ORIGINAL ARTICLE

Antimicrobial Efficacy of Commercial Dentifrices Containing Triclosan, Chlorhexidine, and Herbal Extracts Against Caries-Associated Pathogens

Maleeha Nayyer[1], Asfia Saeed[2], Syeda Ridaa Fatima[3], Fatima Suhaib[4], Saadia Muneer Malik[5], Muhammad Azhar[6]

ABSTRACT

Objective: To evaluate the antimicrobial potential of four commercial dentifrices against caries-associated microbes and to compare the effectiveness of active ingredients within these dentifrices in preventing the onset and progression of dental caries.

Study Design: In vitro Experimental Study

Place and Duration of Study: Army Medical College from 20th January 2023 to 18th August 2023.

Materials and Methods: The study was performed to compare the antimicrobial potential of four commercial dentifrices against microbes responsible for causing Dental Caries i.e. *Streptococcus mutans* (ATCC 25175) and *Lactobacillus casei* (ATCC 393) using agar well diffusion assay.

Incubations of *S. mutans* were accomplished in aerobic and anaerobic conditions, whereas *L.casei* was incubated in microaerophilic and anaerobic environments. After incubation, the zones of inhibition (ZOI) around the tested dilutions were measured and the mean values of ZOI ± SD were calculated. The plate with no visible growth was considered as MIC. The independent t-test was applied to compare the effectiveness of dentifrice against cariogenic microbes. Inter-group comparisons between dentifrices were conducted through one-way analysis of variance (ANOVA) with significant differences further evaluated by Tukey HSD tests. A *p*-value of ≤ 0.05 was considered statistically significant.

Results: Each dentifrice exhibited antibacterial activity against *S. mutans* and *L. casei*. Chlorhexidine was the most effective agent against both *S. mutans* and *L. casei,* while herbal dentifrices exhibited the least efficacy. In addition, for each formulation, *S. mutans* showed significantly higher susceptibility.

Conclusion: All four dentifrices showed antimicrobial effectiveness against key cariogenic pathogens. Chlorhexidine emerged as the most potent in preventing both the initiation and advancement of dental caries due to its strong antibacterial efficacy against *S. mutans* and *L. casei.*

Keywords: *Chlorhexidine, Dentifrice, Herbal, Lactobacillus Casei, Streptococcus Mutans, Triclosan.*

Introduction

Accumulation of plaque on tooth surfaces and crevices is a natural process, representing a complex community of microorganisms and their by-products, embedded within an organized extracellular matrix.[1] Among these, the colonization of cariogenic bacteria, including *Streptococcus mutans* and to a lesser extent *Streptococcus sanguis, Streptococcus sobrinus, Lactobacillus casei* and *Lactobacillus acidophilus,* on the tooth surfaces may lead to demineralization of teeth, cavitation and ultimately pulp necrosis.[2]

Global prevalence of dental caries and periodontal diseases, with caries ranked as first among 328 diseases by Global Burden of Disease (GBD) affecting 2.3 billion adults, is 532 million.[3] Maintenance of good oral hygiene and limiting dietary sugars are essential for preventing the onset and advancement of dental caries. Tooth brushing and flossing are considered standard tools for maintaining oral hygiene. Furthermore, globally, dentifrices containing chemical agents designed to inhibit the initiation of caries and promote remineralization are used.[4] A study conducted in Spain found that maintaining good oral hygiene played a superior role in decreasing caries prevalence than modifying diet.[5] A variety of dentifrices are available worldwide.

[1]*Department of Dental Materials/Oral Pathology[5]/Oral Biology[6] Army Medical College, Rawalpindi*
[2,3]*Department of Dental Materials Shifa College of Dentistry, Islamabad*
[4]*Department of Science of Dental Materials Lahore Medical and Dental College, Lahore*

Correspondence:
Dr. Fatima Suhaib
Assistant Professor Dental Materials
Lahore Medical and Dental College, Lahore
E-mail: fatimasuhaib83@hotmail.com

Received: February 04, 2025; Revised: September 08, 2025
Accepted: September 19, 2025

https://doi.org/10.57234/jiimc.september25.2417

Fluoride was the first and the most widely used therapeutic agents, and is incorporated into most dentifrices.[6] It is usually added in the form of sodium fluoride (NaF), stannous fluoride (SnF_2), or sodium monofluorophosphate (Na_2PO_3F).[7] Fluoride interferes with bacterial metabolism and acid production, preventing demineralization, and promoting remineralization, thereby creating an acid-resistant enamel surface, ultimately reducing the likelihood of developing caries.[8]

