

Antimicrobial Efficacy and Salivary Fluoride Retention Following Toothbrushing with Various Toothpastes: A Comparative Study

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Abstract Introduction: Antimicrobial agents are commonly used in chemotherapeutic treatments to promote oral health. This study aimed to assess the antimicrobial effectiveness of different toothpastes against oral pathogens and salivary fluoride concentration following tooth brushing. **Methods:** This study assesses the antimicrobial effects of various toothpastes against three key oral pathogens: *Streptococcus mutans*, *Lactobacilli*, and *Candida albicans* and Fluoride concentration in the saliva following toothbrushing with various toothpastes such as: Colgate Total 12 clean mint, Colgate Calcium-remin, Galaxy triple protection, ROCS Active Calcium and Splat professional Biocalcium. The antimicrobial properties were evaluated using the agar-well diffusion method to measure inhibition zones after 24-hour incubation. Additionally, the fluoride retention was analyzed among 150 participants randomly divided into 5 groups, who brushed with 1.0 g of toothpaste. Saliva samples were collected at baseline and at intervals (2, 15, 30, 60, and 90 minutes post-brushing). **Results:** Colgate Total 12 clean mint exhibited broad-spectrum antimicrobial activity, especially against *S. mutans*, whereas ROCS Active Calcium and Splat professional Biocalcium were effective solely against *S. mutans*, and Galaxy showed minimal antimicrobial efficacy. Colgate Total 12 clean mint had the strongest inhibitory effect on *S. mutans*, with ROCS Active Calcium and Splat professional Biocalcium showing significantly lower effectiveness ($p < 0.05$). Salivary fluoride retention varied significantly by time, toothpaste type, and post-brushing rinsing routine, with Colgate Total 12 clean mint achieving the highest fluoride concentration ($p < 0.05$). **Conclusion:** Colgate Total 12 clean mint demonstrated superior antimicrobial action against all tested pathogens and sustained higher salivary fluoride levels, suggesting it as an effective alternative to traditional formulations for improved oral health.

Keywords Toothpaste, Fluoride, *Streptococcus mutans*, *Lactobacilli*, *Candida albicans*, Antimicrobial effect

1. Introduction

Dental caries is a localized, progressive, and infectious bacterial disease characterized by the dissolution of dental hard tissues due to acidic byproducts generated from bacterial metabolism [1]. This demineralization process occurs as oral pH levels drop, usually due to the metabolism of dietary sugars by cariogenic bacteria. Caries development relies on multiple factors, such as the presence of cariogenic microflora, availability of fermentable carbohydrates, plaque accumulation, and prolonged exposure to these factors [2]. Dental plaque, a microbial biofilm forming on dental surfaces, supports a diverse microbial ecosystem that can initiate and advance caries [3]. Key bacteria involved in this process include

Streptococcus mutans and *Lactobacilli*, both of which contribute significantly to biofilm development and the deterioration of dental hard tissues [4].

Streptococcus mutans is especially noteworthy for its ability to produce both soluble and insoluble glucans from dietary sucrose, a process facilitated by glucosyltransferase enzymes. These glucans provide an extracellular matrix that enables the bacteria to form a stable biofilm on tooth surfaces, thereby enhancing its cariogenic potential [5]. Meanwhile, *Lactobacilli* contribute to caries progression due to their ability to survive in acidic environments, allowing them to thrive as caries advance into deeper layers of dental tissue. Both *S. mutans* and *Lactobacilli* play a central role in the biofilm community by maintaining an acidic environment that favors demineralization and caries progression [6]. Additionally, *Candida albicans*, a common fungal species, has increasingly been recognized in dental biofilms, especially in individuals with compromised oral immunity or specific

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systemic conditions. While traditionally not a major component of the healthy oral microbiome, *C. albicans* can form biofilms in synergy with *S. mutans* and other bacteria, creating a more resilient biofilm that contributes to cariogenic activity. This fungus is known to produce acidic byproducts that facilitate caries, and its biofilm interactions can contribute to the overall virulence of the biofilm community [7,8].

