Research Article

A Comparative *in vitro* Evaluation of Antibacterial Efficacy of Different Dentifrices against *Streptococcus mutans*

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ABSTRACT

Background: The *in vitro* study was conducted to determine the antibacterial efficiency of commercially accessible dentifrices against strains of *S. mutans*. **Materials and Methods:** The study assessed the 5 commercially accessible toothpastes and designated as M, C, TA, X and PE. The strain of *S. mutans* MTCC 890 was utilized and the agar well diffusion technique was employed to assess antibacterial effectiveness. The diameter of the Inhibition Zone (ZOI) was determined after 24 and 48 hr of incubation. The ANOVA and Tukey's *post hoc* tests were applied to determine the mean variations among the dentifrices using SPSS software. **Results:** Group TA exhibits the highest average diameter against *S. mutans*, measuring 28.37±1.72 (24 hr) and 29.36±1.27 (48 hr). Group M exhibited the minimal zone of inhibition, measuring 23.25±0.43 at 24 hr and 23.14±0.74 at 48 hr. The comparison of various dentifrices indicated a significant difference in ZOI. The *post hoc* test indicated statistically significant differences in the ZOI at 24 hr relative to other groups, except for Group C vs Group PE (p=0.13), Group TA vs Group X (p=0.12) and Group X vs Group PE (p=0.19). The mean ZOI at 48 hr also showed significant variations in mean ZOI values, except for Group C vs. Group X (p=0.056), Group C vs. Group PE (p=0.52) and Group PE vs. Group X (p=0.12). **Conclusion:** The evaluated toothpaste was efficacious against *S. mutans* at 24 and 48 hr. Group TA containing 1450 ppm of sodium fluoride was superior in inhibiting *S. mutans* and Group M had minimal efficacy.

Keywords: Activated charcoal, Dentifrices, Sodium fluoride, *Streptococcus mutans*, Xylitol.

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INTRODUCTION

Dental caries significantly impacts more than one-third of the global population, rendering it a critical public health issue.[1] Caries is a complex disease impacted by the quality of food intake, oral hygiene measures and the oral environment.[2] Highly acidogenic and acidophilic bacteria tend to grow predominantly in acidic conditions, including *Streptococcus mutans (S. mutans), Streptococcus sobrinus* and *Lactobacillus*.[3] The composition of bacterial plaques may alter as caries progress.[4] Dental biofilms can be reduced and managed with consistent dental hygiene, but they are not completely eradicated. Dental products offer a tangible approach to the disintegration of dental biofilms.[3] through the integration of antimicrobial components, including fluoride components, antiseptics, propolis, probiotics, salivary proteins, sodium lauryl sulphate, xylitol and

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arginine, that may inhibit the proliferation of *S. mutans*; consequently, these components are regarded as effective preventive strategies for diminishing the prevalence of biofilm-related diseases.[5]

Fluoride administration is fundamental to caries prevention measures and the reduction in dental caries prevalence in industrialized countries is mostly ascribed to its heightened application.[6] Fluoride at low, sustained levels during an acidic challenge in the oral cavity can adhere to the apatite crystals, preventing demineralization. After restoring the pH, residual fluoride in the oral cavity establishes an intensely supersaturated environment for fluorhydroxyapatite, accelerating remineralization and enhancing enamel's resistance to caries.[7] Nevertheless, it remains the most discussed and controversial subject globally, with scholars holding varying perspectives regarding its utilization and safety. An alternate method for managing microbes in infants and children involves utilizing toothpaste devoid of fluoride and incorporating herbal ingredients or enzymes to elicit antimicrobial actions against cariogenic microbes.[8] The antibacterial efficacy of the herbs is attributed to their incorporation of secondary metabolic products, including alkaloids, flavonoids, lectins and polyphenols.[9] The interactions among the primary components of these herbs synergistically enhance their potency.[8]

The bacteriostatic action of xylitol formulations on *S. mutans* has been substantiated in a clinical study[10] and in vitro studies,[11] suggesting that xylitol toothpaste could prevent dental caries. The charcoal-based dentifrice has gained popularity regardless of its unsubstantiated assertions of possessing antibacterial and antifungal effects. These assertions may induce individuals to embrace the use of such dentifrice as an effective means of caries prevention.[12] The present investigation was conducted to determine the antibacterial efficiency of commercially accessible dentifrices against strains of *S. mutans*.