However, in conditions like xerostomia and hypo salivation, fluoride alone may not be adequate.[9] Therefore, incorporating antimicrobial agents has been suggested to improve plaque control and reduce colonization of cariogenic bacteria. Hashmat *et al*. compared the amount of fluoride released and antimicrobial activity of commercial and experimental dentifrice formulations containing fluoride-doped nano bioactive glass (F-nBG) and zinc oxide nanoparticles. They observed that the combination of F-nBG and ZnO nanoparticles provides plaque control by reducing the growth of *S. mutans* and *L. Casei*.[10] Triclosan is another common antibacterial agent that is commonly used in toothpaste. Triclosan is a non-ionic phenolic derivative with the ability to block fatty acid synthesis in bacterial cells, leading to their death.[11] Similarly, chlorhexidine, a cationic compound which damages the bacterial cell membrane, causing ions' leakage and eventual cell death are often used in dentifrices.[12] It has an antiplaque effect due to its ability to block salivary glycoprotein groups, along with reducing the bacteria's ability to adhere to the tooth structure.[13] Despite the improved antimicrobial properties of these dentifrices, there is an increased demand for the utilization of natural compounds to improve oral health and to avoid the potentially harmful effects of synthetic compounds.[4] These herbal dentifrices usually contain varied ingredients from neem, camomile, mint, meswak, etc.[14]

Serdar *et al.* reported that dentifrice based on natural products exhibits varied antimicrobial efficacy depending on the nature of the herbal extract. In a study comparing herbal dentifrice containing calendula and sage clay extract with a chemically composed dentifrice against *L. bacillus* and *C. albicans*, it was observed that both formulations exhibited similar antimicrobial effects at higher concentrations. However, a reduction in the concentration of the herbal extract resulted in decreased antimicrobial efficacy of the herbal dentifrice.[15]While various in vitro studies have explored the antiplaque effects of experimental dentifrices, limited data is available on the comparative analysis of commercial dentifrices. Considering the availability of abundant toothpaste brands in the market, addressing this gap is crucial for identifying the most effective formulation for patients with increased susceptibility to dental caries. Therefore, this study compared the antimicrobial efficacy of commercial dentifrices containing fluoride, triclosan, chlorhexidine, and plant extracts against caries-associated microbes. The results of this study will make it possible to select an effective dentifrice formulation for high-risk caries patients.

Materials and Methods

This in vitro experimental study was conducted at Army Medical College from 20th January 2023 to 18th August 2023 after obtaining approval from the Ethical Review Board (10/11/22). Four commercial dentifrices were tested for which three dilutions were prepared for each dentifrice, each at a two-fold concentration (1:1, 1:2 and 1:4). Antimicrobial analysis was performed against two lyophilized microbial strains. The microbial strains were obtained from the American Type Culture Collection (ATCC, Manassas, USA). Freeze-dried vials of *Streptococcus mutans (S. mutans)* (ATCC 25175) and *Lactobacillus casei (L. casei)* (ATCC 393) were revived in Brain Heart Infusion (BHI) broth and de Man, Rogosa and Sharpe broth (MRS) broth respectively. Following the incubation period (24 h for *S. mutans* and 48 h for *L. casei*) at 37°C, microbes were cultured on selective culture media, and their growth was confirmed by observing colony morphology and performing biochemical tests and gram staining. Nutrient agar and Chocolate agar were employed for *S. mutans* and *L. casei* respectively.

The agar well diffusion assay was performed according to the guidelines provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Briefly, inoculum was prepared by transferring three to five well-isolated colonies of microbes to test tubes containing 5 ml Normal saline

https://doi.org/10.57234/jiimc.september25.2417

(0.9%) (Sterifluid NS, Frontier Dextrose Ltd, Lahore, Pakistan) to obtain a turbidity of 0.5 grade on the McFarland Turbidity Scale. Within fifteen minutes of inoculum preparation, swabbing was evenly performed on the respective agar plates. On each agar plate, four wells (diameter: 8mm, depth: 4mm) were punched at an equidistance of 20 mm using a sterile cork borer as represented in Figure 1.

A total of six agar plates were used for each dentifrice. Wells were filled with 0.2 ml of prepared dilutions (1:1, 1:2, 1:4) through a micropipette (Genex Beta, Guangdong, China). Distilled water was taken as a negative control. *S. mutans* were incubated in both aerobic and anaerobic conditions, whereas for *L. casei*, a candle-jar (microaerophila) and an anaerobe jar (Gas-Pak sachet-Oxoid AnaeroGen, Thermo Fisher Scientific) were used. Following incubation (24 h for *S. mutans* and 48 h for *L. casei*), the resultant ZOI around the tested dilution were measured using a Vernier caliper. Mean ZOI was calculated by performing triplicate tests. Broth dilution method was adopted to determine the minimum inhibitory concentration as illustrated in Figure 2.[16] Five (two-fold) dilutions, i.e. 1:1, 1:2, 1:4, 1:8 and 1:16 of dentifrice in respective broth (BHI for *S. mutans* and MRS for *L. casei*) were used in the experiment (2 g/2 ml gave a 1:1 dilution). A control test tube containing 2 ml of distilled water with no dentifrice was used as a reference.

