



Original Research Article

Anti microbial effect of prebiotic containing tooth paste against *streptococcus mutans* and lactobacillus: An invitro evaluation

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ABSTRACT

Aim: To evaluate the antimicrobial efficacy of three commercially available toothpastes which are non-fluoridated, fluoridated and the one containing prebiotic ingredient on *Streptococcus mutans* and *Lactobacillus plantarum*.

Materials and Methods: Antimicrobial activities of prebiotic, fluoridated, non-fluoridated toothpastes were assessed at different concentrations (100ug, 200ug, 300ug and 400ug). Since prebiotic toothpaste had other proven antimicrobial agents, 100% inulin extract from chicory (prebiotic) was assessed for its antimicrobial efficacy at varying concentrations. Antimicrobial property was evaluated by determining the zone of inhibition using agar well diffusion method on Luria Bertani (LB) broth media for *S. mutans* and MRS broth media for *L.plantarum*. 100ul of inoculums of *S.mutans* and *L.plantarum* was poured on to the agar plates respectively. Five wells measuring 0.5cm was made, dentifrice at varying concentration i.e; 100ug, 200ug, 300ug and 400ug was loaded into respective wells. 50ul of DMSO was loaded in the well at the centre as control and incubated at 37°C for 24hrs.

Results: All the three tested toothpastes had significant antimicrobial activity against *S.mutans* and *L.plantarum*. The highest antimicrobial activity was exhibited by toothpaste containing prebiotic ingredient with mean zone of inhibition of (18.5+/-0.5) and (20.0+/-1) at 400ug against *S.mutans* and *L.plantarum*. There was no significant difference in antimicrobial activity of fluoridated and non-fluoridated toothpastes against *S.mutans* at 400ug (P=1), but there was a significant difference for *L.plantarum* at 400ug (P<0.001). Antimicrobial activity increased at higher concentrations for all the three tested toothpastes and for 100% Inulin extract.

Conclusion: All the three tested toothpaste were effective against *S.mutans* and *L.plantarum* at varying concentrations, but toothpaste containing prebiotic ingredient was most effective in inhibition of both the micro-organisms.

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1. Introduction

Dental caries is considered as a localized and transmissible pathological infectious process that results in the destruction of hard enamel tissue (Loesche 1986).¹ It is a complex disease which is expressed as an interaction between

various factors including the host, agent, substrate and time.² The primary etiological agents of dental caries are *Streptococcus mutans* and *Lactobacillus species*.³ Their interplay within the dental biofilm is an important feature for the establishment and maintenance of the micro flora and it is also linked to the development of a cariogenic dental plaque.⁴

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Dental plaque is considered as the precursor of dental caries, gingivitis and periodontitis because of it being a reservoir of microbial species.⁵ The oral bacterial species metabolize sucrose to lactic and other acids in the dental plaque and dissolve calcium phosphate in the enamel, consequently giving rise to dental caries.⁶ Therefore these micro-organisms need to be controlled in order to prevent the development of caries and periodontal diseases.⁷

The most effective measure for the prevention of plaque formation is the mechanical removal of dental plaque by regular tooth brushing in conjunction with chemical plaque control agents, such as toothpastes and mouthrinses.⁸ Since toothpaste is by far the most commonly used chemical plaque control agent, it is often used as a mode of delivery system for various chemical agents such as triclosan, chlorhexidine (CHX), metal ions, and essential oils to provide antimicrobial activity.⁹

Fluoride therapy has been the cornerstone of caries preventive strategies ever since the introduction of water fluoridation over five decades ago.¹ Decline in the prevalence of dental caries in developed countries, are mainly attributed to its increased use.¹⁰ The advantages of fluoridated products are well documented in the literature.¹¹ In the 1980s, it was established that fluoride controls caries mainly by its topical effect. Fluoride present in low, sustained concentrations (sub-ppm range) in the oral fluids during an acidic challenge is able to adsorb to the surface of the apatite crystals, inhibiting demineralization. When the pH is re-established, traces of fluoride in solution will make it highly supersaturated with respect to fluorhydroxyapatite, which speeds up the process of remineralization and renders enamel more resistant to caries.¹² However, it is the most spoken and debated topic all over the world with researchers having diverse views about its use and safety. The intensive promotion of fluoridated toothpastes by the oral health-care industry is a major factor in their increased use.¹¹

