

Natural Resources for Human Health 2026, Vol. 6 (7s): 364–376

KEYWORDS:
Pomacea canaliculata; Melastoma malabathricum; enamel remineralization; surface microhardness; Streptococcus mutans; dentine hypersensitivity; circular bioeconomy

Golden apple snail (Pomacea canaliculata L.) Shell as a Biogenic Calcium Source in a Melastoma Malabathricum l.-Enriched Dentifrice: Concentration-Dependent Remineralization Kinetics, Enamel Microhardness Recovery and Anticariogenic Activity

Marlindayanti[1], Ismalayani[2], Nur Adiba Hanum[3], Dhandi Wijaya[4]

[1]Department of Dental Health, Poltekkes Kemenkes Palembang, Jalan Sukabangun 1 No. 1159, Sukajaya, Sukarami District, Palembang City, South Sumatra, Indonesia. Email: marlindayanti@poltekkespalembang.ac.id
[2]Department of Dental Health, Poltekkes Kemenkes Palembang, Jalan Sukabangun 1 No. 1159, Sukajaya, Sukarami District, Palembang City, South Sumatra, Indonesia. Email: ismalayani@poltekkespalembang.ac.id
[3]Department of Dental Health, Poltekkes Kemenkes Palembang, Jalan Sukabangun 1 No. 1159, Sukajaya, Sukarami District, Palembang City, South Sumatra, Indonesia. Email: nuradibahanum@poltekkespalembang.ac.id
[4]Department of Dental Health, Poltekkes Kemenkes Palembang, Jalan Sukabangun 1 No. 1159, Sukajaya, Sukarami District, Palembang City, South Sumatra, Indonesia. Email dhandi@poltekkespalembang.ac.id

ABSTRACT:

Background: Dentine hypersensitivity is associated with an imbalance between enamel demineralization and remineralization, resulting in dentinal tubule exposure and stimulus-induced pain. The shell of the golden apple snail (Pomacea canaliculata L.), an invasive rice pest and abundant agricultural waste in Southeast Asia, is a potential source of biogenic calcium carbonate for sustainable remineralizing dentifrices. Melastoma malabathricum L. fruit (senduduk) provides bioactive phytochemicals with antibacterial potential.

Objective: To determine the effect of P. canaliculata shell concentration on the magnitude, kinetics, and durability of enamel remineralization and identify the optimal dentifrice formulation.

Methods: Four dentifrices containing 5%, 10%, 15%, and 20% (w/w) P. canaliculata shell powder with a fixed M. malabathricum fruit fraction were compared with a commercial sodium fluoride/potassium nitrate dentifrice. One hundred permanent human canines were demineralized at pH 3 for 72 h and allocated to five groups, each subdivided into 90-, 180-, 270-, and 360-min immersion periods (n = 5). Outcomes included Vickers surface microhardness, artificial salivary calcium concentration, antibacterial activity against Streptococcus mutans and Escherichia coli, scanning electron microscopy, pH,

Natural Resources for Human Health
eISSN: 2583-1194

homogeneity, and compliance with SNI 12-3524-1995.

Results: All formulations were homogeneous, pH-neutral (pH 7), free of E. coli, and compliant with SNI requirements. Microhardness differed significantly among groups at all time points ($p < 0.001$; $\eta^2 = 0.70–0.96$). Peak hardness ranged from 306.50 to 341.83 HV, representing a 132–159% increase from baseline. The 5% formulation reached peak hardness at 180 min and showed the strongest early remineralization, whereas higher concentrations required longer exposure. At 90 min, hardness was inversely correlated with shell concentration ($r = −0.947$). The 5% formulation achieved hardness comparable to the commercial control (341.83 vs. 332.00 HV; $p = 0.45$), but demonstrated superior mineral retention and the greatest integrated mineral gain (48,945 vs. 39,583 HV·min). S. mutans inhibition was strongest with the 5% formulation (16 mm) and declined with increasing shell concentration, whereas no formulation inhibited E. coli. Salivary calcium decreased across all groups, indicating net mineral uptake.

Conclusion: Shell concentration primarily influenced remineralization kinetics and mineral retention rather than ultimate remineralization capacity. The 5% formulation demonstrated the most favorable overall performance, combining control-equivalent peak microhardness, superior mineral retention, and the strongest S. mutans inhibition. P. canaliculata shell combined with M. malabathricum fruit therefore represents a promising locally sourced and circular-bioeconomy-based dentifrice candidate.

1. INTRODUCTION

Dentine hypersensitivity (DH) is a prevalent and clinically significant condition in dentistry, affecting an estimated 30% of the global population, with the highest burden reported among adults aged 20–40 years and a greater prevalence among women than men [1–3]. In Indonesia, the burden is similarly substantial, with epidemiological data suggesting that approximately one in five adults experiences DH. Clinically, DH is characterized by a transient, sharp pain elicited by thermal, osmotic, tactile, or evaporative stimuli, including exposure to hot or cold foods and beverages, sweet or acidic substances, toothbrushing, flossing, or cold air. The condition generally occurs when enamel is lost or substantially thinned, exposing dentine and its underlying tubular network to the oral environment and external stimuli[4–6]. Under physiological conditions, dental hard tissues undergo continuous cycles of demineralization and remineralization that remain in dynamic equilibrium. Acid-mediated mineral dissolution is counterbalanced by the precipitation of calcium and phosphate ions derived primarily from saliva, which contribute to the restoration of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], the principal crystalline mineral phase accounting for approximately 90–92% of enamel by volume[7–10]. DH may emerge when this equilibrium is persistently disrupted in favor of mineral dissolution. Such disruption can result from extrinsic dietary acids and acidic beverages, intrinsic acids associated with gastroesophageal reflux or regurgitation, and bacterial acidogenesis within dental biofilms[11–13]. Once mineral loss exceeds the endogenous capacity for repair, spontaneous restoration becomes insufficient, necessitating therapeutic strategies that promote remineralization and reduce dentinal permeability[14–16].

Effective remineralization is governed by a multifactorial physicochemical and biological environment involving adequate salivary flow, maintenance of a near-neutral oral pH, sufficient availability of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions, and control of acidogenic microorganisms responsible for recurrent demineralization[17,18]. Although the mineral component has received considerable attention in dentifrice development, microbial control is equally important because persistent acidogenic activity may compromise mineral deposition and accelerate subsequent mineral loss. Consequently, a formulation capable of simultaneously supplying remineralizing ions and suppressing cariogenic microorganisms may provide a more comprehensive approach to maintaining mineral homeostasis than strategies targeting either process independently.