Microbes were inoculated in test tubes with a sterilized wire loop. *S. mutans* and *L. casei* were incubated at 37°C for 24 h and 48 h, respectively. Following incubation, the test tubes were sub-cultured on selective media in duplicate. The lowest concentration (highest dilution) in which no growth of bacteria was visibly appreciated, was considered the MIC. [17] Data was analyzed using SPSS version 21, IBM, Corp. Armonk, NY, USA. Shapiro-Wilk test *(p* = 0.20) was used to evaluate the normality of data. The mean value ± SD was calculated for the ZOI. Levene's test of equality was checked, and an independent t-test was subsequently applied to compare the effectiveness of dentifrice against cariogenic microbes and assess if different environments impact antimicrobial efficacy. Inter-group comparisons of dentifrice were conducted through a one-way analysis of variance (ANOVA) followed by Tukey HSD tests. A *p* value of ≤ 0.05 was considered significant.

Results

Agar well diffusion method

Figure 3a and b show distinct inhibition zones observed after incubation for *L. casei* and *S. mutans* on the agar plate respectively. No inhibitory effect was observed on either bacterium in the negative control wells. Comparison of mean ZOI for *S. mutans* and *L. casei* in the dentifrices are shown in Figure 3c and d.

Mean values of ZOI for all dentifrices at each dilution are enlisted in Table II. The results indicate that the ZOI inhibition acquired from each dilution for both bacteria across all environments decrease as dilution of the dentifrice increases. This points towards a dose- dependent relationships. Figures 4 and 5 depict graphical comparison of *S. mutans* and *L. Casei* susceptibility in each formulation in different environments. A significant difference in susceptibility between *S. mutans* and *L. casei* is observed. *S. mutans* consistently shows higher susceptibility irrespective of incubation condition or dentifrice formulations.

Table III shows a comparison of dentifrices in inhibiting *S. mutans* in different environments. Chlorhexidine was the most effective while herbal dentifrice exhibited the least efficacy. In aerobic conditions, pair-wise comparisons using a post hoc Tukey's test revealed statistically significant differences among all dentifrice formulations except triclosan and chlorhexidine.

In anaerobic conditions, the ZOI was statistically similar for herbal and fluoride dentifrice, triclosan and fluoride, and triclosan and chlorhexidine, respectively.

A comparative evaluation of the effectiveness of dentifrices in inhibiting *L. casei* in various environments are listed in Table IV. A similar trend was observed, with chlorhexidine being the most effective while herbal dentifrice exhibited the least efficacy. One-way AVOVA (analysis of variance) results showed that herbal and fluoride dentifrice had statistically significant comparable results in each environment, while all other formulations had statistically significant differences.

MIC revealed that Clinica (chlorhexidine) was bactericidal even at the dilution of 1:16 for *S. mutans* and at the dilution of 1:8 for *L. casei*. This finding

correlated with the smallest mean ZOIs observed for microbes using agar well diffusion assay (Tables III and IV). Distinct growth was observed on the control plates for both bacterial strains.

Table I: List of Dentifrices and Their Characteristics

Commercial Dentifrice	Active agent	Conc. of Active Ingredient	Fluoride	Conc. of Fluoride	Manufacturer
Bannet	Triclosan	0.3% w/w	Sodium Monofluorophosphate (MFP)	0.76% w/w	Platinum Pharmaceuticals (Pvt) Ltd. Pakistan
Clinica	Chlorhexidine	0.2% w/w	Not present	-	Platinum Pharmaceuticals (Pvt) Ltd. Pakistan
Colgate Herbal	Mint, clove, Neem and eucalyptus in addition to Fluoride	Not specified	Sodium Monofluorophosphate (MFP)	0.76% w/w	Colgate- Palmolive Pakistan
Colgate Cavity Protection	Fluoride only	No other agent	Sodium monofluorophosphate (MFP)	0.76% w/w	Colgate- Palmolive Pakistan

