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ORIGINAL PAPERS

ANTIMICROBIAL EFFECT OF TOOTHPASTES CONTAINING BIRCH EXTRACT AND NANO-HYDROXYAPATITES

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Abstract. *The study investigates the antimicrobial efficacy of toothpaste formulations incorporating nano-hydroxyapatite (nHAP) and birch extract. Toothpastes were evaluated for their ability to inhibit bacterial growth, with observations revealing varied sensitivities among bacterial species to different formulations. Notably, toothpastes containing both nHAP and zinc exhibited heightened antimicrobial activity, while the addition of birch extract introduced variability in inhibition reactions across species. Further analysis delineated differences in effectiveness between nHAP-only and nHAP-birch extract formulations, suggesting the role of secondary metabolites in enhancing antibacterial properties. The study highlights toothpaste P11 as the most effective inhibitor of bacterial growth, offering a balanced combination of remineralization and antibacterial properties, except for S. mutans strain. Future research directions include time-course tests to assess long-term efficacy and oral microbiome studies to understand broader effects on oral microflora, informing the development of more targeted oral care products.*

Keywords: antimicrobial effect, advanced hydroxyapatites, birch extract, toothpastes

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INTRODUCTION

Plant extracts derived from natural sources are garnering attention due to their biocompatibility [1], biodegradability [2], abundance [3], and therapeutic attributes [4]. However, in comparison to synthetic materials, biomaterials based on plant extracts encounter challenges such as batch-to-batch variability [5], potential allergenicity [6], and limited mechanical characteristics [7]. Consequently, the development of plant extract-based biomaterials for medical applications necessitates thorough characterization [8], standardization [9], and quality control [10].

Modern toothpaste formulations incorporate a diverse array of active ingredients capable of delivering comprehensive oral cleansing while safeguarding enamel integrity. The chemical composition of toothpaste must be meticulously tailored to ensure that its components do not compromise dental enamel and effectively fulfill their intended roles. Typically, a toothpaste's chemical composition aligns with its clinical applications, which encompass abrasive [11], antibacterial [12], anti-calculus [13], anti-inflammatory [14], desensitizing [15], remineralizing [16], whitening [17], and breath freshening properties [18].

Various antimicrobial agents have been developed to combat dental plaque formation facilitated by biofilms. These antimicrobial agents are categorized into three types based on their composition: inorganic, biological, and organic [19]. Inorganic compounds such as nano-calcium fluoride [20], silver nanoparticles (AgNPs) [21], and nano-hydroxyapatite (n-HAP) [22, 23] containing metal ions like zinc (Zn) [24], magnesium (Mg) [25], and strontium (Sr) [26] are commonly utilized in toothpaste formulations.

Plant secondary metabolites have emerged as promising organic antimicrobial agents due to their low toxicity, broad-spectrum antibacterial activity, diverse pharmacological properties, and cost-effectiveness [27-29]. Many plant extracts exhibit significant pharmacological effects attributed to their chemical constituents, including phenols, terpenoids, alkaloids, glycosylated compounds, organic acids, essential oils, vitamins, and sterols [30]. Products containing plant secondary metabolites are widely embraced for their perceived safety, health benefits, and eco-friendliness compared to synthetic chemical additives [31].

The formulation of toothpaste is straightforward and cost-effective, allowing for customization using a plethora of inorganic and organic ingredients. Birch extract, sourced from various parts of birch trees, contains bioactive compounds such as phenolic compounds, flavonoids, and lignans, which possess intriguing biological properties applicable to biomaterials [32, 33].

To enhance the efficacy of enamel recovery, researchers often combine antibacterial agents with diverse matrix materials to develop toothpastes with varied properties. Nanomaterials, renowned for their biocompatibility, are commonly employed as matrix materials. Investigations into the efficacy of nano-hydroxyapatite crystals [34, 35] in toothpaste formulations have demonstrated a significant increase

in the microhardness of human enamel post-treatment, along with notable tubule occlusion competence, hindering mineral disintegration [36].