Commercial desensitizing dentifrices commonly employ fluoride, potassium salts, hydroxyapatite, or arginine–calcium carbonate systems to reduce dentinal permeability and facilitate mineral restoration. However, the availability of affordable, locally sourced, and environmentally sustainable alternatives remains limited, particularly in resource-constrained settings. This has stimulated increasing interest in biogenic calcium carbonate obtained from shell-derived waste as a potential multifunctional ingredient for dental formulations. Calcium carbonate recovered from molluscan and avian shells has demonstrated suitable physicochemical

characteristics for use as a dentifrice abrasive and mineralizing agent, supporting its potential as an alternative source of calcium for oral-care applications[19–21].

Among potential biological sources, the shell of the golden apple snail, Pomacea canaliculata L., represents a particularly promising and contextually relevant feedstock. Introduced into Southeast Asia during the 1980s, P. canaliculata has subsequently become a major invasive agricultural pest, particularly in rice-producing systems, and has been recognized among ecologically and economically consequential alien species[22–24]. Its shell contains a high proportion of calcium carbonate, making it a potentially valuable mineral resource rather than merely an agricultural waste stream. Importantly, population-control activities generate shell biomass continuously, creating an opportunity to transform an invasive-species management burden into a value-added biomedical resource. Previous studies have demonstrated that P. canaliculata shell-derived preparations can increase salivary pH, elevate calcium availability, facilitate calcium deposition at the enamel surface, and enhance enamel surface hardness[25,26]. These findings provide a preliminary biological rationale for incorporating snail-shell-derived calcium carbonate into remineralizing dentifrice formulations and, more broadly, illustrate its potential within a circular bioeconomy framework.

The antimicrobial component of the proposed formulation is derived from Melastoma malabathricum L. (senduduk; Melastomataceae), a widely distributed pantropical shrub with a longstanding ethnomedicinal history throughout the Malay Archipelago[27,28]. Phytochemical investigations have identified multiple bioactive constituents in its fruits and leaves, including flavonoids, tannins, saponins, and alkaloids[29,30]. Previous investigations have reported antibacterial activity of isolated fruit constituents against Staphylococcus aureus and Escherichia coli at concentrations of approximately 1000 ppm, while ethyl acetate fractions of the leaves have demonstrated inhibitory activity against S. aureus, E. coli, and Candida albicans[31–33]. The biological activities of these phytochemical classes are mechanistically complementary: flavonoids can interfere with microbial membrane integrity and cellular motility; tannins can interact with microbial proteins, adhesins, and extracellular enzymes; alkaloids may disrupt cell-wall biosynthesis; and saponins can alter membrane permeability and promote leakage of intracellular components. Accordingly, combining biogenic calcium carbonate with M. malabathricum fruit extract provides a rational formulation strategy that simultaneously addresses mineral replenishment and microbial suppression.

Despite this potential, an important formulation gap remains concerning the concentration-dependent performance of shell-derived calcium carbonate dentifrices. Increasing mineral loading may enhance the availability of calcium and carbonate ions and thereby increase the potential for mineral deposition. However, higher concentrations may simultaneously increase formulation viscosity, alter buffering behavior, and modify the diffusion and release of both mineral ions and phytochemical constituents. Consequently, the relationship between calcium carbonate concentration and remineralization cannot be assumed to be linear. In particular, it remains unclear whether increasing biogenic carbonate concentration primarily affects the magnitude of mineral recovery, the kinetics of remineralization, or the persistence of the resulting mineral gain. Distinguishing among these dimensions is essential because a formulation that produces rapid mineral deposition may not necessarily provide durable protection under subsequent oral challenges. To address this gap, the present study developed four dentifrice formulations containing 5–20% (w/w) P. canaliculata shell-derived calcium carbonate, while maintaining a constant total abrasive load and a fixed proportion of M. malabathricum fruit extract. The formulations were evaluated against a commercially available sodium fluoride/potassium nitrate dentifrice as a reference control over a 90–360 min immersion period. Evaluation encompassed enamel surface microhardness, calcium concentration in artificial saliva, antibacterial activity against Streptococcus mutans and Escherichia coli, enamel surface morphology and ultrastructure using scanning electron microscopy (SEM), and compliance with the Indonesian National Standard SNI 12-3524-1995. By incorporating multiple assessment domains and a time-resolved experimental design, the study seeks to determine not only whether shell-derived calcium carbonate promotes remineralization, but also how its concentration influences the kinetics and persistence of mineral recovery

MATERIALS AND METHODS

Study design

This laboratory-based experimental study employed a post-test-only control group design and was conducted from June to November 2023 at the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, for microhardness testing and scanning electron microscopy (SEM), and at the Medical Laboratory Technology and Pharmacy Laboratories, Poltekkes Kemenkes Palembang, for dentifrice formulation, physicochemical characterization, and microbiological analyses. The dentifrice formulations consisted of *Pomacea canaliculata* L. shell powder, calcium carbonate, gum arabic, sodium saccharin, sodium chloride, menthol, methyl paraben, glycerin, peppermint oil, finely triturated fresh *Melastoma malabathricum* L.

fruit, and *aqua bidestillata*. Artificial saliva was prepared from analytical-grade $NaHCO_3$, Na_2PO_4, KCl, $MgSO_4$, NaCl, and $CaCl_2$ dissolved in *aqua pro injectione*. A commercially available desensitizing dentifrice containing sodium fluoride and potassium nitrate was used as the positive control. Microbiological assays employed pure cultures of *Streptococcus mutans* and *Escherichia coli* cultured on Mueller–Hinton agar and MacConkey agar, respectively. The instruments used included a calibrated pH meter, digital micro-Vickers hardness tester, scanning electron microscope, incubator, analytical balance with four-decimal-place precision, and standard laboratory glassware. *P. canaliculata* shells were thoroughly cleaned to remove soil, adherent tissues, and other organic residues, washed, dried, pulverized, and passed through a 200-mesh sieve to obtain a relatively uniform powder suitable for incorporation into the dentifrice base. Species identification was based on the established morphological and taxonomic characteristics of *P. canaliculata* L. The resulting shell powder was characterized by X-ray diffraction (XRD) to determine its crystalline calcium carbonate phase, scanning electron microscopy to assess particle morphology, and energy-dispersive X-ray spectroscopy (EDS) to determine its elemental composition. Particle-size uniformity was assessed using a 200-mesh sieve, while heavy-metal analysis was performed to assess potential contamination by cadmium (Cd), lead (Pb), and arsenic (As). Ripe *M. malabathricum* L. fruits were selected according to predefined maturity criteria, washed, and finely triturated to obtain a homogeneous pulp, which was incorporated directly into the dentifrice formulations without solvent extraction. Botanical identification was based on established morphological characteristics and documented using a herbarium voucher. Phytochemical characterization of the fruit fraction included quantitative determination of total phenolic, total flavonoid, and total anthocyanin contents using appropriate spectrophotometric methods and reference standards. The *M. malabathricum* fruit fraction was maintained at a constant concentration across all formulations, whereas *P. canaliculata* shell powder was incorporated at concentrations of 5%, 10%, 15%, and 20% (w/w) to evaluate the effect of increasing biogenic calcium carbonate concentration on the physicochemical characteristics and functional performance of the dentifrices.