Table II: Mean ZOI for the Dentifrice Slurries at the Dilution of 1:1, 1:2, 1:4

Dentifrice	Dilution	*S. mutans*				*L. casei*			
		Aerobic		Anaerobic		Microaerophilia		Anaerobic	
		Mean	S.D.	Mean	S.D.	Mean	S.D	Mean	S.D
Colgate Herbal	1:1	24.67	0.57	25.00	1.00	13.33	1.15	16.00	2.00
	1:2	20.00	1.00	19.50	0.50	10.07	2.10	13.00	1.00
	1:4	15.83	0.76	15.83	0.76	10.00	2.00	9.83	0.29
Bannet (Triclosan)	1:1	37.73	0.46	36.00	1.00	19.00	1.00	22.67	1.15
	1:2	34.80	0.72	31.17	1.04	17.33	0.57	20.33	0.57
	1:4	28.83	1.04	25.83	0.28	15.33	0.57	18.16	0.29
Clinica (Chlorhexidine)	1:1	41.26	1.10	40.67	1.15	31.16	1.04	34.83	0.76
	1:2	35.50	0.50	34.16	1.61	24.50	0.50	27.50	1.32
	1:4	34.33	0.57	31.16	0.76	20.33	0.57	25.67	0.57
Colgate Cavity Protection (Fluoride)	1:1	29.83	0.28	29.83	0.28	16.33	1.52	17.67	0.57
	1:2	25.83	1.60	26.50	1.80	11.33	0.76	14.33	0.57
	1:4	21.00	1.00	20.73	1.10	10.40	0.53	11.33	0.57

*Zones of Inhibition (ZOI)

Table III: Comparison of Dentifrices in Inhibiting S. Mutans in Various Environments

Condition	Dentifrice	ZOI (mm)		F(p-value)	Post-Hoc Tukey test	p-value
		Mean	S.D.			
Aerobic	Colgate Herbal	20.17	3.89	37.075 (≤0.001)	Herbal vs Triclosan	≤0.001
					Herbal vs Chlorhexidine	≤0.001
					Herbal vs Fluoride	0.024
	Bannet (Triclosan)	33.80	3.99		Triclosan vs Herbal	≤0.001
					Triclosan vs Chlorhexidine	0.284
					Triclosan vs Fluoride	≤0.001
	Clinica (Chlorhexidine)	37.06	3.32		Chlorhexidine vs Fluoride	≤0.001
	Colgate Cavity Protection (Fluoride)	25.67	4.03			
Anaerobic	Colgate Herbal	20.11	4.04	21.514 (≤0.001)	Herbal vs Triclosan	≤0.001

https://doi.org/10.57234/jiimc.september25.2417

Table IV: Comparison of Dentifrices in Inhibiting L. Casei in Various Environments

Condition	Dentifrice	Zone of inhibition(mm)		F (p-value)	Post-Hoc Tukey test	p-value
		Mean	S.D.			
Aerobic	Colgate Herbal	11.13	2.27	37.155 (≤0.001)	Herbal vs Triclosan	≤0.001
					Herbal vs Chlorhexidine	≤0.001
					Herbal vs Fluoride	0.72
	Bannet (Triclosan)	17.22	1.72		Triclosan vs Herbal	≤0.001
					Triclosan vs Chlorhexidine	≤0.001
					Triclosan vs Fluoride	0.022
	Clinica (Chlorhexidine)	25.33	4.78		Chlorhexidine vs Fluoride	≤0.001
	Colgate Cavity Protection (Fluoride)	12.78	2.86			
Anaerobic	Colgate Herbal	12.94	2.89	51.397 (≤0.001)	Herbal vs Triclosan	≤0.001
					Herbal vs Chlorhexidine	≤0.001
					Herbal vs Fluoride	0.738
	Bannet (Triclosan)	20.44	2.01		Triclosan vs Herbal	≤0.001
					Triclosan vs Chlorhexidine	≤0.001
					Triclosan vs Fluoride	0.002
	Clinica (Chlorhexidine)	29.39	4.37			≤0.001

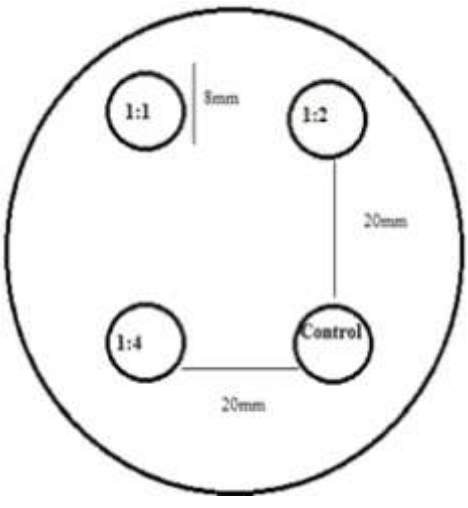

Figure 1: Diagrammatical Representation of Agar Plate for Antimicrobial of Agar Plate for Antimicrobial Susceptibility Testing