Synthetic stoichiometric hydroxyapatite may undergo ionic substitutions such as Mg^{2+}, Na^{+}, and CO_3^{2-}, rendering it well-tolerated by living tissue [37, 38]. Moreover, HAP utilized in toothpastes or other medical applications can be substituted with physiological elements to enhance its bioactivity [39, 40].

This study explores four novel nanomaterials employed in toothpaste production, incorporating various substituted hydroxyapatites containing magnesium (Mg), zinc (Zn), strontium (Sr), and silicon (Si) as replacement components [16,38,41,42]. The rationale for integrating these substitution elements in our toothpaste formulations stems from their beneficial properties, including enhanced biomineralization, antibacterial activity, and remineralization potential [12,16,41]. Through the examination of morpho-structural aspects and surface quality, by using AFM, which is a versatile technique [43-46], and in vitro antibacterial activity [12], we aimed to assess the potential of these toothpastes in treating affected enamel and provide insights for further research in oral disease treatment.

The objective of this study is to optimize the chemical composition of toothpaste formulations with simultaneous remineralizing and antibacterial properties. To this end, we evaluated the efficacy of eleven toothpaste formulations comprising different combinations of pure and substituted nano-hydroxyapatite and birch extract. Given the existing gap in the development of multifunctional toothpastes capable of delivering both remineralizing and antibacterial agents, this study builds upon previous research by incorporating special modified multi-substituted hydroxyapatites (ms-HAPs) that enable structural and crystallinity alterations, nanoparticle shape and size modulation, alongside antibacterial birch extract.

MATERIALS AND METHODS

All reagents utilized in this study were of analytical grade (AR ≥99.7%) and were procured from Sigma-Aldrich. These reagents included: calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, >99%), magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$, 99%), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, >98%), strontium nitrate ($Sr(NO_3)_2$, 99.99%), sorbitol (≥98%), polyethylene glycol (PEG 400), nano-sized silicon dioxide powder (particle size: 10-20 nm, 99.5%), orthophosphoric acid (H_3PO_4, 85 wt. % in H_2O), xanthan gum from Chempur, diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, >99%) and ammonia solution (NH_4OH 25%), and tetraethyl orthosilicate (TEOS, $Si(OEt)_4$, 98%) from Thermo Fischer Scientific.

The formulation of four remineralizing toothpastes involved the utilization of distinct hydroxyapatites, encompassing simple, substituted, and multi-substituted variants denoted as HAP [with the theoretical formula: $Ca_{10}(PO_4)_6(OH)_2$], HAP-5%Zn [theoretical formula; $Ca_{9.22}Zn_{0.78}(PO_4)_6(OH)_2$], HAP-0.23%Mg-3.09%Zn-

2%Si-10%Sr [theoretical formula: $Ca_{8.19}Mg_{0.10}Zn_{0.5}Sr_{1.21}(PO_4)_{5.25}(SiO_4)_{0.75}(OH)_{1.25}$], HAP-2.5%Mg-2.9%Si-1.34%Zn [theoretical formula: $Ca_{8.80}Mg_{1.00}Zn_{0.20}(PO_4)_{5.00}(SiO_4)_{1.00}(OH)_{1.00}$], respectively. The synthesis of these hydroxyapatites was conducted utilizing a methodology established within our research laboratory as documented in prior studies [38,47,48].

A hydro-glycerin-alcoholic extract containing 96% volume ethyl alcohol derived from cereal sources, glycerin, and purified water, sourced from Plant Extract, Romania, was procured. This extract, derived from organically harvested Betula verrucosa sap, exhibited a concentration of 10%, with an ethyl alcohol content of 18% by volume. The previously synthesized multi-substituted nano-hydroxyapatites (ms-HAPs) were incorporated into the toothpaste formulations in accordance with the desired compositions.