Dentifrice formulation

Four experimental formulations were prepared, differing only in the proportion of *P. canaliculata* shell powder (5, 10, 15 and 20% w/w) with a reciprocal reduction in synthetic calcium carbonate, so that total abrasive load remained constant at 50% w/w across all four. All other components, including the *M. malabathricum* fruit fraction, were held constant. Compositions are given in Table 1. This constant-abrasive design is important interpretively: any difference between formulations is attributable to the substitution of biogenic for synthetic carbonate, not to a change in total mineral or abrasive content.

Table 1. Composition of the four experimental dentifrice formulations and the positive control.

Ingredient	Function	F5	F10	F15	F20	Control
P. canaliculata shell powder	Biogenic remineralizing phase	5 g	10 g	15 g	20 g	—
Calcium carbonate (synthetic)	Abrasive	45 g	40 g	35 g	30 g	—
M. malabathricum fresh fruit	Antibacterial / antiplaque	10 g	10 g	10 g	10 g	—
Sodium fluoride + potassium nitrate	Active (control)	—	—	—	—	Marketed formulation
Gum arabic	Binder	12 g	12 g	12 g	12 g	—
Glycerin	Humectant	10 g	10 g	10 g	10 g	—
Methyl paraben	Preservative	0.5 g	0.5 g	0.5 g	0.5 g	—
Sodium chloride	Preservative	0.5 g	0.5 g	0.5 g	0.5 g	—
Sodium saccharin	Sweetener	0.5 g	0.5 g	0.5 g	0.5 g	—
Menthol	Flavour	1 g	1 g	1 g	1 g	—
Peppermint oil	Flavour	1 g	1 g	1 g	1 g	—
Aqua bidestillata	Vehicle	to 100%	to 100%	to 100%	to 100%	—

Total abrasive load (biogenic shell + synthetic carbonate) was held constant at 50 g per 100 g across F5–F20. The discrepancy between the 10 g fruit fraction tabulated here and the 5% figure cited elsewhere in the source documentation requires reconciliation.

Manufacture proceeded as follows. Gum arabic was dispersed in aqua destillata, added incrementally in a mortar until a gel formed. In a separate mortar, sodium saccharin, sodium chloride, menthol and methyl paraben were triturated to a fine powder. Calcium carbonate was incorporated portion-wise with continued trituration, followed by the shell powder, mixing to homogeneity. The gum arabic gel was then added incrementally to the powder blend with further trituration. Glycerin and peppermint oil were introduced slowly with continuous mixing until a smooth, homogeneous, lump-free mass was obtained. Finally the triturated *M. malabathricum* fruit was incorporated and the whole mass triturated again to the target paste consistency.

2.6. Preparation of artificial saliva

Artificial saliva was prepared by sequentially dissolving 19.9 g of $NaHCO_3$, 3.5 g of Na_2PO_4, 0.285 g of KCl, 0.06 g of $MgSO_4$, 0.235 g of NaCl, and 0.02 g of $CaCl_2$ in 500 mL of *aqua pro injectione*. The solution was placed in a closed container and shaken until all components were completely dissolved, after which the pH was measured and verified before use. The artificial saliva was subsequently used as the immersion medium for the enamel specimens. A total of 100 extracted permanent human canines with intact crowns and without caries, cracks, restorations, or other structural defects were used as experimental specimens. To simulate enamel demineralization associated with hypersensitivity, the specimens were immersed in a carbonated beverage adjusted to pH 3 for three consecutive 24-h periods. Following demineralization, Vickers microhardness was measured, yielding a mean baseline hardness of 132.2 HV. The specimens were then allocated into five experimental groups: F5, F10, F15, F20, and a positive-control group treated with a commercially available desensitizing dentifrice. Each group consisted of 20 specimens and was further divided into four immersion-time subgroups of five specimens each, corresponding to 90, 180, 270, and 360 min, resulting in a sample size of $n = 5$ for each group–time combination. The demineralized specimens were coated with the respective dentifrice formulation and subsequently immersed in artificial saliva according to the predetermined immersion period. After 90, 180, 270, or 360 min, the specimens were removed from the immersion medium and prepared for subsequent Vickers microhardness analysis. All specimens were handled using standardized laboratory procedures throughout the experimental period to minimize variation attributable to specimen preparation and treatment conditions.

2.9. Physicochemical and microbiological conformity testing

Physicochemical and microbiological conformity of the dentifrice formulations was assessed based on homogeneity, pH, and absence of *Escherichia coli*. Homogeneity was evaluated by examining samples collected from the upper, middle, and lower portions of each batch, while pH was measured using a calibrated pH meter according to SNI 12-3524-1995 (acceptable range, 4.5–10.5). Microbiological conformity was assessed using MacConkey agar after 24 h of incubation. Antibacterial activity against *Streptococcus mutans* and *E. coli* was determined using the Kirby–Bauer disc diffusion method, with inhibition zones measured after 24 h of incubation at 35–37 °C and interpreted according to Davis and Stout criteria. Calcium concentration in artificial saliva was measured after each immersion period to assess mineral exchange, with changes from baseline indicating net calcium uptake or release. Enamel surface microhardness was determined using a digital micro-Vickers hardness tester under standardized loading conditions, and surface morphology after 180 min of immersion was examined by scanning electron microscopy at 3000× magnification. The independent variable was dentifrice formulation (F5, F10, F15, F20, and positive control), while dependent variables included enamel microhardness, artificial salivary calcium concentration, and bacterial inhibition-zone diameter; immersion time (90, 180, 270, and 360 min) was incorporated as a temporal factor. The null hypothesis stated that the *P. canaliculata* shell-based formulations would not differ significantly from the commercial control in remineralization-related outcomes or antibacterial activity.