Fluoride-containing toothpaste is available in various chemical compositions and formulations. Amine fluoride (AmF), sodium fluoride (NaF), sodium monofluorophosphate (Na₂FPO₃), and stannous fluoride (SnF₂) are the main active fluoride ingredients available in toothpastes. Clinical trials suggested the superiority of NaF composition over SnF₂ and Na₂FPO₃ favouring NaF containing toothpaste [8–10]. Others suggested that AmF toothpaste resulted in higher salivary fluoride concentrations and therefore, marked remineralization of caries compared to NaF and Na₂FPO₃ containing toothpastes [11–14].

Effective management of dental caries requires reducing the abundance and activity of harmful oral microorganisms. Toothpastes are commonly used as part of daily oral hygiene routines to control biofilm formation and maintain oral health [10]. Fluoride, a key ingredient in many toothpastes, has been shown to help reduce the incidence of caries by promoting remineralization of enamel and inhibiting the growth of cariogenic bacteria, such as *Streptococcus mutans* [11]. While some toothpaste ingredients may be associated with localized side effects or systemic reactions, such as irritation or allergies [12], the focus of this study is on comparing the antimicrobial effectiveness of different commercial toothpastes against common oral pathogens and their impact on salivary fluoride concentration.

Furthermore, there is growing interest in the development of oral care products that utilize natural ingredients, such as plant-based compounds, to offer potential therapeutic benefits [14]. While these natural ingredients may have demonstrated antimicrobial or anticariogenic properties in some contexts [15–17], this study focuses on the direct comparison of commercially available toothpaste formulations and their antimicrobial efficacy.

Although many studies have explored the remineralization and anti-plaque benefits of toothpastes, no studies have investigated antimicrobial efficacy and fluoride concentration of toothpastes available in the Uzbek market. Consequently, this study aims to determine and compare the antimicrobial effects and fluoride retention following toothbrushing with various toothpastes, that have become increasingly popular for their health claims and natural formulations.

2. Methodology

In this study, five different toothpastes were analyzed for their antimicrobial efficacy against key oral microorganisms. The tested toothpastes included Colgate Total 12 clean mint and Colgate Calcium-remin (both produced by Colgate-Palmolive Co., Ltd., China), Galaxy triple protection (Dentafill

Plyus, Uzbekistan), ROCS Active Calcium (R.O.C.S., Russia), and Splat professional Biocalcium (Organic pharmaceuticals LLC, Russia). The specific contents of each toothpaste, such as active ingredients, flavoring agents, and other compounds relevant to their therapeutic claims, are detailed in Table 1 to provide a comparative overview of their composition.

2.1. Antimicrobial Properties Analysis

In this research saline was used as a negative control group. The saline, lacking antimicrobial agents, provided a baseline to assess the natural susceptibility of the microorganisms without the influence of active antimicrobial compounds.

Table 1. A comparative overview of the toothpaste composition

Toothpastes	Ingredients
Colgate Total 12 clean mint (Colgate Total)	Aqua, hydrated silica, glycerin, sorbitol, sodium lauryl sulfate, arginine, aroma, zinc oxide, cellulose gum, benzyl alcohol, polaxamer 407, zinc citrate, tetrasodium pyrophosphate, xanthan gum, cocamidopropyl betaine, sodium fluoride, sodium saccharin, phosphoric acid, sucralose, CI 77891
Colgate Calcium-remin	Calcium carbonate, water, sorbitol, sodium lauryl sulfate, hydrated silicon dioxide, arginine, sodium monofluorophosphate, flavor, cellulose gum, sodium carbonate, benzyl alcohol, phosphoric acid, sodium saccharin, sodium bicarbonate.
Galaxy triple protection (Galaxy)	Calcium carbonate, silver microparticles, sorbitol propylenglicol, sodium saccharin, silicon dioxide, SiO ₂ (hydrated silica), purified sodium carboxymethyl, cellulose or gidroxiethyl cellulose, xanthan gum, sodium lauryl sulfate (sodium dodecyl sulphate), sodium benzoate, titanium dioxide, sodium fluoride, flavor (toothpaste aroma), dyes, purified water.
ROCS Active Calcium (ROCS)	Aqua, silica, glycerin, xylitol, sodium lauryl sulfate, xanthan gum, magnesium chloride, aroma, sodium glycerophosphate, calcium glycerophosphate, sodium saccharin, methylparaben, propylparaben, sodium silicate
Splat professional Biocalcium (Splat)	Aqua, hydroxyapatite, titanium dioxide, silica, methylparaben, sodium bicarbonate, sodium coco-sulfate, omega-3 oils, papain, polyvinylpyrrolidone, cellulose gum, limonene, sodium saccharin, flavor, calcium lactate, peg-8, hydrogenated starch hydrolysate.