Figure 2 : Steps to Evaluate Minimum Inhibitory Concentration (MIC) Of Commercial Dentifrice Against Cariogenic Microbes

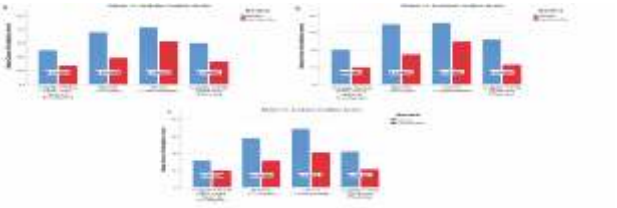

Figure 3: ZOI Following Incubation, (A) L. Casei After 48 Hours of Incubation, (B) S. mutans after 24 Hours of Incubation, (C) Mean ZOI for L. casei, (D) Mean ZOI for S. mutans

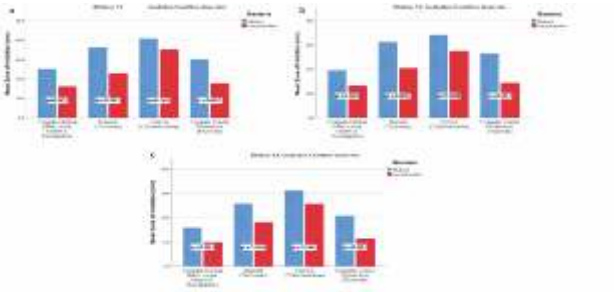

Figure 4: Comparison of Microbial Susceptibility at 37°C Under Aerobic Conditions at Various Dentifrice Dilutions: (A) 1:1 Dilution, (B) 1:2 Dilution, And (C) 1:4 Dilution

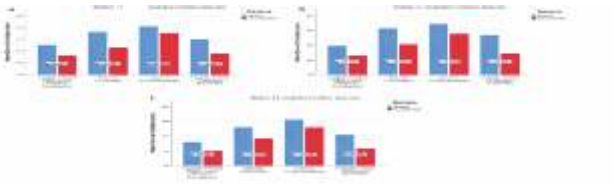

Figure 5: Comparison of Microbial Susceptibility at 37°C Under Aerobic Conditions at Various Dentifrice Dilutions: (A) 1:1 Dilution, (B) 1:2 Dilution, And (C) 1:4 Dilution.

Discussion

Globally, caries is considered one of the most prevalent oral diseases. The primary culprit for dental caries is *Streptococcus mutans* while lactobacilli are considered secondary invaders, mostly involved in the progression of the disease.[18] The first line of defence against dental caries is the maintenance of good oral hygiene. This largely involves tooth brushing and flossing.[19] With so many options of dentifrices available in the market, it is imperative to educate the masses about their caries' preventive potential. This study focuses on the antimicrobial capacity of four commercial dentifrices which are easily available in Pakistan.

A dentifrice mostly contains abrasive, binder, detergent, flavour, and any antibacterial agent.[20] Fluoride is also present in most conventional dentifrices. Herbal dentifrices have also been researched to showcase antibacterial potential against cariogenic bacteria.[21] In this research, Colgate Herbal, Colgate Cavity Protection, Bannet and Clinica toothpaste were evaluated. As mentioned in Table I, the active ingredients in Colgate Herbal include mint, clove, Neem, eucalyptus, and Fluoride. Mint leaves are known to exert an antibacterial effect through their components such as menthol, methyl esters and terpenoids.[22] Eucalyptus is also known to be effective against *S. mutans*. Fluoride is an active agent in both Colgate Herbal and Colgate Cavity Protection. The two toothpastes are well established as having antibacterial activity against several pathogens such as *E. coli, S. aureus,* and *C. albicans.*[23, 24] In addition, research has proven the antimicrobial prowess of triclosan and chlorhexidine, which are the main antibacterial agents in Bannet and Clinica dentifrices.[25,26] Three dilutions for each dentifrice (1:1, 1:2, and 1:4) are prepared and tested for antibacterial activity. This is done to somewhat mimic the salivary dilution of dentifrice in the oral cavity.[27] As depicted in Figure III, a ZOI is formed for both *S. mutans* and *L. casei* for each dilution of each dentifrice.