MATERIALS AND METHODS

This *in vitro* study following the CRIS criteria, assessed the contrast between the effectiveness of fluoride-free and fluoridated toothpaste in diminishing the colonization of *S. mutans*. The research was conducted from April to September 2024 in a dental college in Kerala. Commercially accessible toothpaste was utilized and designated as M, C, TA, X and PE to obscure their identities, hence reducing observer bias and enhancing the internal validity of the investigation. Table 1 presents the dentifrices utilized in the current study, including the group codes and their respective constituents. The sample size was estimated based on the pilot test data, utilizing an effect size of 0.7, at a 5% significance level and 80% statistical power. An estimated sample of 6 per group was found for each of the 5 groups using the G-Power (Version 3.1.9.7., Heinrich Heine-Universität Düsseldorf, Düsseldorf, Germany).

Preparation of the Culture Medium

Two test tubes were filled with 20 mL of Brain Heart Infusion (BHI) broth, which was then autoclaved. The agar was chilled in a water bath at 45ºC to 50ºC before being put into clean Petri dishes. Subsequently, 1200 L of Mueller agar were dispensed into the Petri dishes to achieve a consistent thickness of 4 mM. The agar plate was solidified at ambient temperature. The agar package sustained a pH ranging from 7 to 7.6.

Activation of *S. mutans* strain

The *S. mutans* Microbial Type Culture Collection (MTCC) 890 strains were preserved at 4ºC to 8ºC on BHI agar plates. The bacterial colony was inoculated using a bacteriological loop into tubes containing pure BHI broth and incubated for 24 hr at 37ºC. Following that, turbidity developed, indicating the strain proliferation. The inoculum was derived from BHI broth and cultured for 24 hr at 37ºC.

Inoculum Preparation

The *S. mutans* MTCC 890 strain was inoculated into tubes comprising 10 mL of pure saline for diluting, achieving turbidity equivalent to that of a McFarland scale of 0.5. From the recent preparations, dilutions of 1 in 3 were generated by taking 3 mL of the solutions and diluting it with 9 mL of physiologic serum in capped vials. The resultant solution exhibited a concentration of 1×108 CFU/mL. The methodology employed in our study was consistent with that utilized in a comparable previous investigation concerning anti-microbial actions on specific cultures.[13]

Preparation of the Dentifrice

The toothpaste was supplied to the testing facility completely wrapped and marked with the specified codes to ensure the brands under examination remained unidentifiable. Consequently, the subsequent groupings were established.

Group M: Toothpaste with Miswak extract (devoid of fluoride),

Group C: Activated charcoal toothpaste with 1450 ppm Sodium Fluoride (NaF),

Group TA: Anticavity toothpaste with 1450 ppm NaF,

Group X: Xylitol-enhanced nanotechnology toothpaste with 1000 ppm NaF,

Group PE: Pro-Expert toothpaste with 350 ppm NaF and 1100 ppm stannous fluoride.

Distilled water was utilized to achieve the 50% dilutions of the toothpaste in the following manner: The toothpaste is in 100% concentration, undiluted. For the 50% concentration, 5 g were inserted in a tube with 10 mL of sterile water (1:2 dilution). The dilutions for each of the 5 toothpastes had been centrifuged at 3,500 rpm for 15 min to obtain the supernatant.

Inoculation of Specimens

The agar well diffusion technique was employed to assess antibacterial effectiveness. Forty microlitres of toothpaste concentration dilution were transferred to each of the wells with a diameter of 6 mM. Each petri dish was appropriately marked matching the toothpaste and cultured for 48 hr at 37ºC. After the incubation process, the diameter of the Zone of Inhibition (ZOI) of the microbial growth was determined (mm). The recorded measurements were conducted in triplicate and descriptive statistics were computed. A 0.12% chlorhexidine solution and distilled water functioned as the positive and negative controls respectively.