A safer approach to control microbial species among infants and children is the use of other alternative toothpaste which does not contain fluoride in their composition but has herbal products or enzymes to produce antiseptic or antimicrobial effects against cariogenic and opportunistic micro-organisms in the oral environment.¹³ The antimicrobial activity of the herbs is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins.¹⁴ Synergistic interactions between the principal components of these herbs play a vital role in improving their efficacy.¹³

Prebiotics have a proven complementary action with probiotics in the treatment of oral diseases. Prebiotics are non-digestible dietary supplements. Their function is to enhance the growth and activity of beneficial organisms (probiotics) and simultaneously suppress the growth and activity of potentially deleterious bacteria. The characteristic feature of prebiotic ingestion is to

alter the density of microbial population.¹⁵ Some of the commonly known prebiotics are Lactose, Inulin, Fructo oligosaccharides, Galacto oligosaccharides and Xylo oligosaccharides.¹⁶

Inulin is a generic term that includes all straight chain fructans consisting of fructosyl units linked by β -D^{1,2,17,18} and it is one of the most studied compounds, the prebiotic effect of which has been proved through in vitro and in vivo tests.¹⁹ This compound, which is a carbohydrate reserve of many plants, was first extracted from the root of Inula helenium by German scientist Rose in 1804 and was later called inulin in 1918.²⁰

Inulin is considered within the so-called fructans compounds and is present in fruits and vegetables, with its most common sources being wheat, onions, bananas, asparagus, chicory, garlic, leek. When tested at higher doses on animals there were no reported toxic effects.²¹ The plant that has higher amount of inulin-type fructan is chicory, a plant from the Compositae family, whose fresh root gives unfractionated inulin consisting of glucose, fructose, sucrose and small oligosaccharides.¹⁷ In chicory, inulin is stored as reserve carbohydrate in the fleshy taproot, which is equal to 15–20% inulin; thus, it can be considered as concentrated source of inulin. Chicory inulin contains up to 10% mono and disaccharides, mainly sucrose and fructose, and an oligosaccharide content of approximately 30%.¹⁸

The main and probably the most important prebiotic action of inulin on gut microflora is its ability to selectively stimulate the growth of health beneficial bacteria at the expense of pathogenic groups. This means that inulin completely alters the diversity and population of gut microbiota within few weeks of consumption.²² The effect of inulin consumption on the oral flora has not been investigated. Therefore it is important to determine the efficiency of antibacterial effect of different toothpaste brands which have different ingredients to reduce bacterial load in human mouth and contribute to dental health.²³ As Herbal formulations can provide an option for safe and long term use, the interest in alternative toothpaste based on plant extracts has increased recently.²⁴ Because of the popularity of natural ingredients in medicines and dentifrices continues to rise, dental professionals are in a position to provide information to patients about safety and efficacy of these products.⁷ Therefore we have attempted to evaluate the efficacy of Fluoride containing toothpaste, Non-Fluoridated toothpaste and Prebiotic containing toothpaste which are commercially available (over-the counter) on common caries inducing organisms like *Streptococcus mutans* and *Lactobacillus plantarum*.

2. Materials and Methods

Three toothpastes were selected for assessment of their in vitro antimicrobial activities. They were purchased from e-stores. The composition of these dentifrices is given in

below.

Tooth paste	Ingredients
Fluoridated toothpaste(Kidodent)	Sodium Monofluorophosphate + Triclosan + Xylitol
Non-fluoridated toothpaste (Mamaearth)	Water, glycerin, hydrated silica, sorbitol, xylitol, aloe barbadensis, lauryl glucoside, cellulose gum, natural strawberry, stevia.
Prebiotic toothpaste(Dessert essence)	Calcium carbonate, glycerin, aloe barbadensis leaf juice, melaleuca alternifolia (tea tree) leaf water, sodium lauroyl sarcosinate, cellulose gum, caprylic/capric triglyceride (mct oil), mentha piperita (peppermint) flower/leaf/stem oil, zingiber officinale extract (ginger), hydrolyzed pea protein, cichorium intybus (chicory) root extract, inulin (agave), perilla frutescens extract (perilla seed), glycyrrhiza glabra (licorice) root extract, equisetum avense leaf extract (horsetail), quillaja saponaria bark extract (soapbark), melia azadirachta (neem) bark extract, melaleuca alternifolia (tea tree) leaf oil, hamamelis virginiana (witch hazel) extract, glutathione, sodium bicarbonate (baking soda), zinc citrate, calcium ascorbate (vitamin C), phytic acid, sea salt, stevia rebaudiana leaf/stem powder.