The experimental design encompassed three groups denoting the independent variable, with 11 levels. Specifically, the P1-P4 group denoted the basic formula supplemented with varied compositions of nano-hydroxyapatite (nHAP); the P5-P8 group contained the basic formula along with nHAP and 1.3% birch extract; and the P9-P11 group comprised toothpaste variants devoid of nHAP but containing varying concentrations of birch extract, as follows: P9 0.25%, P10 – 0.70% and P11 1.3% birch extract. As remineralization agents, variants P1 and P5 featured HAP-5%Zn, variants 2 and 6 contained pure nHAP, variants 3 and 7 incorporated HAP-0.23%Mg-3.09%Zn-2%Si-10%Sr, while variants 4 and 8 included HAP-2.5%Mg-2.9%Si-1.34%Zn. The dependent variables of the experiment encompassed the remineralization capacity and antibacterial efficacy of the toothpastes. The experimental constants comprised temperature, working techniques, and the reagents employed.

The microorganisms examined in this study included *Streptococcus mutans* ATCC 25175, *Porphyromonas gingivalis* ATCC 33277, *Enterococcus faecalis* ATCC-29212, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923, sourced from the Microbiology Lab, Faculty of Biology and Geology, UBB, Cluj-Napoca. The antibacterial activity was evaluated using the Agar Disk Diffusion Test, a standard method for antimicrobial susceptibility testing, involving the measurement of inhibitory zone diameters [12, 49-51].

Bacterial cultures were prepared in Nutrient Broth and diluted to 1% (V/V) in culture medium. Portions of 500 µL from the diluted suspensions were spread onto Petri plates containing sterile swabs to cover the entire surface of the solid culture medium (Mueller-Hinton agar). For *S. mutans*, an overnight culture grown in Brain Heart Infusion (BHI) at 37 °C was mixed with 3.5 mL of soft Mueller-Hinton agar and evenly spread over the plates surrounding the discs. Round samples measuring 5 mm in diameter and 1 mm in thickness were directly applied to the culture media in the Petri plates. Following incubation at 37 °C for 48 hours, the inhibition zones were measured using an inhibition zone measuring scale. All experiments were conducted

in triplicate under aseptic conditions. After 48 hours of incubation at 37 °C, inhibition zones were measured for the tested bacterial strains.

Bacterial growth inhibition was statistically analyzed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) for Windows. Significant differences among groups consisting by toothpastes and bacterial strains were determined using One-Way ANOVA analysis followed by post-test Bonferroni's Multiple Comparison Test with a significance level set for p value between 0.01-0.001.

RESULTS AND DISCUSSION

The toothpaste samples were inoculated in Petri dishes containing 80 µL of cultivation medium. The majority of the bacterial species under investigation are indigenous to the oral cavity or inadvertently introduced therein, contributing to various pathological conditions. Distilled water served as the control sample.

Following the assessment of the toothpaste's antimicrobial activity, the ensuing observations can be delineated: each bacterial strain exhibited varying degrees of sensitivity to the different test samples (Fig 1).