Statistical Analysis

The descriptive statistics were computed and one-way ANOVA and Tukey's *post hoc* tests were applied using IBM SPSS Version 26.0 to assess the average deviations among the 5 dentifrices.

RESULTS

Table 1 presents the formulations of the various toothpastes utilized in the current investigation. The kinds of fluoridated toothpaste brands include groups C, TA, X and PE, which comprise sodium fluoride; group M consists of meswak extract as an antibacterial component. In addition to antibacterial content, all kinds of toothpaste contain silica, sorbitol, tetra- or pentasodium pyrophosphate, sodium lauryl sulphate and zinc citrate. Group PE contains both sodium fluoride (350 ppm) and stannous fluoride (1100 ppm) and group X consists of both xylitol and sodium fluoride (1000 ppm). The groups C and TA consist of 1450 ppm of sodium fluoride. In addition to fluoride, group C also contains activated charcoal. Further, groups X and TA also comprise titanium dioxide which is regarded to possess an antibiofilm effect. Among the toothpastes, group M with plant extract, devoid of fluoride, exhibits the least inhibitory activity and henceforth, the antibacterial efficacy was relatively inferior to that of the other toothpastes (Table 2).

Table 1: The ingredients of the toothpaste samples utilized in the study.

Toothpaste sample used	Ingredients
Miswak extract (Group M).	Calcium carbonate, sorbitol, silica, Na lauryl sulphate, miswak extract, cellulose gum, carrageenan, PVM/MA copolymer, Na silicate, saccharin, Zn gluconate, Na benzoate, benzyl alcohol, thymol.
Activated Charcoal (Group C).	NaF (1450 ppm), hydrated silica, sorbitol, calcium pyrophosphate, glycerin, arginine, Zn oxide, pentasodium triphosphate, benzyl alcohol, tetrapotassium pyrophosphate, poloxamer, Na lauryl sulphate, cellulose gum, Na saccharin, charcoal powder, phosphoric acid, mica, xanthan gum, eugenol, Zn citrate, sucralose, cocamidopropyl betaine
Anticavity toothpaste (Group TA).	Glycerin, Silica, Na Lauryl Sulphate, Arginine, Cocoamidopropyl Betaine, Zn Oxide, Na Carboxymethyl Cellulose, Titanium Dioxide, Poloxamer 407, Zn Citrate Trihydrate, Tetrasodium Pyrophosphate, Xanthan Gum, Benzyl Alcohol, Phosphoric Acid, Na Saccharin, NaF (1450 ppm), Titanium Dioxide Coated Mica, Sucralose.
Xylitol advanced nanotechnology toothpaste (Group X).	Sorbitol, Silica, Glycerine, Potassium nitrate, Na Lauryl Sulphate, Nano-hydroxyapatite, Xylitol, Na carboxymethyl cellulose,, Titanium Dioxide, Na Saccharin, NaF (1000 ppm), Na Methyl Hydroxy Benzoate, Methyl Salicylate, Disodium Hydrogen phosphate dihydrate, Disodium EDTA, Na Propyl Hydroxy Benzoate.
Pro-Expert toothpaste (Group PE).	Glycerin, Hydrated Silica, Na Hexametaphosphate, Na Gluconate, Na Lauryl Sulphate, Aroma, Carrageenan, Trisodium Phosphate, Stannous Fluoride (1100 ppm), Na Saccharin, PVP, Stannous Chloride, Xanthan Gum, Cocamidopropyl Betaine, Cinnamal, NaF (350 ppm), Eugenol, Na Hydroxide, CI 74160, Na Benzoate.

Na-Sodium; NaF-Sodium fluoride; Zn-Zinc.