2.1. Procedure

2.1.1. Well diffusion method to check the Minimum Inhibition Concentration (MIC)

The sample – fluoride containing toothpaste Kidodent(KD), non-fluoridated toothpaste Mamaearth (ME) and Prebiotic containing dessert essence (PRE) toothpaste, and as the major ingredient used in the prebiotic toothpaste was inulin, 100% Inulin(IN) extract from chicory were assessed for their MIC property against organisms viz. *Lactobacillus plantarum* and *Streptococcus mutans*.

2.2. Culture preparation

MRS broth media (Peptone 10g, Beef extract 10g, Yeast extract 5g, Dextrose 20g, Polysorbit 1g, Ammonium citrate 2g, Sodium acetate 5g, Magnesium Sulphate 0.1g, Manganese sulphate 0.05g, Dipotassium phosphate 2g, Distilled water 1000mL) 30mL was prepared by setting pH 6.5 and autoclaved at 121°C for 15 mins. Later, *Lactobacillus plantarum* strain NCIM 5396 was inoculated and incubated for 24h at 37°C.

Luria Bertani (LB) broth media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared and autoclaved at 121°C for 15

mins. Later, *Streptococcus mutans* strain MTCC 497 was inoculated and incubated for 24h at 37°C.

2.3. Sample preparation

10 mg of the sample (KD, ME and PRE and IN) was dissolved in 1mL of Dimethyl sulfoxide (DMSO) respectively. Different aliquots of the sample containing 100µg, 200µg, 300µg, and 400µg was prepared by pipetting 10µL, 20µL, 30µL and 40µL of the sample and the final volume was made up-to 50µL by adding DMSO.

2.4. Media preparation

Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 15g, Distilled water 1000mL) 500mL was prepared and autoclaved at 121°C for 15 mins.

2.5. Plate preparation

Approximately 25mL of the sterile media was poured into the sterilized petriplates and allowed it to solidify. Later, 24hrs cultured 100µL inoculum of *Lactobacillus plantarum* and *Streptococcus mutans* were poured on to the agar plates and spread throughout the plate using a spreader. Five wells measuring 0.5cm were made using well borer and the samples containing.

100µg, 200µg, 300µg, and 400µg are loaded into the respective wells and 50µL of DMSO loaded in the center well as control blank and incubated at 37°C for 24hrs.

2.6. Statistical analysis

The obtained data were tabulated and subjected to statistical analysis using one-way ANOVA test and post hoc-Tukey test.

3. Results

When we observed the effect of all 3 tested toothpastes on inhibition of *S.mutans* and *L.plantarum* growth, all 3 tested toothpastes were effective in inhibiting the growth of both the organisms, the zone of inhibition was greater for prebiotic containing toothpaste at all tested concentrations, with maximum of (18.5+/-0.5) and (20.0+/-1) for *S.mutans* and *L.plantarum* respectively at 400ug (Table 2), and the effect was statistically significant (p<0.001) when compared with fluoridated and non-fluoridated toothpaste. However, there was no statistically significant difference between fluoridated (15.5+/-0.5) and non-fluoridated toothpastes (15.5+/-0.5) (p=1) against *S.mutans*. There was a statistically significant difference (p<0.001) between fluoridated (17.5+/-0.5) and non-fluoridated toothpastes (12.5+/-0.5) in inhibition of *L.plantarum* at 400ug.(Table 4). The effectiveness of toothpastes increased with increasing concentration. Table 5

shows the zone of inhibition of 100% inulin against *S.mutans* and *L.plantarum*, which was also concentration, dependent and had its highest zone of inhibition of 11mm for *S.mutans* and 7 mm for *L.plantarum* at 400ug.