The study indicates that chlorhexidine shows the highest antimicrobial activity across all formulations. It is widely used in dentifrices and is the gold standard of antiplaque agents.[28] De Rossi *et al.* in their research proved that dentifrice containing chlorhexidine showed comparable antibacterial activity to those containing triclosan or natural components.[29] Racheli *et al.* published research concerning chlorhexidine's biocidal zombie effect in 2019. This effect involves the biocidal effect of bacterial cells that have been killed by chlorhexidine by acting as a reservoir for the antibacterial agent.[30]

Colgate Herbal exerted the least antibacterial effect on the tested bacteria. Another study comparing Colgate Herbal with other herbal dentifrices has documented relatively diminished antibacterial activity.[31] A study conducted in Iran found that chemical and herbal dentifrices exert the same antibacterial effect, however, the efficacy of herbal dentifrice is highly sensitive to its concentration.[15] Similar results were found from Patil *et al.* who proved that the herbal dentifrices containing neem

https://doi.org/10.57234/jiimc.september25.2417

have the same antibacterial potential as fluoride-containing dentifrice.[32]

It is evident from the results that the antimicrobial activity varies in a dose-dependent manner. Sarembe *et. al.,*[27] explored the effect of dentifrice's concentration on oral hygiene, concluding that increased concentration leads to higher antimicrobial activity. Another study found that the ZOI for any dentifrice diminishes as its dilution is increased.[33] It is further noted from Figures 4 and 5 that *S. mutans* was much more susceptible to all dentifrice formulations as compared to *L. casei.* This finding was consistent irrespective of dentifrice's dilution or bacteria's incubation conditions. Several studies have indicated the increased sensitivity of *S. mutans* against antibacterial agents.[34] Although there is data that *L. casei* is more sensitive to Neem-containing dentifrice than *S. mutans*, the results of this study indicated that Colgate Herbal containing Neem also exerted a much more pronounced antibacterial effect against *S. mutans* than *L. Casei.*[35] Another research conducted in India suggests that the Neem-containing dentifrices are just as effective against *S. mutans* as Fluoride-containing ones.[32]

It is evident from the intergroup comparison between active ingredients of dentifrices that both chlorhexidine and triclosan were more effective antimicrobial agents against *S. mutans* as compared to herbal ingredients or fluoride. This is in line with previous research that has shown comparable antimicrobial potential of the two active ingredients.[29] Therefore, both can be used in patients where the goal is to prevent the onset of dental caries. Although triclosan is an effective antibacterial agent, its use in soaps specifically has been banned by FDA since 2016.[36] This decision came after raising public concerns over the toxicity of triclosan and its extended effect on the human body. It is now classified as 'not generally recognized as safe and effective (GRASE)'. Despite this, its use in toothpastes is not banned as many products containing triclosan slip past the regulation process.[37] Therefore, further research exploring health effects of triclosan in toothpaste will add valuable information.

For *L. casei*, chlorhexidine showed greater antimicrobial activity than all other active components. These results suggest that dentifrices containing chlorhexidine (Clinica) as the active ingredient is most effective against both onset and progression of dental caries. This finding is in line with previous research that has proven chlorhexidine to be either just as effective or more effective than triclosan.[38] However, research has shown that antiplaque efficacy of chlorhexidine is reduced when used with Sodium Lauryl Sulphate (SLS) containing toothpaste.[13,39] This is due to the anionic nature of SLS surfactant that inhibits the antiplaque effect of the cationic chlorhexidine. Although most commercial toothpastes contain SLS, it is recommended to use a non-ionic surfactant to maintain the antibacterial activity of chlorhexidine.[40]

As the study was done to compare commercial dentifrices, it would be interesting to note the effects of antimicrobial agents in novel materials compared with commercial products. The authors suggest that the new formulations may be experimented with lower concentrations of the antimicrobial agents to eliminate any toxic effects associated with these agents.

Conclusion

Thus, in conclusion, the four dentifrices used in this study all showed antimicrobial activity against *S. mutans* and *L. casei* in both aerobic and anaerobic conditions across all formulations. However, the antimicrobial effect of the dentifrices was found to be dose dependent. Moreover, Chlorhexidine containing dentifrice was found to be best suited to prevent the onset and progression of dental caries.

Acknowledgement: None

Disclaimer: None

Conflict of Interest: The authors have no conflict of interest to disclose and no financial assistance was taken. Data has not been presented previously in any conference.

Funding Disclosure: The authors have no funding to disclose.

REFERENCES

1. Gasmi Benahmed A, Gasmi A, Dadar M, Arshad M, Bjørklund G. The role of sugar-rich diet and salivary proteins in dental plaque formation and oral health. *J oral Biosci*. 2021;63(2):134-141. doi:10.1016/j.job.2021.01.007.
2. P SM, Mallya S. Microbiology and Clinical Implications of Dental Caries – A Review. *J Evol Med Dent Sci*. 2020;9(48):3670-3675. doi:10.14260/jemds/2020/805.
3. Qin X, Zi H, Zeng X. Changes in the global burden of