All the groups of toothpaste employed in this study exhibited inhibitory action against *S. mutans* ranging from 23.25±0.43 mM to 28.37±1.72 mM at 24 hr and 23.14±0.74 mM to 29.36±1.27 mM at 48 hr. The comparative analysis of ZOI at 24 hr revealed that the reduction of *S. mutans* in the group TA (anticavity toothpaste) (28.37±1.72 mM) was much greater than that observed in the other groups using xylitol (group

X) (26.89±1.22 mM), Pro-Expert toothpaste (PE) (25.97±1.01 mM), activated Charcoal (C) (24.89±1.22 mM) and Miswak extract (M) (23.25±0.43 mM). Table 2 illustrates the average ZOI exhibited by the 5 readily accessible dentifrices. Similarly, findings were observed concerning ZOI at 48 hr. Group TA exhibits the highest average diameter against *S. mutans*, measuring 28.37±1.72 mM at 24 hr and 29.36±1.27 mM at 48 hr. Group M exhibited the minimal ZOI, measuring 23.25±0.43 mM at 24 hr and 23.14±0.74 mM at 48 hr, respectively. The comparison of various dentifrices indicated a significant difference in microbial ZOI among all 5 dentifrices against *S. mutans*, as seen in Table 2.

Table 2: Comparisons of the diameter of the zone of inhibition (mm) for *S. mutans* among the study samples.

Groups	N	24 hr			48 hr		
		Mean±SD	F-test	*p*-value	Mean±SD	F-test	*p*-value
M	6	23.25±0.43	15.89	0.000**	23.14±0.74	28.16	0.000**
C	6	24.89±1.22			25.08±0.96		
TA	6	28.37±1.72			29.36±1.27		
X	6	26.89±1.22			26.51±1.31		
PE	6	25.97±1.01			25.43±0.87		

** Highly significant.

The findings of our study indicate that the usage of anticavity toothpaste and xylitol toothpaste resulted in a comparable reduction of bacterial count in saliva after 24 hr (p=0.12). Similarly, brushing with xylitol toothpaste is equally effective in diminishing *S. mutans* levels compared to pro-expert toothpaste (p=0.19) at 24 hr. On the other hand, at 48 hr, while the variations in efficacy between groups X and PE were statistically insignificant (p=0.12), a significant variation was observed between groups X and TA (p=0.003). Similarly, while a significant difference was noted between groups X and C at 24 hr (p=0.02), the difference in the efficacy was statistically insignificant at 48 hr (p=0.056). Furthermore, activated charcoal exhibited comparable effectiveness in reducing the *S. mutans* levels as opposed to pro-expert toothpaste at 24 hr (p=0.13) and 48 hr (p=0.52).

The *post hoc* tests were employed for mean comparisons among the various dentifrices at 24 and 48 hr (Table 3). The *post hoc* multiple comparisons indicate statistically significant differences in the average ZOI at 24 hr relative to other groups, except for Group C versus Group PE, Group TA versus Group X and Group X vs Group PE. The mean ZOI at 48 hr also showed significant variations in mean ZOI values when compared between the groups, except for Group C vs. Group X, Group C vs. Group PE and Group PE vs. Group X.

DISCUSSION

The present study evaluated the antibacterial efficacy of 5 distinct toothpaste brands, labeled M, C, TA, X and PE, against *S. mutans*. All 5 toothpastes showed efficacy against *S. mutans*, with group TA (28.37±1.72 mM at 24 hr and 29.36±1.27 mM at 48 hr) exhibiting the greatest average inhibition zone, followed by groups X (26.89±1.22 mM at 24 hr and 26.51±1.31 at 48 hr), PE (25.97±1.01 mM at 24 hr and 25.43±0.87 at 48 hr), C (24.89±1.22 mM at 24 hr and 25.08±0.96 at 48 hr) and M (23.25±0.43 mM at 24 hr and 23.14±0.74 mM at 48 hr). Group M displayed the least activity.