4. Discussion

Dental caries involves interactions between the tooth structure, the microbial biofilms formed on the tooth surface and sugars, as well as salivary and genetic influences.²⁵ The oral microbiota grows on surfaces as structurally and functionally organised communities of interacting species, termed as dental plaque.²⁶ The microbial community of dental caries is diverse and contains many obligate and facultative anaerobic bacteria.²⁷

The initiation of dental caries has close association with *S.mutans*. On the other hand, further progression of carious lesions has been attributed to *Lactobacillus* species.²⁸ Throughout history, brushing and flossing have been considered as the gold standard in controlling the plaque and preventing dental caries.²⁹ Since tooth brushing as an isolated effect, i.e., without therapeutic effect of antimicrobial agent, it has only a limited effect on caries control.^{30,31} Thus tooth brushing should be accompanied with chemotherapeutic agents such as toothpastes and mouth rinses which act as an ideal vehicle for daily delivery of antibacterial agents such as fluoride, CHX etc.

Fluoridated dentifrices are considered most effective caries control products and most commonly employed technique for caries reduction, and the effectiveness of fluoride toothpastes as an antimicrobial agent is concentration dependent. Excessive ingestion of fluoride may cause toxic and harmful effects. It is important to note that oral hygiene products are the major source of fluoride toxicity, with tooth paste being the main source. More than 80% of fluoride toxicity was reported in children below the age of 6 years as swallowing reflex is not completely developed. Some natural alternatives are available which is as efficient as Fluoride in preventing dental caries, gingivitis and plaque accumulation with no adverse effects even if they are accidentally swallowed.³²

According to recent developments, the oral microbial ecology can be modulated for the purpose of controlling diseases like dental caries and gingivitis that occurs due to micro-organisms. Oral microbial ecology can be effectively altered by use of prebiotic and probiotics. FAO/WHO defines prebiotics as a non-viable food component that confer health benefit(s) on the host, associated with modulation of the microbiota. The term prebiotics was coined in 1995 by Gibson and Roberfroid as a “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon”.³³ This definition has been adapted multiple times, with the most recent update into a “substrate that

is selectively utilized by host microorganisms conferring a health benefit”.³⁴ In caries management, these would include nutrients for microbial taxa that either inhibit acidogenic and aciduric microbes and/or enhance the pH recovery by generating alkali from these nutrients.^{35,36}

Some of the sources of prebiotics include: breast milk, soybeans, inulin sources (like Jerusalem artichoke, chicory roots etc.), raw oats, unrefined wheat, unrefined barley, yacon, non-digestible carbohydrates, and in particular non-digestible oligosaccharides. Among prebiotics only bifidogenic, non-digestible oligosaccharides particularly inulin, fulfil all the criteria for prebiotic classification.³⁷

Prebiotics like inulin and pectin exhibit several health benefits like reducing the prevalence and duration of diarrhea, relief from inflammation and other symptoms associated with intestinal bowel disorder and protective effects to prevent colon cancer.³⁸ They are also implicated in enhancing the bioavailability and uptake of minerals, lowering of some risk factors of cardiovascular disease, and promoting satiety and weight loss thus preventing obesity.³⁷ But there are only limited studies on prebiotics and its effect on oral health. No documented trials have been reported with its focus on prebiotic toothpaste use, especially in pediatric population.

In the present study, three commercially available toothpastes were used in four concentrations (100, 200, 300 and 400ug) to be tested for their antimicrobial efficacy against *S.mutans* and *L.plantarum* by agar-diffusion method. The antimicrobial efficacy of any natural or synthetic agent can be evaluated using broth dilution method, agar dilution method, disc diffusion method, agar well-diffusion method, and ditch-plate method. Nevertheless, agar well-diffusion method was used in the present study as it depends on the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing the test material.³⁹

Data from the present study exhibited wide variations in their effectiveness against two tested micro-organisms i.e. *S.mutans* and *L.plantarum*. This is largely due to the antimicrobial active ingredients used in toothpastes. Among all the investigated toothpastes, prebiotic containing toothpaste emerged as the most effective, based on the mean diameter of the zone of microbial inhibition produced in agar well diffusion method, against *S.mutans* and *L.plantarum*. This may be due to the presence of inulin in its formulation. As the prebiotic containing toothpaste used in the present study also had other proven antimicrobial agents, 100% inulin extract from chicory root was tested at various concentrations (100, 200, 300 and 400ug) (Table 5) using agar well diffusion method against both the organisms. This revealed that antimicrobial activity of inulin was also concentration dependent. Inulin exhibited maximum zone of inhibition of 11mm for *S.mutans* and 7mm for *L.plantarum*