To assess the antimicrobial effectiveness of the toothpastes, microorganisms from dental plaque of a volunteer were received. *Streptococcus mutans*, *Lactobacilli*, and *Candida albicans* were isolated from dental plaque. Prior to testing, each strain was inoculated into separate media, followed by activation and multiplication in an incubator set at 37 ± 1 °C for a duration of 24 hours to ensure an adequate growth phase for antimicrobial testing.

The antimicrobial activity was evaluated using Agar plates, with plate dimensions of 9 cm in diameter and 5 mm in thickness, in alignment with guidelines provided by the European Antibiotic Susceptibility Committee (EUCAST). The agar-well diffusion method was employed for this purpose, with a separate agar plate designated for each

microorganism and toothpaste combination. The wells were spaced equidistantly from one another and the edges of the plate to ensure consistent measurement. Each well was allocated for one sample: a well for the toothpaste under testing, one for the negative control.

The antibacterial properties of toothpaste were studied using the paper disc method to determine the sensitivity of microorganisms (*Str. Mutans*, *Lactobacilli*, *Candida Albicans*) to toothpastes. The study was conducted in two stages. In the first stage, microorganisms collected from dental plaque were cultured on a cotton swab in "nutrient broth" and incubated at 37°C in a thermostat for 24 hours. In the second stage, the liquid sample was inoculated using the lawn method on "nutrient agar" in Petri dishes. The Petri dishes with the inoculated samples were dried at room temperature for 30-40 minutes, after which discs impregnated with toothpaste suspensions were placed on them. A saline solution was used as a negative control sample. The dishes with the discs were incubated for 24 hours at 37°C. The antibacterial activity of the toothpaste was evaluated by measuring the diameter of the zone around the paper discs where microorganisms did not grow.

2.2. Fluoride Analysis

The salivary fluoride concentration following toothbrushing was determined in five toothpaste brands available on the market in Uzbekistan to assess their therapeutic and preventive properties. The analysis took into account information on the concentration and type of fluoride listed on the packaging (e.g., sodium fluoride, sodium monofluorophosphate, ammonium fluoride). Attention was also given to the product's manufacturing date and expiration date.

To determine the fluoride concentration, all participants were instructed to refrain from brushing their teeth on the day of sample collection (the last time they could brush their teeth was the night before), and fast for at least two hours before their appointment and throughout the entire appointment. On the day of the experiment, each participant was asked to drool (unstimulated saliva sample) into a 15 ml sterile tube for two minutes to determine the salivary flow rate and the suitability of the participant to be included in the study. Participants were then asked to brush with a pre-weighed toothpaste (1.0 g) of one toothpaste for two full minutes. A timer was used to record the start and finish times of the brushing.

Each saliva sample tube was labelled with the participant's screening number, date of collection, and time interval. The fluoride ion content in the samples was determined using a fluoride-sensitive electrode (Mettler Toledo, Switzerland), which was connected to a digital ion analyzer (Symphony SB70P, VWR Avantor, USA). Before each analysis, the electrode was calibrated using four standard fluoride solutions with concentrations of 0.1, 1.0, 10.0, and 100.0 mg/l (Thermo Scientific, USA). A calibration curve was constructed based on these standard solutions to ensure measurement accuracy.

The prepared solution samples were diluted at a 10% V/V ratio using a buffer solution (TISAB III, Thermo Scientific, USA), which helped regulate the overall ionic strength in the samples, ensuring measurement stability. This method allowed for accurate determination of fluoride concentrations in toothpaste, enabling an objective evaluation of their potential in caries prevention.

2.3. Statistical Analysis

The statistical analysis of the data collected in this study was conducted using SPSS software version 22.0. Initially, the distribution of the data was assessed for normality using the Shapiro-Wilk test. For datasets that did not follow a normal distribution, intergroup comparisons were performed using the Kruskal-Wallis test along with post-hoc analyses to identify specific group differences. Descriptive statistics for the findings are presented as mean values accompanied by standard deviations (mean \pm SD). Statistical significance was defined as a p-value less than 0.05, indicating that differences at this level were considered statistically meaningful.