Antibacterial testing

Antibacterial activity was evaluated using the Kirby–Bauer disc diffusion method against *Streptococcus mutans* and *Escherichia coli*. Sterile paper discs were saturated with each dentifrice suspension in sterile *aqua destillata* for 30 min, placed on inoculated Mueller–Hinton agar, and incubated at 35–37 °C for 24 h. The diameter of the inhibition zone was measured using a vernier caliper and classified according to Davis and Stout criteria as weak (<5 mm), moderate (5–10 mm), or strong (>10–20 mm) inhibition. Calcium concentration in artificial saliva was measured after each immersion period to evaluate mineral exchange between the enamel specimens and immersion medium; a decrease from baseline was interpreted as net calcium uptake by enamel, whereas an increase indicated net calcium release. Calcium was quantified using a validated analytical method with appropriate calibration standards and replicate measurements. Surface microhardness was determined using a digital micro-Vickers hardness tester after embedding the specimens in resin while leaving the enamel surface exposed. Vickers indentations were made under standardized loading conditions, and hardness values were calculated from the mean lengths of the indentation diagonals. Representative

enamel specimens from each group were further examined by scanning electron microscopy (SEM) after 180 min of immersion at 3000× magnification to evaluate the distribution and morphology of deposited material. The independent variable was dentifrice formulation (F5, F10, F15, F20, and positive control), while the dependent variables were enamel surface microhardness, artificial salivary calcium concentration, and bacterial inhibition-zone diameter. Immersion time (90, 180, 270, and 360 min) was incorporated as a temporal factor. The null hypothesis stated that the *P. canaliculata* shell-based formulations would not differ significantly from the commercial control in their effects on enamel microhardness, calcium exchange, or antibacterial activity.

Statistical analysis

Data are presented as mean ± standard deviation. Homogeneity of variance was assessed by Levene test before parametric analysis. Microhardness and salivary calcium were compared between formulations at each immersion time by one-way ANOVA with Bonferroni-corrected pairwise comparisons; inhibition zone diameters, which were not normally distributed, were compared by Kruskal–Wallis test. Significance was set at $\alpha = 0.05$. To characterize the time course beyond the individual time points, three derived measures were computed for each formulation from the group means: peak hardness and the time at which it occurred; the post-peak decline rate, expressed in HV per minute and as a percentage of peak lost by 360 min; and the area under the hardness–time curve above the demineralized baseline of 132.2 HV, calculated by the trapezoidal rule over the 90–360 min interval and expressed both as HV·min and as the equivalent mean hardness elevation sustained across that interval. Partial eta-squared (η^2) is reported as the effect size for each ANOVA, and Cohen's d for comparisons against the positive control. Analyses were performed in SPSS (version [insert version]); derived measures and effect sizes were computed from the group summary statistics.

RESULT

Physical characteristics and standards conformity

All four experimental formulations were produced as smooth, homogeneous pastes. Sampling from the upper, middle and lower thirds of each batch showed uniform texture with no air entrapment, no aggregates and no separated particles at any sampling position, confirming homogeneous dispersion of both the shell powder and the fruit fraction throughout the matrix. All four experimental formulations recorded a pH of 7.0, while the marketed positive control recorded pH 6.0 (Table 2). All five pastes therefore fall within the SNI 12-3524-1995 range of 4.5–10.5, but the experimental formulations are neutral whereas the control is mildly acidic — a distinction with mechanistic relevance discussed below, since the solubility of hydroxyapatite is steeply pH-dependent. Notably, pH was invariant across a fourfold range of shell concentration, indicating that within this compositional window the carbonate buffer system rather than the loading determines the equilibrium pH. No formulation, and not the control, supported growth of *E. coli* after 24 h on agar, satisfying the microbiological requirement of SNI 12-3524-1995.

Table 2. pH and conformity to SNI 12-3524-1995.

Formulation	pH	SNI pH 4.5–10.5	E. coli (24 h)	Homogeneity
F5 (5% shell)	7.0	Complies	Not detected	Homogeneous
F10 (10% shell)	7.0	Complies	Not detected	Homogeneous
F15 (15% shell)	7.0	Complies	Not detected	Homogeneous
F20 (20% shell)	7.0	Complies	Not detected	Homogeneous
Positive control	6.0	Complies	Not detected	Homogeneous

Antibacterial activity

Against *S. mutans*, all five pastes produced measurable inhibition, and the differences between them were significant on Kruskal–Wallis testing ($p < 0.001$). Inhibition was strongest for F5 at 16 mm — "strong" by the Davis and Stout criteria — and fell as shell content rose, to 10 mm at both 10 and 15% and 8 mm at 20%, the latter equalling the marketed control (Table 3). The relationship between shell concentration and inhibition zone was strongly negative and monotonic (Spearman $\rho = -0.949$, $p = 0.051$; Pearson $r = -0.894$), the borderline p value reflecting the four concentration levels available rather than any weakness of the trend. This inverse relationship is mechanistically informative precisely because the *M. malabathricum* fruit fraction was held

constant across all four formulations. The decline in activity therefore cannot be attributed to dilution of the antibacterial constituent, and must instead reflect a property of the paste matrix that changes with carbonate loading — most plausibly reduced diffusion of the phytochemical fraction out of an increasingly viscous, densely packed matrix, together with the greater buffering capacity of the higher-carbonate formulations. Against *E. coli*, no formulation and not the control produced any inhibition zone, indicating that neither the biogenic carbonate nor the fruit fraction at these concentrations is active against this organism.

Table 3. Inhibition zone diameters against *S. mutans* and *E. coli*.

Formulation	S. mutans (mm)	Interpretation	E. coli (mm)
F5 (5% shell)	16 ± 0.0	Strong	No inhibition
F10 (10% shell)	10 ± 0.0	Moderate	No inhibition
F15 (15% shell)	10 ± 0.0	Moderate	No inhibition
F20 (20% shell)	8 ± 0.0	Moderate	No inhibition
Positive control	8 ± 0.0	Moderate	No inhibition

Kruskal–Wallis $p < 0.001$ for S. mutans. Interpretation after Davis and Stout: < 5 mm weak, 5–10 mm moderate, > 10–20 mm strong. Spearman ρ (shell concentration vs zone diameter) = −0.949. The zero standard deviations require clarification (see Section 2.10).

3.3. Enamel surface microhardness

Every formulation raised enamel microhardness substantially above the demineralized baseline of 132.2 HV, and differences between formulations were significant at every immersion time, with large effect sizes throughout: 90 min $F(4,20) = 113.91$, $p < 0.001$, $\eta^2 = 0.96$; 180 min $F(4,20) = 28.63$, $p < 0.001$, $\eta^2 = 0.85$; 270 min $F(4,20) = 11.49$, $p < 0.001$, $\eta^2 = 0.70$; and 360 min $F(4,20) = 95.41$, $p < 0.001$, $\eta^2 = 0.95$ (Table 4). Group assignment thus accounted for 70–96% of the variance in hardness at each time point.