untreated dental caries from 1990 to 2019: A systematic analysis for the Global Burden of Disease study. *Heliyon*. 2022;8(9):e10714. doi:10.1016/j.heliyon.2022.e10714.
4. Marinho VT, dos Reis AC, da Costa Valente ML. Efficacy of Antimicrobial Agents in Dentifrices: A Systematic Review. *Antibiotics*. 2022;11(10). doi:10.3390/antibiotics 11101413.
5. Pita-Fernández S, Pombo-Sánchez A, Suárez-Quintanilla J, Novio-Mallón S, Rivas-Mundiña B, Pértega-Díaz S. [Clinical relevance of tooth brushing in relation to dental caries]. *Aten primaria*. 2010;42(7):372-379. doi:10.1016/j.aprim. 2009.10.014.
6. Gupta A, Gallagher JE, Chestnutt IG, Godson J. Formulation and fluoride content of dentifrices: a review of current patterns. *Br Dent J*. Published online 2021. doi:10.1038/ s41415-021-3424-y.
7. Stovell AG, Newton BM, Lynch RJM. Important considerations in the development of toothpaste formulations for children. *Int Dent J*. 2013;63 Suppl 2:57-63. doi:10.1111/idj.12083.
8. Clark MB, Keels MA, Slayton RL. Fluoride Use in Caries Prevention in the Primary Care Setting. *Pediatrics*. 2020;146(6). doi:10.1542/peds.2020-034637.
9. Levy SM. An Update on Fluorides and Fluorosis . Published online 2003.
10. Gul H, Nayyer M, Gilani M, et al. Comparative Fluoride Release and Antimicrobial Analysis of Commercial and Experimental Bioactive Glass/Nano-Oxide-Based Dentifrices. *Eur J Dent*. 2020;14(1):38-44. doi:10.1055/s-0040-1701292.
11. Alfhili MA, Lee MH. Triclosan: An update on biochemical and molecular mechanisms. *Oxid Med Cell Longev*. 2019;2019. doi:10.1155/2019/1607304.
12. Poppolo Deus F, Ouanounou A. Chlorhexidine in Dentistry: Pharmacology, Uses, and Adverse Effects. *Int Dent J*. 2022;72(3):269-277. doi:10.1016/j.identj.2022.01.005.
13. Martu M alexandra, Stoleriu S, Pasarin L, Tudorancea D. TOOTHPASTES COMPOSITION AND THEIR ROLE IN Compoziţia clasică a pastei de dinţi. 2021;10(3).
14. Sunitha J, Ananthalakshmi R, Jeeva JS, Jeddy N, Dhakshininamoorthy S, Muthu Meenakshi RM. Antimicrobial effect of herbal dentifrices: An in vitro study. *J Pharm Bioallied Sci*. 2015;7(Suppl 2):S628-31. doi:10.4103/0975-7406.163575.
15. Kooshki F, Tabatabaei F, Tajik S, Aayan A. The comparison of antimicrobial effects of herbal and chemical agents on toothpaste: An experimental study. *Dent Res J (Isfahan)*. 2018;15(4):289-294. doi:10.4103/1735-3327.237247.
16. Haraszthy VI, Zambon JJ, Sreenivasan PK. Evaluation of the antimicrobial activity of dentifrices on human oral bacteria. *J Clin Dent*. 2010;21(4):96-100.
17. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*. 2021;10(2):1-21. doi:10.3390/pathogens10020165.
18. Abdul-Hussain Y, Juma'a Z, Fadhil R. Correlation between Caries Related Microorganisms in the Dental Plaque and Saliva with Dental Caries Level in the Upper and Lower Jaws in 5 - 9 Years Old Children in Baghdad City. *J Baghdad Coll Dent*. 2016;28(3):132-136. doi:10.12816/0031120.
19. Olczak-Kowalczyk D, Gozdowski D, Turska-Szybka A. Protective Factors for Early Childhood Caries in 3-Year-Old Children in Poland. *Front Pediatr*. 2021;9(March):1-10. doi:10.3389/fped.2021.583660.
20. Botoacă O, Albu CC, Bogdan-Andreescu C, Albu ŞD, Grama A, Defta C. Comparison of Antimicrobial Activity of two Commercial Toothpastes. 2025;15:356-371.
21. Karadağlıoğlu Öİ, Ulusoy N, Başer KHC, Hanoğlu A, Şık İ. Antibacterial activities of herbal toothpastes combined with essential oils against streptococcus mutans. *Pathogens*. 2019;8(1). doi:10.3390/pathogens8010020.
22. Banavar Ravi S, Nirupad S, Chippagiri P, Pandurangappa R. Antibacterial Effects of Natural Herbal Extracts on Streptococcus mutans: Can They Be Potential Additives in Dentifrices? *Int J Dent*. 2017;2017. doi:10.1155/2017/ 4921614.
23. Mbabazi I, Wangila P, K'owino IO. Antimicrobial activity of Euclea divinorum hern (ebenaceae) leaves, tender stems, root bark and an herbal toothpaste formulated from its ethanolic root bark extract. *Int J Res Reports Dent*. 2020;3(3):8-16. http://ir-library.kabianga.ac.ke/handle/ 123456789/151.
24. Vi V, Mamdapure S V. Evaluation of Antimicrobial Activity of Different Pepsodent and Colgate Toothpastes on Oral Flora of ' Lactobacillus acidophilus .' 2019;(51).
25. Poger D, Mark AE. Effect of Triclosan and Chloroxylenol on Bacterial Membranes. *J Phys Chem B*. 2019;123(25):5291-5301. doi:10.1021/acs.jpcb.9b02588.
26. Noor S. Chlorhexidine: Its properties and effects. *Res J Pharm Technol*. 2016;9(10):1755-1760.
27. Sarembe S, Ufer C, Kiesow A, et al. Influence of the Amount of Toothpaste on Cleaning Efficacy: An in Vitro Study. *Eur J Dent*. 2023;17(2):497-503. doi:10.1055/s-0042-1747953.
28. Meenakshi Rajendiran, Harsh M Trivedi, Dandan Chen, Praveen Gajendrareddy, Lin Chen. Recent Development of Active Ingredients in Mouthwashesand Toothpastes for Periodontal Diseases. *Molecules*. 2009;14(2):738-754.
29. de Rossi AD, Cunha Araújo Ferreira D, Assed Bezerra da Silva R, Mussolino de Queiroz A, Assed Bezerra da Silva L, Nelson-Filho P. Antimicrobial activity of toothpastes containing natural extracts, Chlorhexidine or triclosan. *Braz Dent J*. 2014;25(3):186-190. doi:10.1590/0103-6440201300027.
30. Ben-Knaz Wakshlak R, Pedahzur R, Avnir D. Antibacterial Activity of Chlorhexidine-Killed Bacteria: The Zombie Cell Effect. *ACS Omega*. 2019;4(25):20868-20872. doi:10.1021/acsomega.9b00297.
31. Peck MT, Africa CW, Stephen LX, Marnewick J, Majeed A. An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers. 2011;2(6):28-32.
32. Patil S, Venkataraghavan K, Anantharaj A, Patil S. Comparison of two commercially available toothpastes on the salivary streptococcus mutans count in urban preschool children-an in vivo study. *Group*. 2010;1(50):53720.
33. Roopavathi KM, Gopal SV, Pushpalatha G, Bennadi D, Renushri BV, Madhura AS. Antimicrobial efficacy of commercially available toothpastes - An in vitro study. *J Young Pharm*. 2015;7(3):187-193. doi:10.5530/jyp.