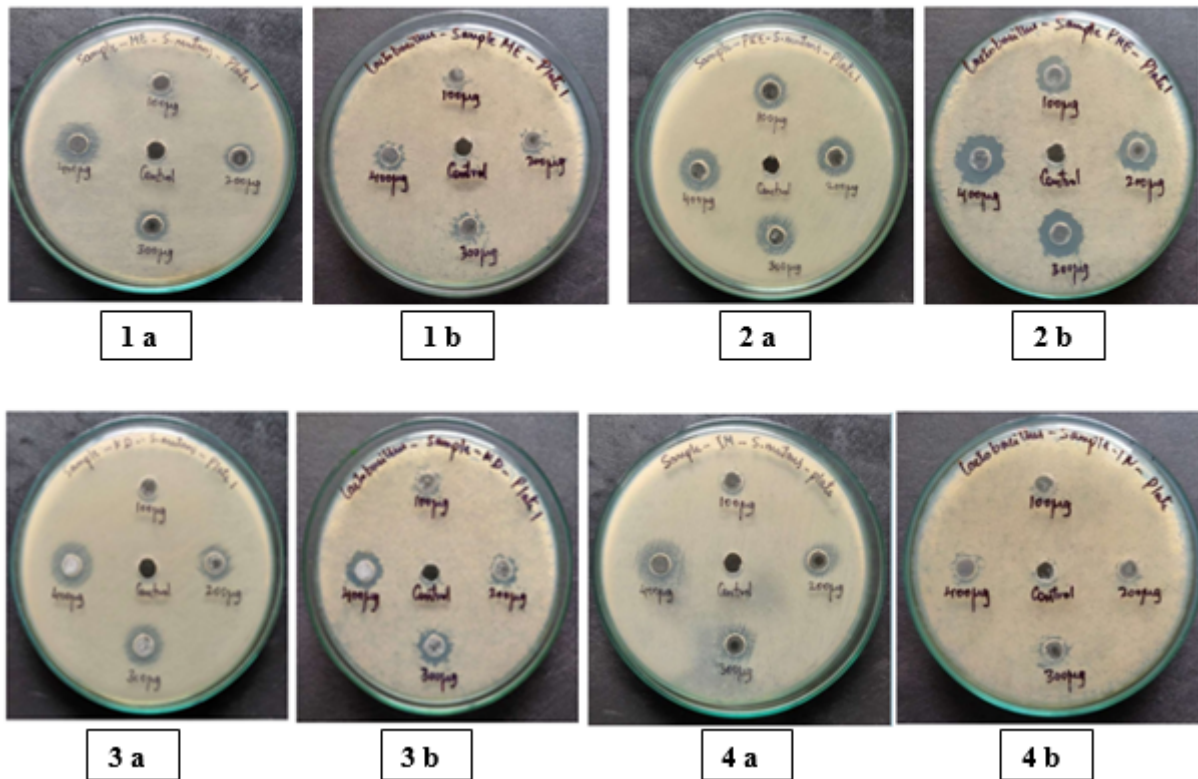


Fig. 1: ZOI of (1a) ME on *S.mutans* (1b) ME on *L. plantarum* (2a) PRE on *S.mutans* (2b) PRE on *L. plantarum* (3a) KD on *S.mutans* (3b) KD on *L. plantarum* (4a) IN on *S.mutans* (4b) IN on *L.plantarum*

Table 1: Comparison of mean ZOI among the study groups at various concentrations against s.mutans

		N	Mean	Std. Deviation	F	P
Sm100	KD	3	10.5	.50	153.9	0.0001*
	ME	3	.0	.0		
	PRE	3	12.0	.0		
Sm200	KD	3	12.0	1.0	36.6	0.0001*
	ME	3	10.0	.0		
	PRE	3	14.5	.50		
Sm300	KD	3	13.5	.50	33.5	0.0001*
	ME	3	12.5	.50		
	PRE	3	17.0	1.0		
Sm400	KD	3	15.5	.50	36.0	0.0001*
	ME	3	15.5	.50		
	PRE	3	18.5	.50		

at 400ug. Although inulin had antimicrobial property against both the organisms, other antimicrobial ingredients in the prebiotic toothpaste may also have contributed in enhancing the action against both the organisms.