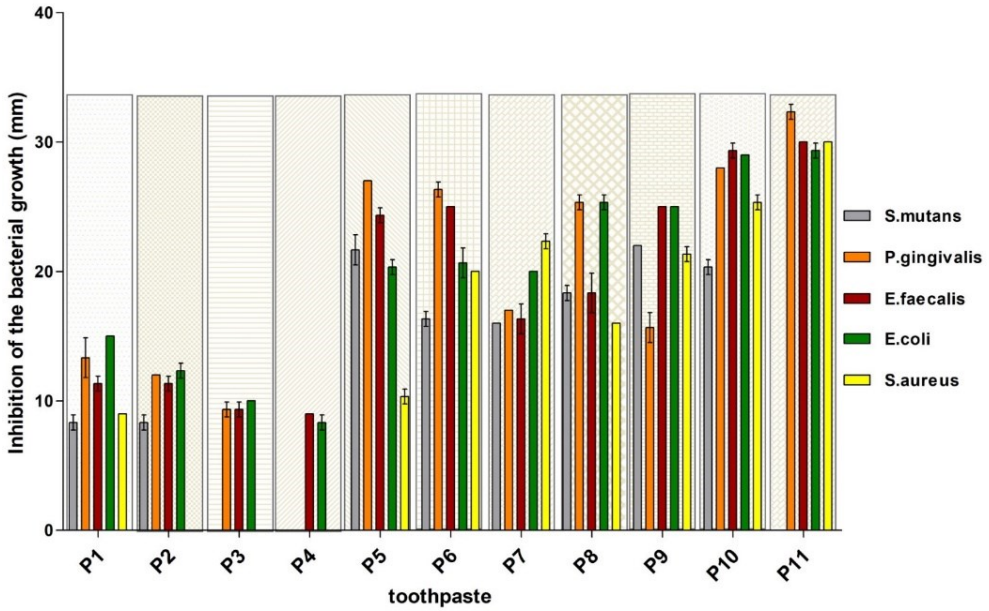

Fig.1 Effect of toothpastes (P1-P11) on bacterial strain inhibition

As a general observation when analyzing the data in Fig. 1 is the increased bacterial inhibition response by up to 2-3 folds of the toothpaste group P5-P8 (nHAP and birch extract) and the group P9-P11 (toothpastes containing different concentrations of birch extract. The bacterial strains of *Streptococcus mutans* and

Porphyromonas gingivalis, known to be the most involved in oral cavity pathology, proved to be among the most resistant, especially to the first group of P1-P4 toothpastes. *Streptococcus mutans* and *Porphyromonas gingivalis* are the main microorganisms implicated in caries formation and periodontitis, especially in immunodeficiency conditions. *S. mutans* grow in acidic conditions, becoming the main bacterium in the environment with permanently reduced pH, and is present in dental biofilms [52]. *Porphyromonas gingivalis* is a Gram-negative anaerobic, and asaccharolytic bacterium, that modulates host immune inflammatory responses and is able to infect gingival epithelial cells, periodontal ligament fibroblasts, and alveolar osteoblasts [53].

Upon scrutinizing the data within the P1-P4 group, comprising toothpastes solely containing nano-hydroxyapatite (nHAP), discernible antibacterial activity was noted, particularly in the P2 variant consisting of pure nHAP. Notably, within this group, toothpastes P1 and P2 demonstrated superior efficacy in impeding bacterial growth in the culture medium. The most significant inhibition across multiple bacterial species within this group was evident in experimental variant P1, encompassing nHAP-5%Zn. Moreover, it is noteworthy that toothpaste variants containing zinc (P1, P3, and P4) exhibited heightened antimicrobial activity, correlating generally with the concentration of this element, consistent with documented literature indicating zinc's antimicrobial properties [51].

In the P5-P8 group of toothpastes containing both nHAP and 1.3% birch extract, a considerable variability in bacterial inhibition reaction was observed depending on the species. Overall, toothpaste P5, comprising nHAP-Zn5%, elicited the most pronounced inhibition within the group across all bacterial species except *Staphylococcus aureus*. Particularly notable is variant P7, comprising HAP-0.23%Mg-3.09%Zn-2%Si-10%Sr, which significantly inhibited the growth of this species. In a previously published study of our team in which the ability of P1-P4 and P5-P8 toothpaste groups to restore the artificially demineralized enamel was evaluated by AFM measurements of mean arithmetic roughness (Ra), the effectiveness of first group was ranked in the following order: Ctrl = P2 = P3 > P4 > P1. P2 toothpaste, represented by small-sized HAP nanoparticles were able to restore the microstructure of enamel by an increased ability to penetrate the pores and cracks of damaged enamel. Similarly, a synergic action of substitute ions released from ms-HAP (P1, P3 and P4 and P5-P8 group) enhanced the remineralization process. Incorporation of birch extract in nHAP formulation of P5-P8 toothpastes seems to not exert an obvious influence on the enamel remineralization.

Upon comparison between the P1-P4 group containing only nHAP and the P5-P8 group with added birch extract, significant disparities in terms of bacterial growth inhibition were evident. This inhibition could be attributed to the secondary metabolites present in the birch extract. A noteworthy instance is *Staphylococcus aureus,* which exhibited resistance to toothpaste variants P2-P4 but demonstrated

growth inhibition in the presence of birch extract. Similarly, *Streptococcus mutans* exhibited resistance to variants P3-P4 but was inhibited by the addition of birch extract.