2015.3.8.
34. Guven Nilufer; Tuna, Elif Bahar; Aktoren, Oya YU. Antimicrobial Effect of Newly Formulated Toothpastes and a Mouthrinse on Specific Microorganisms: An In Vitro Study. *Eur J Dent*. 2019;13(02):172-177. doi:10.1055/s-0039-1695655.
35. Srichan R, Thaweboon B, Thaweboon S, Mala S. Antimicrobial activity of neem toothpaste against caries-associated microorganisms. 2021;41(July):213-218.
36. Papavasilopoulos RK, Kang S. Bibliometric Analysis: The Effects of Triclosan on Human Health. *Toxics*. 2022;10(9). doi:10.3390/toxics10090523.
37. Cosme-Silva L, Silveira A, Gasque K, Moretti-Neto R, Sakai V, Moretti AB. Antimicrobial activity of various brands of children's toothpastes formulated with Triclosan, Fluoride and Xylitol. *Brazilian Dent Sci*. 2019;22:344-348. doi:10.14295/bds.2019.v22i3.1739.
38. Paasanth M. Antimicrobial Efficacy of Different Toothpastes and Mouthrinses : An In Vitro Study. 2011;8(2):85-94.
39. Sheen S, Owens J, Addy M. The effect of toothpaste on the propensity of chlorhexidine and cetyl pyridinium chloride to produce staining in vitro: A possible predictor of inactivation. *J Clin Periodontol*. 2001;28(1):46-51. doi:10.1111/j.1600-051x.2001.280107.x.
40. Venu V, Prabhakar AR, Basappa N. Comparative evaluation of antibacterial property and substantivity of chlorhexidine containing dentifrices with sodium lauryl sulfate and Tween as surfactants: An in vivo study. *Indian J Dent Res*. 2013;24(4):521. doi:10.4103/0970-9290.118367.

CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.