Oral cavity is a complex ecosystem, which has a rich and diverse microbiota. The wide range of pH, nutrient availability, shedding and non shedding surfaces, salivary and crevicular fluids are the contributing elements. These fluids select localized, discrete microbial climax communities during the metabolic activity⁴⁰ and this reaches a homeostasis, inspite of fluctuations in

the composition of these fluids. But any change in the environment disturbs the homeostasis and causes diseases. A dental disease is observed even with a slight change in the local environment. This leads the potential pathogens to gain competitive advantage under appropriate conditions. The function of prebiotics is to enhance the growth and activity of beneficial organisms and simultaneously suppress the growth and activity of potentially deleterious bacteria.⁴¹ Nunpan et al in 2019 showed that GOS (galactooligosaccharides) and FOS (fructooligosaccharides), which are polysaccharide

Table 2: Pair wise comparison for s.mutans

Dependent variable	(I) grp	(J) grp	Mean Difference	STD. Error	Sig.
Sm100	KD	ME	10.50*	0.23	0.001*
		PRE	-1.500*	0.23	0.002*
	ME	PRE	-12.00*	0.23	0.001*
Sm200	KD	ME	2.0*	0.52	0.021*
		PRE	-2.50*	0.52	0.008*
	ME	PRE	-4.50*	0.52	0.001*
Sm300	KD	ME	1	0.57	0.269
		PRE	-3.50*	0.57	0.002*
	ME	PRE	-4.50*	0.57	0.001*
Sm400	KD	ME	0	0.40	1
		PRE	-3.00*	0.40	0.001*
	ME	PRE	-3.00*	0.40	0.001*

Table 3: Comparison of mean zoi among the study groups at various concentrations against l.plantarum

		N	Mean	Std. Deviation	F	P
Ip100	KD	3	11.5	.50	868.5	0.001*
	ME	3	.0	.00		
	PRE	3	12.5	.50		
Ip200	KD	3	14.5	.50	1345	0.001*
	ME	3	.0	.00		
	PRE	3	15.5	.50		
Ip300	KD	3	17.0	.00	199.5	0.001*
	ME	3	11.5	.50		
	PRE	3	17.5	.50		
Ip400	KD	3	17.5	.50	87.5	0.001*
	ME	3	12.5	.50		
	PRE	3	20.0	1.0		

Table 4: Pairwise comparison for *lactobacillus plantarum*

Dependent Variable	(I) grp	(J)grp	Mean Difference (I-J)	Std. Error	Sig.
Ip100	KD	ME	11.5*	0.33	0.001*
		PRE	-1	0.33	0.054
	ME	PRE	-12.5*	0.33	0.001*
Ip200	KD	ME	14.5*	0.33	0.001*
		PRE	-1	0.33	0.054
	ME	PRE	-15.5*	0.33	0.001*
Ip300	KD	ME	5.50*	0.33	0.001*
		PRE	-0.5	0.33	0.355
	ME	PRE	-6.0*	0.33	0.001*
Ip400	KD	ME	5.0*	0.58	0.001*
		PRE	-2.50*	0.58	0.012*
	ME	PRE	-7.50*	0.58	0.001*

Table 5: Antimicrobail activity of inulin against s.mutans and l.plantarum at various concentrations

Organism Plates	Zone of Inhibition against Pathogens in Miliimeter			
	100 Plate1	Plate1	Plate1	Plate1
L. Plantarum	NIL	NIL	5mm	7mm
S.Mutans	NIL	6mm	8mm	11mm

Table 6: Antimicrobial activity of three toothpastes (KD)(Kidodent) ME (Mama earth) and PRE (Prebiotic) used in the study