In the subsequent experimental group (P9-P11) where nHAP was removed and the concentration of birch extract in the toothpaste was variable, significantly greater inhibition of bacterial growth was observed compared to the previous variants, particularly at 1.3% extract concentrations.

The efficacy of the toothpaste varied depending on the bacterial species, with toothpaste P11, comprising the basic formulation with 1.3% birch extract, emerging as one of the most effective inhibitors of growth across all studied bacterial species, excepting *Streptococus mutans.* In natural conditions *S. mutans* survives at very low pH producing enzymes that induce the formation of dental plaque with the accumulation of metabolic acids [54].

Toothpaste P10 was frequently cited as highly effective, although its efficacy varied by species. Toothpastes P5 and P6 demonstrated effectiveness against several species, notably *Porphyromonas gingivalis*. Conversely, toothpastes P1–P4, while inhibitory in some instances, generally exhibited lower effectiveness compared to others.

According to the data, toothpaste P11 exhibited the highest effectiveness against bacterial strains, ranking first in three out of the five bacterial species studied. It consistently outperformed other bacterial inhibitors across a wide range of bacterial species, establishing it as the preferred option for inhibiting bacterial growth. The decrease in the antimicrobial action of P9-P11 toothpaste on *S. mutans* strain as the concentration of birch extract increases can be attributed to the simultaneous increase in the carbohydrate content that is used as a source of carbon and energy. *S. mutans* is characterized by metabolic versatility, particularly in its capacity to use various carbon sources. This plasticity is crucial for its survival and pathogenicity. Understanding the metabolic processes of *S. mutans* is critical for understanding its virulence mechanisms and potential interventions in dental caries [55]. The expression of genes and phenotypic properties of *S. mutans* undergo profound changes in response to carbohydrate availability. ManL, a component likely involved in carbohydrate metabolism, appears to play a significant role in modulating key virulence attributes based on carbohydrate availability [56].

Carbohydrates serve as energy sources for dental plaque bacteria, contributing to their virulence and cariogenic potential. Notably, *S. mutans* can produce extracellular polysaccharides (EPS) from various carbohydrates, including sucrose, fructose, and glucose. EPS production, particularly from sucrose, is a significant factor in the cariogenicity of *S. mutans* [57].

The carbohydrate composition of birch sap, containing high quantities of fructose and glucose, suggests its potential impact on the growth and virulence of *S. mutans*.

This highlights the importance of considering environmental carbohydrate sources in dental caries risk assessment [58].

Statistical analysis highlighted highly significant differences for P1 versus all toothpastes and for all bacterial strains excepting S. *mutans* (P2) and *E. faecalis* (P2) (Table 1). Significant differences were also found in P2, with the exception of *S. aureus* in response to P3 and P4. The effects of P3 toothpaste were significantly lower than all other toothpastes excepting P4 on *S. mutans, E. faecalis* and *S. aureus,* as well as P11 on *S. mutans.* P4 had the lowest antimicrobial activity compared to all other toothpastes except P11 on *S. mutans.*

Table 1. Statistical analysis GraphPad Prism 5: "Two-way ANOVA" with "Bonferroni posttests" comparing the toothpastes, using the data from Fig.1; P1 versus P2-P11, P2 versus P3-P11, P3 versus P4-P11, and P4 versus P5-P11, as function of bacterial strains.