Numerous clinical research has proven the inhibitory effects of antimicrobial dentifrice on oral health.[14] The current data supports this claim, as all examined dentifrices demonstrated significant variation in their efficacy against *S. mutans*, likely attributable to their antimicrobial active ingredients, including sodium fluoride, xylitol, stannous fluoride and activated charcoal. These observations align with the findings of Okpalugo *et al.*,[15] who indicated that toothpastes containing sodium fluoride and triclosan, resulted in a 20% greater reduction in oral bacterial flora compared to toothpastes without triclosan. Additionally, the report indicated that fluoridated toothpaste is, on average, related to a 24% decrease in tooth decay.[16] Prior studies have shown that rinsing with antimicrobial toothpaste and mouthwash substantially decreases bacterial levels in saliva and mucosal surfaces.[14,17,18] The overall results of the present study indicate that diverse toothpastes contain many active and inactive components that exhibit differing amounts of antibacterial activity. This is likely attributable to variations in preparations, the levels of the active ingredient and its interactions with other components. This finding substantiates the antibacterial assertions of the mouthwashes stated by previous researchers.[16,19,20]

The antimicrobial activity of herbal or synthetic drugs could be assessed using the broth dilution method,

Table 3: Mean intergroup comparisons among the study samples using Tukey's *post hoc* tests.

Groups	Intergroup comparisons	24 hr		48 hr	
		Mean difference	*p*-value	Mean difference	*p*-value
M	C	–1.64	0.011*	–1.94	0.003**
	TA	–5..12	<0.001**	–6.22	<0.001**
	X	–3.64	<0.001**	–3.37	<0.001**
	PE	–2.72	<0.001**	–2.29	<0.001**
C	M	–1.64	0.011*	1.94	0.003**
	TA	–3.48	0.002**	–4.28	<0.001**
	X	–2	0.02*	–1.43	0.056
	PE	–1.08	0.13	–0.35	0.52
TA	M	5..12	<0.001**	6.22	<0.001**
	C	3.48	0.002**	4.28	<0.001**
	X	1.48	0.12	–2.85	0.003**
	PE	2.4	0.014*	3.93	<0.001**
X	M	3.64	<0.001**	3.37	<0.001**
	C	2	0.02*	1.43	0.056
	TA	–1.48	0.12	–2.85	0.003**
	PE	0.92	0.19	1.08	0.12
PE	M	2.72	<0.001**	2.29	<0.001**
	C	1.08	0.13	0.35	0.52
	TA	–2.4	0.014*	–3.93	<0.001**
	X	–0.92	0.19	–1.08	0.12

** Highly significant; * Significant.

agar dilution approach, disc diffusion technique, agar well-diffusion assay and ditch-plate test. Nonetheless, the agar well-diffusion technique was employed in this investigation, since it relies on the diffusion of the substance being studied to the degree that the proliferation of the introduced microbe is completely inhibited in a zone surrounding the well holding the sample.[21] School-based trials by Sintes *et al.*[22] using bi-daily brushing with toothpaste comprising 10% xylitol combined with 0.243% sodium fluoride or 0.836% sodium mono-fluorophosphate determined a substantial decrease in caries after three years. In contrast to the present study findings, Chi *et al.*[23] revealed that brushing teeth with xylitol toothpaste yielded no therapeutic advantage over fluoride-only toothpaste, since a decrease in *S. mutans*, levels was not observed and further a rise in DMFS index was observed in the high-risk group. Numerous *in vitro* and animal experiments showed that fluoride is more efficient than xylitol, whether used alone or in conjunction with fluoridated toothpaste, in combating *S. mutans*.[23] Randall *et al.*[24] determined that the antimicrobial efficacy of commonly used dentifrices against *S. mutans* may be attributed to constituents other than fluoride, such as triclosan and sodium lauryl sulphate, which exhibit superior antibacterial properties compared to fluoridated dentifrices.[24] Xylitol must be applied in complete contact with the tooth surface consistently and directly to achieve optimal efficacy.[6,11] Autio *et al.*[10] examined preschoolers' salivary *S. mutans* levels after xylitol gum, three times a day for three weeks. The decrease in the level of *S. mutans* score was larger in the xylitol group than in the control group. According to this study, chewing xylitol gum may lower salivary *S. mutans* levels and preschoolers may benefit from xylitol gum for caries prevention.