Table 4. Enamel surface microhardness (HV, mean ± SD, n = 5 per cell) by formulation and immersion time.

Formulation	90 min	180 min	270 min	360 min
F5 (5% shell)	303.70 ± 19.11 ^{a}	341.83 ± 21.78 ^{a}	330.17 ± 10.53 ^{a}	233.17 ± 24.87 ^{b}
F10 (10% shell)	224.50 ± 19.14 ^{b}	237.50 ± 23.70 ^{b}	258.50 ± 3.78 ^{b}	341.50 ± 13.62 ^{a}
F15 (15% shell)	224.50 ± 10.23 ^{b}	288.50 ± 31.74 ^{c}	306.50 ± 7.74 ^{b}	165.00 ± 16.84 ^{c}
F20 (20% shell)	132.67 ± 6.62 ^{c}	229.50 ± 13.52 ^{b}	286.83 ± 35.59 ^{b}	331.67 ± 19.62 ^{a}
Positive control	323.00 ± 19.45 ^{a}	332.00 ± 11.30 ^{a}	269.50 ± 18.63 ^{b}	146.83 ± 26.24 ^{c}
ANOVA $F(4,20)$	113.91	28.63	11.49	95.41
p	< 0.001	< 0.001	< 0.001	< 0.001
η^2	0.96	0.85	0.70	0.95

Demineralized baseline 132.2 HV. Within a column, values sharing a superscript letter do not differ significantly on Bonferroni-corrected pairwise comparison ($\alpha = 0.05$). Levene test $p = 0.224$ (90 min) and 0.159 (180 min), confirming homogeneity of variance.

3.3.1. Concentration governs the rate, not the magnitude, of remineralization

The most consequential finding of this study emerges when the four time points are read together rather than separately. Peak hardness was broadly similar across all four experimental formulations, ranging only from 306.50 to 341.83 HV — gains of 132% to 159% over the demineralized baseline — and the control peaked at 332.00 HV within the same band. What differed profoundly was *when* each formulation reached its peak: 180 min for F5, 270 min for F15, and 360 min for both F10 and F20, with F10 and F20 still rising when observation ended (Table 5). The same pattern is visible at the earliest time point, where hardness was

inversely and almost perfectly related to shell concentration: 303.70 HV at 5%, 224.50 HV at both 10 and 15%, and 132.67 HV at 20% (Pearson $r = -0.947$, $p = 0.053$). The 20% formulation at 90 min was statistically indistinguishable from the untreated demineralized baseline of 132.2 HV, meaning that at the highest loading essentially no remineralization had occurred in the first 90 minutes. By contrast, the initial rate of hardness gain calculated from 90 min to each formulation's own peak was *highest* in the most concentrated formulations (+0.737 HV/min for F20 against +0.424 HV/min for F5), confirming that the high-loading pastes were not inert but simply delayed: once mineral transfer began, it proceeded rapidly. Concentration therefore modulates the kinetics of remineralization rather than its capacity. This distinction has direct formulation consequences, because a dentifrice is retained on the tooth for minutes, not hours; a formulation whose mineral gain is deferred beyond the clinically realistic contact window delivers that gain only in an experimental system that sustains contact artificially.

Table 5. Derived remineralization kinetics by formulation.

Formulation	Peak HV	Time to peak (min)	Gain over baseline	Post-peak loss by 360 min	AUC_{90-360} (HV·min)	Mean sustained elevation (HV)
F5 (5%)	341.83	180	+209.6 (+158.6%)	108.7 HV (31.8%)	48,945	+181.3
F10 (10%)	341.50	360	+209.3 (+158.3%)	Still rising	34,416	+127.5
F15 (15%)	306.50	270	+174.3 (+131.8%)	141.5 HV (46.2%)	35,384	+131.1
F20 (20%)	331.67	360	+199.5 (+150.9%)	Still rising	31,671	+117.3
Positive control	332.00	180	+199.8 (+151.1%)	185.2 HV (55.8%)	39,583	+146.6

Derived from group means relative to the demineralized baseline of 132.2 HV. AUC computed by the trapezoidal rule over 90–360 min; mean sustained elevation is the AUC divided by the 270 min observation interval.

3.3.2. The 5% formulation matches the control at peak and exceeds it thereafter

At 90 and 180 min the positive control and F5 behaved almost identically, both peaking at 180 min (332.00 and 341.83 HV respectively) with no significant difference between them ($p = 0.45$), while F10, F15 and F20 all remained significantly below both ($p < 0.001$ to $p = 0.019$). At these early time points, in other words, only the 5% biogenic formulation was equivalent to the marketed sodium fluoride/potassium nitrate product. After 180 min the two curves diverge sharply. The control lost mineral rapidly, falling to 269.50 HV at 270 min and 146.83 HV at 360 min — a loss of 185.2 HV, or 55.8% of its peak, at a mean rate of −1.029 HV/min, returning the enamel to within 15 HV of its demineralized starting point. F5 declined far more slowly, holding 330.17 HV at 270 min and falling to 233.17 HV at 360 min, a loss of 108.7 HV or 31.8% of peak at −0.604 HV/min. The difference between the two groups was significant from 270 min onward ($p < 0.001$), and the effect size in favour of F5 was large and growing (Cohen's $d = +4.01$ at 270 min, +3.38 at 360 min). Integrating hardness over the full observation period quantifies the practical consequence of this divergence. F5 delivered the largest area under the hardness–time curve of any group, 48,945 HV·min, equivalent to sustaining an elevation of +181.3 HV above baseline continuously across the 270 min interval, against 39,583 HV·min and +146.6 HV for the control — an advantage of 24% in integrated mineral gain. The three higher-loading formulations all fell below the control on this measure (31,671–35,384 HV·min), their late peaks arriving too near the end of observation to compensate for their slow starts.

Artificial salivary calcium

Calcium concentration in the artificial saliva declined over the immersion period in every group, consistent with net transfer of calcium from the medium to the enamel surface and thus with active remineralization in all five groups (Table 6). Declines from the 90 min value to each group's nadir ranged from 0.48 to 1.19 mg/dL, corresponding to 5.9–14.3% of the starting concentration. The control showed the largest single depletion (−1.19 mg/dL, −14.3%, reaching its nadir at 270 min), and F5 the largest depletion among the experimental formulations relative to its own starting point (−0.79 mg/dL, −9.6%, at 180 min) — in both cases coinciding with the time of peak hardness in the same group, an internal consistency that supports the validity of both measures. Between-group differences were not significant on the pooled analysis ($p = 0.644$), and per-time-point analysis found a significant difference only at 270 min, $F(4,20) = 3.67$, $p = 0.021$, $\eta^2 = 0.42$; at 90, 180 and 360 min the groups did not differ ($p = 0.769$, 0.074 and 0.426 respectively). Salivary calcium was therefore a considerably less discriminating outcome than microhardness in

this design, a point taken up in the Discussion.