3. Results

The inhibition zone diameters observed for the tested toothpastes against *Streptococcus mutans*, *Lactobacilli*, and *Candida albicans* after 24 hours of incubation are summarized in Figure 1. Among the toothpastes tested, Colgate Total 12 clean mint demonstrated antimicrobial activity against all three microbial strains, whereas the Splat toothpaste exhibited no observable antimicrobial effects.

The control group showed no antimicrobial activity against any of the tested microorganisms. Colgate Total 12 clean mint, formulated with traditional ingredients, was found to have the highest antimicrobial efficacy against *S. mutans*. It was followed by other toothpastes, including Colgate Calcium remin, Galaxy, Splat and Rocs. Notably, no statistically significant difference was observed between Splat and Rocs toothpastes against *S. mutans* ($p > 0.05$). For *Lactobacilli*, both the Colgate Calcium-remin and Galaxy toothpastes showed highest inhibitory effects followed by Rocs, Galaxy and Splat ($p < 0.05$). For *Candida albicans*, the toothpaste brands Colgate Total 12 clean mint, Colgate Calcium-remin, Galaxy, and Splat demonstrated comparable antimicrobial effectiveness, with no significant differences in their performance, as indicated by zone diameters ranging from 0.4 to 0.45 mm ($p < 0.05$). These results suggest a similar level of efficacy in inhibiting the growth of *Candida albicans*. In contrast, Rocs exhibited a significantly lower antimicrobial effect, with a much smaller zone of inhibition measuring only 0.18 mm, indicating a markedly reduced ability to combat this microorganism. Differences in antimicrobial efficacy among the formulations highlight the varying potential of natural agents as alternatives in oral hygiene products (Figure 1).

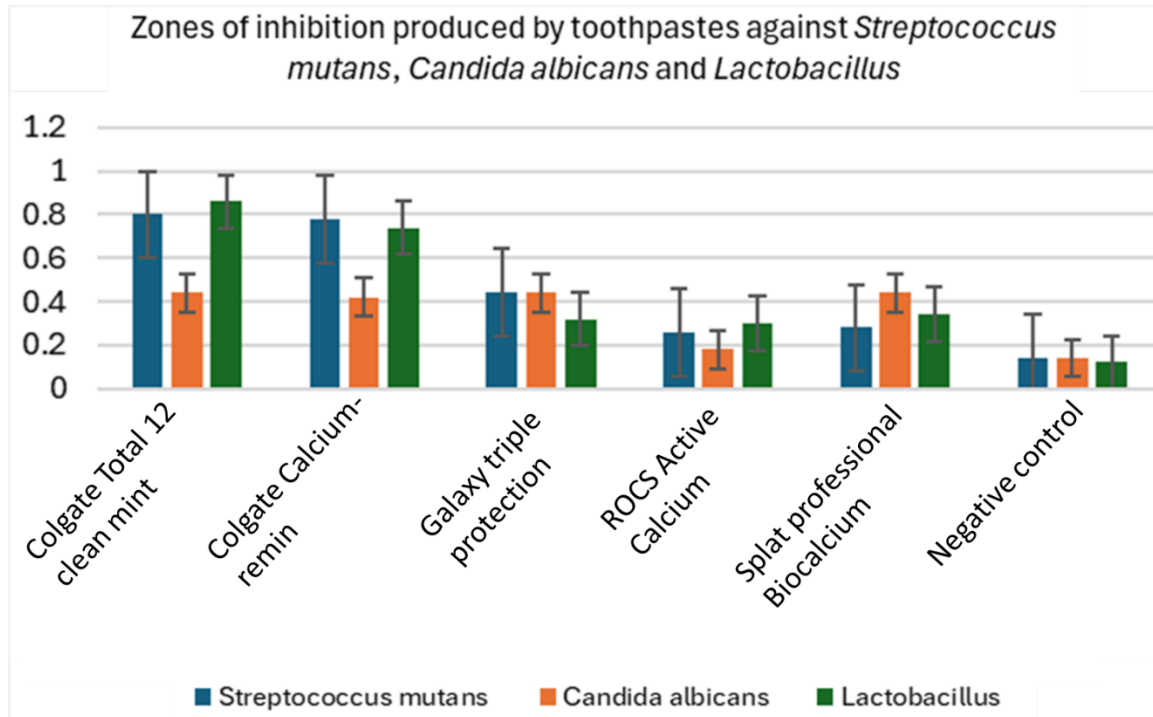


Figure 1. Means (\pm standard error of the mean) of microorganism growth inhibition zones of each toothpaste