Sano *et al.* established that the amalgamation of 500 ppm fluoride and 5% xylitol in the dentifrice significantly improved both lesion size and fluorescence loss integrated across lesion size, evaluated by using quantitative light-induced fluorescence. These data suggested that xylitol in fluoridated toothpaste may improve *in vivo* remineralization.[11] In another similar study, children who were given instructions to use 0.2 g of xylitol toothpaste twice a day showed reduced levels of *S. mutans* in their saliva and plaque after six months.[25] Although xylitol is incorporated into numerous dental preventive programs as a recognized

anticaries agent, there exists a paucity of empirical evidence demonstrating its efficacy, except in studies involving fluoridated toothpaste supplemented with xylitol.[26] Complex formulations have been developed for toothpaste, resulting in a variety of products. Nanotechnology has made toothpaste with calcium phosphate salts effective in remineralizing tooth enamel. Calcium and phosphate penetrate micropores, create crystalline nuclei and incorporate salivary ions.[27] Nanotechnology has improved toothpaste by adding nanohydroxyapatite, nanoparticles of silver, calcium phosphate and nanocalcium. All of this has upgraded toothpaste by reducing cavities and controlling acidic pH or inhibiting microbial growth.[28]

S. mutans is closely associated with the onset of dental caries. Conversely, *Lactobacillus* species have been implicated in the further advancement of carious lesions.[6] Among the several methods for reducing dental cavities, the most popular and effective is the use of fluoride-containing dentifrices; nevertheless, the antibacterial effects of fluoride toothpaste are influenced by concentration.[6] It competes with glycolysis, the mechanism by which cariogenic microbes metabolize carbohydrates to generate acid, inhibits demineralization and fosters the remineralization of early enamel lesions when observed in dental plaque and saliva. It exerts a bactericidal effect on cariogenic and other microbes at elevated doses.[29] Our research demonstrated that fluoridated toothpaste was more beneficial than non-fluoridated toothpaste in inhibiting *S. mutans*. The two most common forms of fluoride found in fluoridated toothpaste are sodium fluoride and sodium mono-fluorophosphate. When comparing sodium fluoride to sodium mono-fluorophosphate, the former is superior at preventing cavities in children.[17] When comparing fluoridated and miswak toothpaste, it is possible that the higher zone of inhibition against the *S. mutans* was due to the sodium fluoride content of the former.

Kurian *et al.*[8] assessed the antibacterial properties of both herbal and fluoride toothpaste, demonstrating that fluoridated toothpaste is the benchmark standard in terms of antimicrobial performance. Toothpaste containing fluoride, owing to the presence of triclosan/copolymer and 1000 ppm fluoride, offers superior protection against plaque and periodontal health compared to regular toothpaste.[30] The antibacterial efficacy of fluoridated toothpaste accounts for the inhibition of lipid production by triclosan through selectively impeding the enzyme enoyl-acyl carrier protein reductase.[8] Tonguc-Altin *et al.*[3] showed that dentifrices incorporating aloe vera, propolis, sorbitol, sodium lauryl sulphate and fluoride significantly impeded the establishment of *S. mutans* biofilm over 24 hr. In our investigation, all fluoride-containing toothpastes showed comparable efficacy in preventing biofilm formation. Nonetheless, it is essential to recognize that the mechanical process of brushing, along with toothpaste, would significantly diminish the viability of *S. mutans*.[31] Panareillo *et al.*[12] observed that charcoal dentifrices have comparable antimicrobial properties and reduced enamel demineralization compared to regular dentifrices, with no discernible benefit in caries prevention or management when substituting ordinary fluoride-containing dentifrices. Thamke *et al.* [32] also assessed bacterial contamination and antibacterial activity of charcoal bristles versus non-charcoal bristles, revealing a statistically significant variance after one week of usage, indicating that charcoal had antibacterial properties. These studies align with our study findings indicating that the activated charcoal can reduce bacterial viability. However, additional definitive evidence obtained from clinical studies is required to validate the current results.