	P1 vs P2	**P1 vs P3**	**P1 vs P4**	**P1 vs P5**	**P1 vs P6**	**P1 vs P7**	**P1 vs P8**	**P1 vs P9**	**P1 vs P10**	**P1 vs P11**
S. mutans	ns	***	***	***	***	***	***	***	***	***
P. gingivalis	*	***	***	***	***	***	***	***	***	***
E. faecalis	ns	***	***	***	***	***	***	***	***	***
E. coli	***	***	***	***	***	***	***	***	***	***
S. aureus	***	***	***	*	***	***	***	***	***	***
		P2 vs P3	**P2 vs P4**	**P2 vs P5**	**P2 vs P6**	**P2 vs P7**	**P2 vs P8**	**P2 vs P9**	**P2 vs P10**	**P2 vs P11**
S. mutans		***	***	***	***	***	***	***	***	***
P. gingivalis		***	***	***	***	***	***	***	***	***
E. faecalis		***	***	***	***	***	***	***	***	***
E. coli		***	***	***	***	***	***	***	***	***
S. aureus		ns	ns	***	***	***	***	***	***	***
			P3 vs P4	**P3 vs P5**	**P3 vs P6**	**P3 vs P7**	**P3 vs P8**	**P3 vs P9**	**P3 vs P10**	**P3 vs P11**
S. mutans			ns	***	***	***	***	***	***	ns
P. gingivalis			***	***	***	***	***	***	***	***
E. faecalis			ns	***	***	***	***	***	***	***
E. coli			**	***	***	***	***	***	***	***
S. aureus			ns	***	***	***	***	***	***	***
				P4 vs P5	**P4 vs P6**	**P4 vs P7**	**P4 vs P8**	**P4 vs P9**	**P4 vs P10**	**P4 vs P11**
S. mutans				***	***	***	***	***	***	ns
P. gingivalis				***	***	***	***	***	***	***
E. faecalis				***	***	***	***	***	***	***
E. coli				***	***	***	***	***	***	***
S. aureus				***	***	***	***	***	***	***

Table 2. Statistical analysis GraphPad Prism 5: "Two-way ANOVA" with "Bonferroni posttests" comparing the toothpastes, using the data from Fig.1; P5 versus P6-P11, P6 vs P7-P11, P7 vs P8-P11, P8 vs P9-P11, P9 vs P10-P11, and P10 vs P11, as function of bacterial strains.

	P5 vs P6	**P5 vs P7**	**P5 vs P8**	**P5 vs P9**	**P5 vs P10**	**P5 vs P11**
S. mutans	***	***	***	ns	*	***
P. gingivalis	ns	***	**	***	ns	***
E. faecalis	ns	***	***	ns	***	***
E. coli	ns	ns	***	***	***	***
S. aureus	***	***	***	***	***	***
		P6 vs P7	**P6 vs P8**	**P6 vs P9**	**P6 vs P10**	**P6 vs P11**
S. mutans		ns	***	***	***	***
P. gingivalis		***	ns	***	**	***
E. faecalis		***	***	ns	***	***
E. coli		ns	***	***	***	***
S. aureus		***	***	*	***	***
			P7 vs P8	**P7 vs P9**	**P7 vs P10**	**P7 vs P11**
S. mutans			***	***	***	***
P. gingivalis			***	*	***	***
E. faecalis			***	***	***	***
E. coli			***	***	***	***
S. aureus			***	ns	***	***
				P8 vs P9	**P8 vs P10**	**P8 vs P11**
S. mutans				***	***	***
P. gingivalis				***	***	***
E. faecalis				***	***	***
E. coli				ns	***	***
S. aureus				***	***	***
					P9 vs P10	**P9 vs P11**
S. mutans					**	***
P. gingivalis					***	***
E. faecalis					***	***
E. coli					***	***
S. aureus					***	***
						P10 vs **P11**
S. mutans						***
P. gingivalis						***
E. faecalis						ns
E. coli						ns
S. aureus						***

Table 2 shows the comparative analysis of the nHAP-birch extract toothpaste group (P5-P8) and the P9-11 group with different concentrations of birch extract. Very similar high values of bacterial growth inhibition were observed in the P5-P8 group, without statistical differences between P5vs.P6 (*P.gingivalis, E.faecalis, E.coli*), P5 vs. P7 and P6vs. P7 (*E.coli*) and P6vs. P8 (*P.gingivalis*). A very high inhibition response for *P.gingivalis* was observed in this group, with somewhat lower

inhibition values only at P7. The differences were significant between the two toothpaste groups for most pathogens. Toothpastes P9-P11 proved to be the most active in terms of antibacterial effect, with the highest bacterial growth inhibition values for P10 and P11 for *P.gingivalis*, *E.faecalis, E.coli* and *S.aureus.*