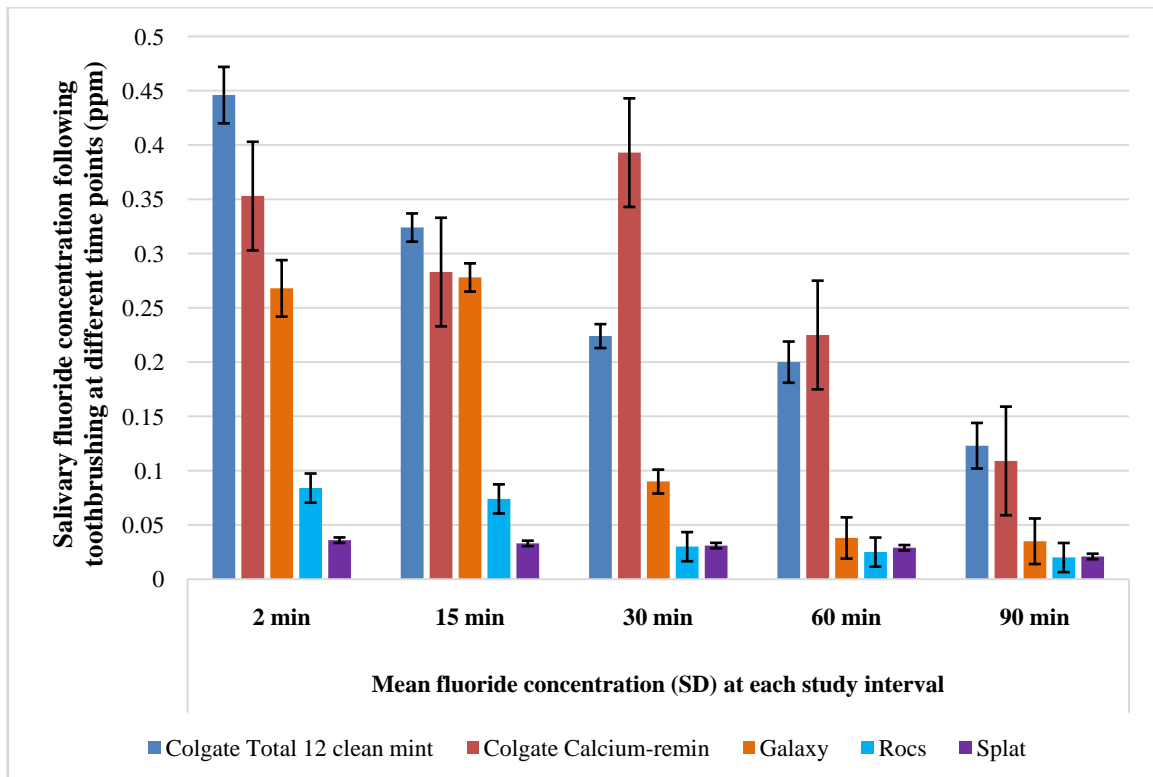


Figure 2. Salivary fluoride concentration following toothbrushing at different time points

Figure 2 demonstrates the analysis of fluoride ion concentration in various toothpaste formulations revealed substantial differences in fluoride content among the tested products. The fluoride concentrations (mean \pm standard deviation) for the different brands are as follows: Colgate Total 12 clean mint exhibited the highest fluoride concentration

at 0.446 ± 0.02 ppm. This was followed by Colgate Calcium Remin, which had a fluoride concentration of 0.353 ± 0.01 ppm, and Galaxy Triple protection, with a concentration of 0.268 ± 0.02 ppm. On the other hand, Rocs and Splat displayed significantly lower fluoride concentrations of 0.084 ± 0.01 ppm and 0.036 ± 0.01 ppm, respectively.