The pH employed in laboratory investigations of bacterial growth can affect both the bacterial generation time and the lag phase before bacterial division, potentially influencing the bacterial population at the periphery of the cleared zone in the agar diffusion assay.[33] The agar diffusion technique employed in the study is effective for screening and evaluating the potential antibacterial efficacy. Diverse mixtures and solutions of pure compounds exhibit variations in solubility and other physical qualities, which can influence the diffusion of the agent through the agar, therefore affecting the observed growth inhibition.[24] However, utilizing standardized bacterial inocula, constant storage conditions and uniform well dimensions and quantities per plate has yielded insights into the comparative efficacy of the dentifrices under study.

Ericsson and Forsman[34] proposed that toothpaste containing either sodium fluoride or sodium monofluorophosphate would have a fluoride concentration of less than 0.1% for usage by children of the tooth mineralizing stage. The increased bioavailability of fluoride in toothpaste significantly elevates the risk of dental fluorosis. Consequently, daily fluoride consumption must be approached with prudence. Since regular fluoridated toothpaste may raise the incidence of dental fluorosis, studies on toothpaste with less than 1000 ppm fluoride are significant. Thus, evaluating fluoridated toothpaste to maximize dental caries prevention with low dental fluorosis threat

is crucial. Sano *et al.*[11] found that 500 ppm sodium fluoride and 5% xylitol in dentifrices enhanced early caries lesions remineralization for 14 days in contrast to toothpaste with 500 ppm of fluoride in vitro. Though in vitro findings cannot be directly translated to *in vivo* conditions, an amalgamation of 500 ppm sodium fluoride and 5% xylitol in dentifrices may suppress caries and reduce dental fluorosis in pediatric patients undergoing enamel remineralization.[11]

In most cases, saliva dilutes the effectiveness of toothpaste when used in living organisms. This leads to the possibility of antimicrobial compounds being mitigated in saliva at different concentrations. As a result, it is not necessarily true that the dentifrices with the widest zone of inhibition have the most antimicrobial components. In addition, the antibacterial components found in various dentifrices may diffuse at distinct rates. As a result, the antibacterial activity of toothpaste cannot be accurately determined in *in vivo* contexts based on in vitro findings. The varying antibacterial effects of toothpastes are due to their differing formulations, key components and how they integrate with other compounds.

CONCLUSION

While all of the evaluated toothpastes were efficacious against *S. mutans* at 24 and 48 hr, the group TA that contained 1450 ppm of NaF was superior in inhibiting *S. mutans* than the other toothpastes. Group M had the least efficacy against the *S. mutans*. The therapeutic utilization of these findings may suggest the usage of the most efficacious toothpaste to enhance the management of the pathogenic strain of *S. mutans*. Subsequent examination of the associations between fluoride and non-fluoride constituents would enhance the chemical makeup of dentifrices to maximize their anti-cariogenic efficacy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BHI: Brain heart infusion; **CFU/mL:** Colony Forming Units per Milliliter; **CRIS:** Checklist for reporting in vitro studies; MTCC: Microbial type culture collection; NaF: Sodium fluoride; ***S. mutans:*** *Streptococcus* mutans; ppm: Parts per million; ZOI: Zone of inhibition.

SUMMARY

This in vitro study elucidated the impact of fluoride and fluoride-free toothpaste on *S. mutans*. The study evaluated 5 commercially available toothpastes using *S. mutans* MTCC 890 strain, assessing their antibacterial effectiveness. The ZOI diameter was determined and mean differences were analyzed using SPSS software by applying ANOVA and *post hoc* tests. Group TA had the largest average diameter compared to *S. mutans*, measuring 28.37±1.72 (24 hr) and 29.36±1.27 (48 hr). Group M had the lowest inhibition zone, measuring 23.25±0.43 at 24 hr and 23.14±0.74 at 48 hr. Comparing dentifrices showed significant ZOI differences. Except for Group C compared Group PE, Group TA versus Group X and Group X versus Group PE, the *post hoc* test showed statistically significant differences in ZOI at 24 hr. Except for Group C vs. Group X, Group C vs. Group PE and Group PE vs. Group X, the mean ZOI at 48 hr varied significantly. Thus, it was concluded that the toothpastes tested were effective against *S. mutans* at 24 and 48 hr intervals, with the TA group showing superior inhibition, while Group M had minimal efficacy.

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