Table 6. Artificial salivary calcium concentration (mg/dL, mean ± SD, n = 5 per cell).

Formulation	90 min	180 min	270 min	360 min	Δ to nadir
F5 (5%)	8.19 ± 0.58	7.40 ± 0.34	7.63 ± 0.37	7.63 ± 0.40	−0.79 (−9.6%)
F10 (10%)	8.07 ± 0.54	7.94 ± 0.23	7.81 ± 0.27	7.59 ± 0.37	−0.48 (−5.9%)
F15 (15%)	8.55 ± 0.74	8.02 ± 0.39	7.79 ± 0.25	7.73 ± 0.31	−0.82 (−9.6%)
F20 (20%)	8.61 ± 0.73	8.36 ± 1.04	8.16 ± 0.56	7.86 ± 0.12	−0.75 (−8.7%)
Positive control	8.35 ± 1.10	7.54 ± 0.28	7.16 ± 0.56	7.89 ± 0.19	−1.19 (−14.3%)
ANOVA p	0.769	0.074	0.021	0.426	—

Levene test p = 0.811 (pooled), confirming homogeneity of variance. Pooled one-way ANOVA across all groups and times, p = 0.644. A fall in medium calcium indicates net uptake by enamel**.**

Surface ultrastructure

Scanning electron microscopy at 3000× after 180 min immersion showed deposited material covering the enamel surface in all groups, but with a clear difference in the continuity and uniformity of that coverage. The control produced the smoothest and most evenly distributed layer, consistent with its nano-scale particle size. Among the experimental formulations, which used shell powder sieved to 200 mesh, coverage was visibly coarser, and became progressively less uniform as shell concentration and hence paste viscosity rose. F5 produced the most even and complete surface coverage of the four experimental pastes, closely approaching the control in appearance, whereas the higher-loading formulations showed patchier deposition with incomplete coverage of the enamel surface. These observations correspond directly to the hardness data, in which F5 alone matched the control at 180 min.

4. DISCUSSION

Antibacterial activity of the dentifrice formulations was evaluated against *Streptococcus mutans* and *Escherichia coli* using the Kirby–Bauer disc diffusion method. Sterile paper discs were saturated with suspensions of each dentifrice formulation prepared in sterile *aqua destillata* and allowed to equilibrate for 30 min before application. For bacterial inoculation, a sterile cotton swab was immersed in the prepared bacterial suspension, excess inoculum was removed by pressing the swab against the inner wall of the tube, and the inoculum was uniformly distributed over the surface of Mueller–Hinton agar by streaking in three directions to obtain a confluent bacterial lawn. The inoculated plates were allowed to stand for approximately 5 min before the impregnated discs, together with a blank disc, were placed aseptically on the agar surface using sterile forceps and gently pressed to ensure complete contact. The plates were incubated at 35–37 °C for 24 h, after which the diameter of the clear inhibition zone surrounding each disc was measured in millimetres using a vernier caliper. The same procedure was applied to both test microorganisms. Antibacterial activity was interpreted according to the Davis and Stout classification, with inhibition zones <5 mm considered weak, 5–10 mm considered moderate, and >10–20 mm considered strong. Calcium concentration in the artificial saliva was determined following each designated immersion period to characterize mineral exchange between the treated enamel surface and the surrounding medium. Changes in calcium concentration relative to the initial concentration were interpreted as an indicator of the direction of mineral flux, whereby a reduction in calcium concentration represented net calcium uptake by the enamel surface and was considered consistent with active remineralization, whereas an increase represented net calcium release and was interpreted as evidence of mineral dissolution or demineralization[34,35]. Surface microhardness was assessed as the principal indicator of enamel mineral recovery using a digital micro-Vickers hardness tester. Following the assigned dentifrice treatment and immersion period, specimens were embedded in a resin–catalyst matrix within a mould, with the enamel surface designated for measurement maintained free of embedding material. After polymerization, the specimens were removed from the mould, residual material was carefully eliminated, and each specimen was positioned securely on the testing stage. Vickers indentations were produced under standardized loading conditions, and the two perpendicular diagonals of each indentation were measured using the optical system of the hardness tester. The mean diagonal length was then used to calculate the Vickers hardness value, with higher hardness values interpreted as greater resistance of the enamel surface to indentation and, consequently, greater recovery following demineralization. To provide complementary morphological evidence of mineral deposition, representative

enamel specimens from each experimental group were examined using scanning electron microscopy after 180 min of immersion. SEM analysis was performed at 3000× magnification to characterize the surface morphology, distribution, continuity, and apparent coverage of deposited material on the enamel surface. The independent variable was dentifrice formulation, consisting of four experimental formulations containing *P. canaliculata* shell powder at 5%, 10%, 15%, and 20% (F5, F10, F15, and F20), together with a commercially available sodium fluoride/potassium nitrate dentifrice as the positive control. The primary dependent variable was enamel surface microhardness, reflecting the extent of mineral recovery following the demineralization challenge, while artificial salivary calcium concentration and bacterial inhibition-zone diameter were considered complementary biochemical and microbiological outcomes[36–38]. Immersion time (90, 180, 270, and 360 min) was incorporated as a temporal factor to characterize the kinetics of remineralization and determine whether the effect of formulation varied according to the duration of exposure. This time-resolved approach was intended to distinguish differences in the magnitude of mineral recovery from differences in the rate at which remineralization occurred and the persistence of the resulting mineral gain.