Statistical analysis using one-way analysis of variance (ANOVA) confirmed that the differences in fluoride concentrations among the toothpaste groups were statistically significant ($p < 0.05$). To further delineate the differences, a Tukey post-hoc test was conducted. The results of this test categorized the toothpaste brands into three distinct groups based on their fluoride content. Colgate Total 12 clean mint demonstrated a significantly higher fluoride concentration than all the other tested products. Colgate Calcium Remin and Galaxy Triple Protection exhibited similar fluoride levels, with no statistically significant difference between them. Similarly, Rocs and Splat also showed no significant difference in their fluoride concentrations, which were the lowest among all the products tested.

The observed differences in fluoride concentrations likely reflect the intended purposes of these toothpaste formulations. Colgate Total 12 clean mint, with its high fluoride content, is marketed as a standard fluoride toothpaste and is expected to provide robust protection against dental caries. The intermediate fluoride levels in Colgate Calcium Remin and Galaxy triple protection suggest that these products are designed for consumers who seek moderate fluoride levels, possibly for remineralization purposes or reduced fluoride exposure while maintaining some degree of caries prevention. In contrast, Rocs and Splat, with their minimal fluoride concentrations, may cater to users with specific preferences for low-fluoride or fluoride-free oral care products. These could include individuals concerned about overexposure to fluoride, those in areas with fluoridated water, or individuals with specific health conditions or age-related considerations, such as young children.

The standard deviations reported for each product indicate some variability in fluoride concentrations within the tested samples. This variability may be attributed to manufacturing processes, storage conditions, or variations in batch formulations. However, the consistent grouping of products into statistically distinct categories suggests that these differences are unlikely to impact their clinical utility within their respective fluoride concentration ranges.

These findings highlight the importance of understanding the fluoride content in toothpaste formulations to align with the oral health needs and preferences of different populations. Products with higher fluoride content, such as Colgate Total, may be more appropriate for individuals at higher risk of dental caries, whereas products with low fluoride content, such as Rocs and Splat, may serve a niche market that prioritizes minimal fluoride exposure. The significant differences observed in fluoride concentrations across the tested toothpaste brands emphasize the need for consumers to select toothpaste based on their specific oral health requirements and risk factors.

4. Discussion

Findings indicated that Colgate total 12 clean mint exhibited antimicrobial effects on all three microorganisms specifically effective against *S. mutans*. Interestingly, toothpastes

containing enzymes did not demonstrate antimicrobial efficacy against any of the tested bacteria.

In other studies for comparative purposes, a 0.2% chlorhexidine digluconate solution was employed as a positive control, given its widely accepted status as the “gold standard” in antimicrobial agents due to its strong binding to negatively charged microbial cell walls, disruption of osmotic balance, broad-spectrum effectiveness, and plaque-prevention capabilities [21]. Among all groups, the positive control demonstrated the highest antimicrobial efficacy against *S. mutans*, *S. sanguinis*, and *E. faecalis*, with traditional toothpaste following in effectiveness. Additionally, in both the positive control and toothpaste groups, *S. sanguinis* and *E. faecalis* exhibited greater resistance compared to *S. mutans*, a finding supported by previous studies that suggest this resistance may stem from the ability of these bacteria to create an impermeable barrier using outer cell layers, which limits antimicrobial penetration [22,23].

Colgate total 12 clean mint toothpaste demonstrated broad antimicrobial efficacy, corroborating findings from Lakshmi *et al.* [27], who reported its effectiveness against *S. mutans*, *Lactobacillus acidophilus*, *E. faecalis*, and *Candida albicans*. Additionally, he investigated that theobromine, containing in cocoa beans, tea, guarana, and mate leaves, contains active components such as anti-glycosyltransferase, oleic and linoleic acids, flavonoids, and polyphenols, which contribute to its anticariogenic properties. Furthermore, glycotransferase inhibition, attributed to its anti-glycosyltransferase components, reduces plaque formation by preventing *S. mutans* from adhering to dental surfaces [25] and flavonoids and polyphenols provide a bacteriostatic effect by inhibiting biofilm formation, which in turn diminishes caries development [26].

This study also found that toothpastes including Splat and Galaxy triple protection were selectively effective against *S. mutans*. This is also align with this study’s finding of efficacy against *S. mutans*. For example, one study reported high efficacy of toothpaste against *S. mutans*, *C. albicans*, *S. sanguinis*, and *Actinomyces viscosus* [34], although other formulations with varying active agents.