Zone of inhibition of Pathogens in Millimeter					
Sample KD in μg	L. plantarum		S.mutans		
	Plate 1	Plate 2	Plate 1	Plate 2	
100	11	12	11	10	
200	15	14	13	11	
300	17	17	14	13	
400	18	17	15	16	
Zone of inhibition of Pathogens in Millimeter					
Sample ME in μg	L. plantarum		S.mutans		
	Plate 1	Plate 2	Plate 1	Plate 2	
100	-	-	-	-	
200	-	-	10	10	
300	11	12	12	13	
400	12	13	16	15	
Zone of inhibition of Pathogens in Millimeter					
Sample PRE in μg	L. plantarum		S.mutans		
	Plate 1	Plate 2	Plate 1	Plate 2	
100	12	13	12	12	
200	16	15	14	15	
300	18	17	18	16	
400	19	21	18	19	

prebiotics, did not support the growth rate of *S. mutans* and *L. acidophilus* making it useful in treatment of high caries-risk patients.⁴² Inulin is a type of FOS. The added advantage of using prebiotic toothpaste is that, Prebiotics enhances the growth and/or increase the activity of the probiotic organisms in the host, thereby preventing the risk of dental caries.

Fluoride is effective at controlling caries because it acts in several different ways. When present in dental plaque and saliva, it delays the demineralization and promotes the remineralization of incipient enamel lesions, and it also interferes with glycolysis, the process by which cariogenic bacteria metabolize sugars to produce acid. In higher concentrations, it has a bactericidal action on cariogenic and other bacteria.⁴³ Our study showed that fluoridated toothpaste was more effective than non-fluoridated toothpaste in inhibition of *S.mutans* and *L.plantarum*. The gradual decrease in caries has been long attributed to the regular use of fluoride in toothpaste.⁴⁴ In fluoridated dentifrice, fluoride is mostly available in the form of sodium fluoride or sodium mono-fluorophosphate. Sodium fluoride is more effective in preventing caries in children when compared with the sodium mono-fluorophosphate.⁴⁵ The fluoridated toothpaste we used contained sodium mono-fluorophosphate which may have resulted in the decreased zone of inhibition against the tested organisms when compared to prebiotic toothpaste.

Based on school based trials of twice tooth brushing with toothpaste containing 10% xylitol added to 0.243% sodium fluoride or 0.836% sodium mono-fluorophosphate concluded that there was a significant reduction of caries

after 3years.⁴⁶ On the contrary Chi et al, in 2014 reported, that tooth brushing with a xylitol toothpaste resulted in no therapeutic benefit compared to fluoride-only toothpaste, as there was no reduction in *S.mutans*, and increase in dmfs was seen in high risk group.⁴⁷ Various in-vitro and animal studies have demonstrated that fluoride was more effective than xylitol alone or in combination with fluoride toothpaste against *S.mutans*.⁴⁷ Randall et al in 2015, concluded that antimicrobial activity of commercial dentifrices against *S. mutans* may be due to the presence of components other than fluoride, such as triclosan and sodium lauryl sulphate which have higher antimicrobial effects than fluorides.⁴⁸

Xylitol is used in food as a sugar substitute and does not lead to decrease in *S.mutans* salivary level.⁴⁹ It must be used in full contact with the surface of the teeth in a frequent and direct manner to obtain the maximum effect.^{49,50} Though xylitol is included in many dental prophylactic programs as an acknowledged anticariogenic agent, there is a shortage of evidence based data indicating the effectiveness of xylitol, apart from those pertaining to fluoride toothpastes with the addition of xylitol.⁵¹ When children, were instructed to brush their teeth twice a day with xylitol toothpaste (0.2g xylitol/day), they had low levels of salivary (0.81ml) and plaque (0.89 CFU/sample) *S.mutans* after 6 months.⁵² But the toothpaste also had triclosan along with xylitol, which would have added to its efficacy. We found similar results, as the surfactant– sodium lauroyl sarcosinate in the xylitol toothpaste must have caused it to become less active.⁵³

Among the three tested toothpaste, the one containing prebiotic proved to have higher antimicrobial action. Prebiotic components promotes the growth of probiotics, which has an anticaries effect. In in-vivo conditions, the

action of prebiotic may work in synergy with probiotics thereby enhancing the antimicrobial efficacy. Therefore, there is a need to further explore this action in in-vivo conditions.

5. Conclusion

This study was aimed to reveal the influence of prebiotic, fluoride and non-fluoride toothpastes on *S.mutans* and *L.plantarum*. All the three tested toothpastes were effective against *S.mutans* and *L.plantarum* at varying concentrations, but prebiotic containing toothpaste was more effective in inhibition of *S.mutans* and *L.plantarum*. Antimicrobial activity increased with increased concentration for all the three tested toothpastes.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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