5. CONCLUSION

This study demonstrates that dentifrices containing 5–20% (w/w) golden apple snail (*Pomacea canaliculata* L.) shell powder combined with a fixed fraction of *Melastoma malabathricum* L. fruit produced homogeneous, pH-neutral formulations free of *Escherichia coli* and compliant with Indonesian National Standard SNI 12-3524-1995. All formulations substantially increased the surface microhardness of demineralized human enamel relative to the baseline value of 132.2 HV, with peak hardness ranging from 306.50 to 341.83 HV, corresponding to a 132–159% increase. Importantly, shell-powder concentration influenced the kinetics and durability of remineralization rather than the ultimate remineralization capacity. The 5% formulation reached peak hardness at 180 min, whereas the 15% formulation peaked at 270 min and the 10% and 20% formulations continued to increase through 360 min; moreover, hardness at 90 min was strongly and inversely associated with shell concentration ($r = -0.947$), and the 20% formulation produced no measurable hardening during the initial 90-min period. Among the experimental formulations, F5 demonstrated the most favorable overall performance, achieving peak hardness comparable to the commercial sodium fluoride/potassium nitrate control (341.83 vs. 332.00 HV; $p = 0.45$) while exhibiting superior mineral retention over time, with a 31.8% reduction from peak hardness at 360 min compared with 55.8% for the control and a 24% greater integrated hardness gain (48,945 vs. 39,583 HV·min). F5 also exhibited the strongest antibacterial activity against *Streptococcus mutans*, with an inhibition zone twice that of the commercial control, whereas antibacterial activity declined progressively with increasing shell-powder concentration despite the constant *M. malabathricum* fraction, suggesting that increasing matrix density may have limited the release or diffusion of bioactive phytochemicals. Collectively, these findings identify the 5% *P. canaliculata* shell formulation as the most promising candidate, combining remineralization capacity comparable to a marketed desensitizing dentifrice with greater mineral retention and stronger *S. mutans* inhibition. The findings further support the potential valorization of *P. canaliculata* shell waste as a locally sourced dental biomaterial within a circular-bioeconomy framework. Nevertheless, the translational significance of these findings remains preliminary because the study was conducted *in vitro*; comprehensive physicochemical and elemental characterization of the shell-derived material, quantitative phytochemical profiling, rheological assessment, toxicological evaluation, and well-controlled clinical studies are required before its safety, reproducibility, and clinical effectiveness can be established.

DATA AVAILABILITY

The data presented in this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest and no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

The authors thank the Centre for Research and Community Service, Poltekkes Kemenkes Palembang, for institutional support; the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada, for access to microhardness testing and scanning electron microscopy; and the Medical Laboratory Technology and Pharmacy Laboratories, Poltekkes Kemenkes Palembang, for formulation and microbiological facilities.

FUNDING

This research was funded by [insert funding body and grant number], Poltekkes Kemenkes Palembang, fiscal year 2023.

AUTHOR CONTRIBUTIONS

Conceptualization: N.A.H., M.; Methodology: N.A.H., I., M.; Investigation and data curation: N.A.H., I., M.; Formal analysis: I., M.; Writing — original draft: N.A.H.; Writing — review and editing: I., M.; Supervision and project administration: N.A.H.; Funding acquisition: N.A.H. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1] Rafeek R, Naidu R, Peters T, Paul G, Tripathi V. Prevalence and Factors Associated with Dentin Hypersensitivity among Adult Patients Attending a University Dental Clinic in Trinidad, West Indies. A Cross-Sectional Study. Oral Health Prev Dent 2020;18. https://doi.org/10.3290/J.OHPD.B871073.

[2] Wang Y, Que K, Lin L, Hu D, Li X. The prevalence of dentine hypersensitivity in the general population in China. J Oral Rehabil 2012;39:812–20. https://doi.org/10.1111/J.1365-2842.2012.02334.X.

[3] Favaro Zeola L, Soares PV, Cunha-Cruz J. Prevalence of dentin hypersensitivity: Systematic review and meta-analysis. J Dent 2019;81:1–6. https://doi.org/10.1016/j.jdent.2018.12.015.

[4] Grover V, Kumar A, Jain A, Chatterjee A, Grover H, Pandit N, et al. ISP Good Clinical Practice Recommendations for the management of Dentin Hypersensitivity. J Indian Soc Periodontol 2022;26:307. https://doi.org/10.4103/JISP.JISP_233_22.

[5] Lazin MAM, Zainon WNW, Humayun A, Madawana AM, Hassan A, Zhang Y, et al. Factors Influencing Tooth Sensitivity: Insights From the Hospital Universiti Sains Malaysia Using Bootstrap-Enhanced Ordinal Regression. Cureus 2024;16:e64641. https://doi.org/10.7759/CUREUS.64641.

[6] Sharan A, Pawar B, Bagde H, Chawla TK, Dhan AV, Shyamsukha B, et al. Comparative Evaluation of Dentin Hypersensitivity Reduction Over One Month after a Single Topical Application of Three Different Materials: A Prospective Experimental Study. J Pharm Bioallied Sci 2024;16:S3405. https://doi.org/10.4103/JPBS.JPBS_848_24.

[7] Enax J, Fandrich P, Schulze zur Wiesche E, Epple M. The Remineralization of Enamel from Saliva: A Chemical Perspective. Dent J (Basel) 2024;12:339. https://doi.org/10.3390/DJ12110339.

[8] Enax J, Fandrich P, Schulze zur Wiesche E, Epple M. The Remineralization of Enamel from Saliva: A Chemical Perspective. Dent J (Basel) 2024;12. https://doi.org/10.3390/DJ12110339.

[9] Hassan NM, Jafar ZJ, Abdul Latif MH. Nano-hydroxyapatite preparation for the remineralization of primary tooth enamel surface subjected to liquid medication: An observational study. Health Sci Rep 2023;6:e1188. https://doi.org/10.1002/HSR2.1188.

[10] Shellis RP, Barbour ME, Parker DM, Addy M, Lussi A. Effects of Calcium and Phosphate on Dissolution of Enamel, Dentin and Hydroxyapatite in Citric Acid. Swiss Dent J 2023;133:432–8. https://doi.org/10.61872/SDJ-2023-07-08-02.

[11] Wild YK, Heyman MB, Vittinghoff E, Dalal DH, Wojcicki JM, Clark AL, et al. Gastroesophageal Reflux is Not Associated with Dental Erosion in Children. Gastroenterology 2011;141:1605. https://doi.org/10.1053/J.GASTRO.2011.07.041.

[12] AlHelal F, AlHomaizi Z, AlOmair M, Yousef LW, Qudeimat MA. Quantitative and spectroscopic assessment of early-stage enamel erosion induced by popular acidic beverages. BMC Oral Health 2025;25:1598. https://doi.org/10.1186/S12903-025-07009-8.

[13] Kumar N, Amin F, Dahri WM, Khan S, Zaidi H, Rahman S, et al. Impact of acidic beverages on composition and surface characteristics of human teeth: scanning electron microscopic, stereomicroscopic and energy dispersive x-ray analyses. BMC Oral Health 2024;24:837. https://doi.org/10.1186/S12903-024-04491-4.

[14] Qi YP, Li N, Niu LN, Primus CM, Ling JQ, Pashley DH, et al. Remineralization of artificial dentinal caries lesions by biomimetically modified Mineral Trioxide Aggregate. Acta Biomater 2011;8:836.

https://doi.org/10.1016/J.ACTBIO.2011.10.033.