Fluoride levels between 1000–1500 ppm are considered optimal for caries prevention in most populations [31,32]. Previous studies by Twetman *et al.* [33] have demonstrated that toothpastes with fluoride concentrations above 1,000 ppm effectively reduce enamel demineralization and promote remineralization. Comparatively, our findings suggest that Colgate Total aligns with this standard, supporting its efficacy in high-caries-risk individuals. In contrast, intermediate fluoride levels like those in Colgate Calcium Remin and Galaxy may be less effective but can still provide adequate protection for individuals with lower caries risk, similar to findings reported by Petersson *et al.* [34].

The low fluoride concentrations in Rocs and Splat are consistent with other low-fluoride or fluoride-free products analyzed in studies by Buzalaf *et al.* [35]. These products often target niche markets, including children, individuals in fluoridated areas, or those avoiding fluoride due to health concerns. However, their fluoride concentrations are far

below the effective threshold for caries prevention, as established by Zero et al. [36-39]. These findings raise concerns about their ability to protect against caries effectively, especially in high-risk populations.

Comparing these findings to other studies, variations in fluoride concentration between products can be attributed to differences in their intended purposes and formulations. For instance, a study by Whelton et al. [40] found that toothpastes marketed as "natural" or "low-fluoride" often contain less fluoride to cater to consumer preferences but may compromise clinical efficacy. Similarly, low-fluoride toothpastes analyzed in our study likely aim to meet these preferences but may require complementary caries prevention strategies.

Statistical analysis in this study confirmed significant differences in fluoride concentrations ($p < 0.05$), with distinct groupings observed between high-, intermediate-, and low-fluoride products. These differences are critical for regulatory and consumer decision-making. While high-fluoride products like Colgate Total are better suited for high-risk individuals, low-fluoride products such as Rocs and Splat may only be appropriate in specific scenarios, such as for populations with sufficient fluoride exposure through drinking water.

However, while this study focused on fluoride concentration, other factors, such as fluoride bioavailability and the role of additional active ingredients, should be considered in future research. For instance, studies by Attin et al. [41-44] and Lynch et al. [45] have emphasized the importance of fluoride-release profiles and the potential synergistic effects of other remineralizing agents, such as calcium and phosphate, which were not explored in this study.

This study also has several limitations. Toothpastes are complex products with multiple ingredients, and variations in the concentration of active ingredients, surfactants, or other antimicrobial agents can impact efficacy. Although toothpastes with only one active ingredient listed by the manufacturer were chosen for this study, additional components were present upon closer examination. For example, chamomile was found alongside propolis, magnolia with aloe, and mint with miswak, each potentially enhancing antimicrobial activity. Furthermore, ingredients like sodium bicarbonate [46], titanium dioxide [47], glycerin [48], and xylitol [49] are commonly added to toothpastes and may also contribute to antimicrobial effects.

These additional components, whether listed by the manufacturer or not, could lead to discrepancies in antimicrobial efficacy results across studies. Thus, to ensure oral health, it is essential to evaluate the antimicrobial properties of these natural and alternative products, which are increasingly used as substitutes for traditional toothpastes.

5. Conclusions

Toothpaste plays a crucial role in maintaining oral health by preventing dental caries and supporting gum health. This study evaluated the antimicrobial effectiveness of various toothpastes against common oral pathogens and examined

fluoride retention in saliva. The results revealed that Colgate Total 12 clean mint exhibited broad-spectrum antimicrobial activity against all tested pathogens, positioning it as a promising alternative to traditional formulations. Furthermore, Colgate Total 12 clean mint demonstrated significantly higher fluoride retention at multiple time intervals, suggesting its potential to provide longer-lasting protection. However, despite these promising findings, further research is needed to fully assess the practical efficacy and long-term safety of these formulations in everyday oral care routines.

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Declaration of Competing Interests

All the authors declare no conflict of interest.

CRedit Authorship Contribution Statement

SA contributed to the methodology, conducted the investigation, curated the data, wrote the original draft, and participated in manuscript preparation. FA was involved in the conceptualization, methodology, original draft writing, and manuscript preparation. SO and FA both contributed to the conceptualization, methodology and participated in writing through review and editing. BK contributed to the conceptualization, methodology, conducted the investigation and participated in writing through review and editing. All authors collaboratively wrote and revised the manuscript and read and approved the final version.

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Ethics Approval and Consent to Participate

Not applicable.

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