Considering antibacterial effectiveness, as expressed by the rankings: P11 > P10 > P9 > P5 > P6 > P8 > P7 > P1 > P2 > P3 > P4 toothpaste P11 emerges as a suitable choice for achieving good antibacterial activity, offering a balanced combination of remineralization and antibacterial properties. Even if the antibacterial effect is higher for the toothpaste group P9-P11, the formulation of the toothpaste group P5-P8 with close antibacterial effects will allow a simultaneous restoration of the damaged enamel associated with pathologies of the oral cavity. Another advantage of the combination of nHAP and birch extract is the delayed release of secondary active metabolites from the birch extract adsorbed on the nanoparticles of HAPs. This layer-by-layer structures of P5-P8 toothpaste group similar to micelles, enhance the molecular interactions at fluid interface with high biocompatibility and thus maintaining an adequate microbiome and preventing the occurrence of oral cavity diseases.

Among the bacterial species examined, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 exhibited the highest sensitivity, closely followed by *Porphyromonas gingivalis* ATCC 33277. *Streptococcus mutans* fell within the mid-range of bacterial species tested. *Staphylococcus aureus* ATCC 25923 demonstrated the lowest degree of growth inhibition under the applied treatment conditions.

For future research directions of this experiment, time-course tests could be conducted to ascertain the longevity of toothpaste efficacy in preventing bacterial growth over varying time intervals. Additionally, oral microbiome studies may provide insights into the broader effects of toothpaste variants on oral microflora. Exploring other oral health parameters such as anti-inflammatory or anti-adhesive properties of toothpastes and investigating the mechanisms underlying specific toothpaste variants' inhibition of bacterial growth could inform the development of targeted oral care products. By integrating these forthcoming discoveries, a deeper understanding of toothpaste effectiveness can be attained, thereby enhancing oral health outcomes and contributing to the development of more efficacious oral care products worldwide.

In summation, based on the results obtained under uniform conditions, the toothpaste samples can be ranked in terms of effectiveness in inhibiting growth for each bacterial species (from highest to lowest) as follows: P11 > P10 > P9 > P5 > P6 > P8 > P7 > P1 > P2 > P3 > P4 toothpaste.

CONCLUSIONS

Oral health maintenance constitutes a critical component of overall well-being and quality of life. Functional toothpaste is increasingly preferred for routine oral care

enhancement. The objective of this investigation is to formulate functional toothpaste formulations possessing dual properties of enamel remineralization and antibacterial efficacy, leveraging nano-hydroxyapatite and birch extract.

The surface morphology analysis of the examined samples depicts a homogeneous dispersion of globular nano-hydroxyapatite (nHAP) nanoparticles across enamel surfaces, exhibiting varied sizes corresponding to distinct treatments. Statistical scrutiny of surface roughness delineates notable alterations among experimental iterations, underscoring the efficacy of toothpaste interventions in enamel remineralization. Notably, the ranking order of effectiveness in this process elucidates those formulations characterized by smaller-sized nHAP nanoparticles, notably Ctrl = P2 = P3 > P4 > P1, demonstrate a capacity to infiltrate enamel pores and ameliorate microstructural damage. Furthermore, the release of substituted ions facilitated by the modified hydroxyapatite structure contributes to enamel restoration, enhancing its durability.

Regarding antimicrobial activity, the experiments unveil differential responses of various bacterial strains to diverse toothpaste compositions. Notably, even toothpastes exclusively containing nHAP exhibit antimicrobial efficacy, which is augmented by the inclusion of zinc as a substitution element in nHAP. Comparative assessment across different toothpaste variant groups underscores substantial disparities in bacterial growth inhibition, attributable to the presence of secondary metabolites inherent in birch extract.

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