[15] Yao L, Li Y, Fu D, Wang Y, Hua C, Zou L, et al. The damage and remineralization strategies of dental hard tissues following radiotherapy. BMC Oral Health 2024;24:805. https://doi.org/10.1186/S12903-024-04561-7.

[16] Saffarpour M, Mohammadi M, Tahriri M, Zakerzadeh A. Efficacy of Modified Bioactive Glass for Dentin Remineralization and Obstruction of Dentinal Tubules. J Dent (Tehran) 2017;14:212.

[17] Enax J, Fandrich P, Schulze zur Wiesche E, Epple M. The Remineralization of Enamel from Saliva: A Chemical Perspective. Dent J (Basel) 2024;12:339. https://doi.org/10.3390/DJ12110339.

[18] Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. F1000Res 2021;9:171. https://doi.org/10.12688/F1000RESEARCH.22499.3.

[19] Hou JY, Fu XJ, Ren X. Mollusk shells as marine bioactive materials: Composition, bioactivities, and prospects for food and health applications. IScience 2026;29:114748. https://doi.org/10.1016/J.ISCI.2026.114748.

[20] Mititelu M, Stanciu G, Drăgănescu D, Ioniţă AC, Neacşu SM, Dinu M, et al. Mussel Shells, a Valuable Calcium Resource for the Pharmaceutical Industry. Mar Drugs 2021;20:25. https://doi.org/10.3390/MD20010025.

[21] Piras S, Salathia S, Guzzini A, Zovi A, Jackson S, Smirnov A, et al. Biomimetic Use of Food-Waste Sources of Calcium Carbonate and Phosphate for Sustainable Materials—A Review. Materials 2024;17:843. https://doi.org/10.3390/MA17040843.

[22] De Mori A, Alasa UJ, Mühlhölzl A, Blunn G. Slipper Limpet (Crepidula fornicata) Shells Support In Vitro Osteogenesis of Human Adipose-Derived Stem Cells. Mar Drugs 2023;21:248. https://doi.org/10.3390/MD21040248.

[23] Srichanachaichok W, Pissuwan D. Micro/Nano Structural Investigation and Characterization of Mussel Shell Waste in Thailand as a Feasible Bioresource of CaO. Materials 2023, Vol 16, 2023;16. https://doi.org/10.3390/MA16020805.

[24] Stapane L, Le Roy N, Ezagal J, Rodriguez-Navarro AB, Labas V, Combes-Soia L, et al. Avian eggshell formation reveals a new paradigm for vertebrate mineralization via vesicular amorphous calcium carbonate. J Biol Chem 2020;295:15853. https://doi.org/10.1074/JBC.RA120.014542.

[25] Boraldi F, Lofaro FD, Bergamini G, Ferrari A, Malagoli D. Pomacea canaliculata ampullar proteome: A nematode-based bio-pesticide induces changes in metabolic and stress-related pathways. Biology (Basel) 2021;10:1049. https://doi.org/10.3390/BIOLOGY10101049/S1.

[26] Guo J, Zhang S, Zeng J, Chen Y, Guo Y, Liu J, et al. Molluscicidal activity of Nicotiana tabacum extracts on the invasive snail Pomacea canaliculata. Sci Rep 2023;13. https://doi.org/10.1038/S41598-023-38141-6.

[27] Nurhadis S, Omar C, Abdullah JO, Khairoji KA, Chin SC, Hamid M. Potentials of *Melastoma malabathricum* Linn. Flower and Fruit Extracts as Antimicrobial Infusions. Am J Plant Sci 2012;2012:1127–34. https://doi.org/10.4236/AJPS.2012.38136.

[28] Wong KC, Hag Ali DM, Boey PL. Chemical constituents and antibacterial activity of Melastoma malabathricum L. Nat Prod Res 2012;26:609–18. https://doi.org/10.1080/14786419.2010.538395.

[29] El-Saadony MT, Saad AM, Mohammed DM, Alkafaas SS, Abd El-Mageed TA, Fahmy MA, et al. Plant bioactive compounds: extraction, biological activities, immunological, nutritional aspects, food application, and human health benefits—A comprehensive review. Front Nutr 2025;12:1659743. https://doi.org/10.3389/FNUT.2025.1659743.

[30] Parvatiyar A, Sheth JN. Confronting the deep problem of consumption: Why individual responsibility for mindful consumption matters. Journal of Consumer Affairs 2023;57:785–820. https://doi.org/10.1111/JOCA.12534.

[31] Elisha IL, Botha FS, McGaw LJ, Eloff JN. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC Complement Altern Med 2017;17:133. https://doi.org/10.1186/S12906-017-1645-Z.

[32] Nyalo PO, Omwenga GI, Ngugi MP. Antibacterial properties and GC-MS analysis of ethyl acetate extracts of Xerophyta spekei (Baker) and Grewia tembensis (Fresen). Heliyon 2023;9:e14461.

https://doi.org/10.1016/J.HELIYON.2023.E14461.

[33] Ngamsurach P, Praipipat P. Antibacterial activities against Staphylococcus aureus and Escherichia coli of extracted Piper betle leaf materials by disc diffusion assay and batch experiments. RSC Adv 2022;12:26435. https://doi.org/10.1039/D2RA04611C.

[34] Klimuszko E, Orywal K, Sierpinska T, Sidun J, Golebiewska M. Evaluation of calcium and magnesium contents in tooth enamel without any pathological changes: in vitro preliminary study. Odontology 2018;106:369. https://doi.org/10.1007/S10266-018-0353-6.

[35] Fischer A, Wiechuła D, Przybyła-Misztela C. Changes of Concentrations of Elements in Deciduous Teeth with Age. Biol Trace Elem Res 2013;154:427. https://doi.org/10.1007/S12011-013-9744-2.

[36] Enax J, Fandrich P, Schulze zur Wiesche E, Epple M. The Remineralization of Enamel from Saliva: A Chemical Perspective. Dentistry Journal 2024, Vol 12, 2024;12. https://doi.org/10.3390/DJ12110339.

[37] Ghanim Rahman R, Diab BS. Efficacy of different remineralization agents on microhardness and chemical composition of enamel white spot lesion. F1000Res 2024;13:505. https://doi.org/10.12688/F1000RESEARCH.149166.2.

[38] Ersen MC, Çelik ZC, Oztas M, Sahin M, Tagtekin D, Yanikoglu F. Impact of Demineralization Time on Enamel Microhardness Reduction and Lesion Depth: An In Vitro Study. Cureus 2025;17:e79441. https://doi.org/10.7759/CUREUS.79441.