AN ANTIMICROBIAL EVALUATION OF COMMERCIALLY AVAILABLE PEDIATRIC DENTAL HERBAL DENTIFRICES AGAINST STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS: AN INVITRO STUDY.

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ABSTRACT: Aims and objectives: To investigate the antimicrobial efficacy of different dentifrices and to study the variations in their effectiveness against the test microorganisms.

Materials and methods: The study groups were divided based on the compound present in these dentifrices: (I) Calcium sucrose phosphate (II)Sorbitol (III)Maltitol (IV)Fennel (V) positive control (VI) negative control. The antimicrobial efficacy of one herbal and three commercially available toothpastes with different ingredients were evaluated against S. mutans and L. acidophilus by the zone of inhibition on the culture plates were measured.

Results: The data reflected differential response by the bacteria to the dentifrices. The size of the zone of inhibition against S. mutans were in order: positive control >Calcium sucrose phosphate= Sorbitol > Maltitol > Fennel > negative control. L. acidophilus response showed the order: positive control > Calcium sucrose phosphate= sorbitol > Maltitol > Fennel = negative control.

Conclusion: The results of this study have revealed the differences in antimicrobial efficacy of different dentifrices. It has been demonstrated that Calcium sucrose phosphate containing dentifrice formulations are more effective in control of oral microflora.

Keywords:
Toothpaste, antimicrobial, S.mutans, Calcium sucrose phosphate

Conflict of interest: Nil
No conflicts of interest : Nil

INTRODUCTION: Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental tissue. It results from accumulation of plaque on the surface of the teeth and biochemical activities of complex micro-communities.1Streptococcus mutansis one of the main opportunistic pathogens of dental caries, which plays a central role in fermenting carbohydrates resulting in acid production and leading to the demineralization of the tooth enamel.2

The dentifrices are one of the viable options which can be of great help in caries reduction. Dental plaque forms continuously on tooth surfaces in allage groups and must be removed to promote oral hygiene. Streptococcus mutans and Lactobacillus acidophilus are important bacterial species implicated in dental caries.3

Toothpastes contain active ingredients or additives that perform specific functions,

herbal formulations and calcium sucrose phosphate formulations have been introduced in certain dentifrices.4The aim of this invitro study was to investigate the antimicrobial efficacy of different dentifrices and to study the variations in their effectiveness against the test microorganisms.

MATERIAL AND METHODS: In the present invitro study Streptococcus mutans and Lactobacillus acidophilus were selected as test microorganisms against which different dentifrices were tested. The procedure included the evaluation of dentifrices, antimicrobial assay by modified agar well diffusion method.

The study groups were divided based on the compound present in these dentifrices: (I) Calcium sucrose phosphate

(II) Sorbitol (III) Maltitol (IV) Fennel (V) Positive control (VI) Negative control. The antimicrobial efficacy of one herbal and three commercially available toothpastes with different ingredients were evaluated against S. mutans and L. acidophilus by the zone of inhibition.

Table 1: Groups and ingredients of dentifrice

Number	Ingredients	Other ingredients in dentifrice
I	Calcium sucrose phosphate	-
II	Sorbitol	Glycerine, silica, purified water, sodium lauryl sulphate, sodium carboxymethyl cellulose, sodium monoflourophosphate, saccharin, sodium methyl hydroxybenzoate, sodium propyl hydroxybenzoate.
III	Maltitol	Dicalcium phosphate dihydrate, purified water, glycerin, trimagnesium phosphate, carrageenan, flavour, sodium lauryl sulfate, methylparaben, propylparaben, tartrazine, carmoisine
IV	Saunf	Saunf, mulethi, meswak, neem, babool, pudina, laung, tomar, triclosan, sorbitol, silica, glycerine, sodium benzoate, sls powder
V	Positive control	
VI	Negative control	1% hydroxyethylcellulose gel

The microorganism Streptococcus mutans and Lactobacillus acidophilus were inoculated in the Muellar Hilton agar and rogosa agar plates respectively. Two petri plates were prepared with each microorganism. A lawn culture of the bacterial isolate was made on both the petri plates. The dentifrice and controls were mixed with saline to prepare a solution with 1:1 dilution. The prepared solution was placed into the three wells dug/plate as group 1,2 and 3 and group 4, 5 and 6 of two petri plates. After incubation at 37° C for 48 hrs the zones of inhibition were measured in mm. The zones of inhibition indicate the antimicrobial activity and generally, higher diameter of zones of inhibition indicated better antibacterial efficacy.

Data was entered in Microsoft excel 2016 for Windows. Mean, standard deviation (SD), minimum and maximum values of zone of inhibitions of different toothpastes and control against Lactobacillus acidophilus and Streptococcus mutans were calculated. Shapiro-Wilk test showed that zone of inhibitions did not follow normal distribution. Hence, non-parametric test- Kruskal-Wallis test was applied for comparison. When Kruskal-Wallis test showed significant results, Mann-Whitney U test was applied for pairwise comparison. P value <0.05 was considered statistically significant. Data analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA).

RESULTS: The data reflected differential response by the bacteria to the dentifrices. The size of the zone of inhibition against S. mutans were in order: positive control > Calcium sucrose phosphate= Sorbitol > Maltitol >Fennel> negative control. L. acidophilus response showed the order: positive control > Calcium sucrose phosphate= Sorbitol > Maltitol >Fennel = negative control. The calcium sucrose phosphate and positive control were comparable & significant. This investigation showed that toothpaste having naturalformulation gave maximum zones of inhibition against Streptococcus mutansat all dilutions and Lactobacillus acidophilus at 1:1 dilution.

Kruskal-Wallis test showed significant difference between different toothpastes and control for antimicrobial activity against Lactobacillus acidophilus. After this Mann-Whitney U test was applied for pairwise comparison, which showed that largest zone of inhibition was observed with group 5, followed by group 1 and group 2. There was no significant difference between group 1 and group 2. Zone of inhibition with group 3 was significantly larger than group 4 and group 6 but smaller than group 1 and group 2. There was no effect of group 4 and group 6 (zone of inhibition was zero mm).

Kruskal-Wallis test showed significant difference between different toothpastes and control for antimicrobial activity against Streptococcus mutans. After this Mann-Whitney U test was applied for pairwise comparison, which showed that largest zone of inhibition was observed with positive control, followed by group 1 and group 2. There was no significant difference between group 1 and group 2. Zone of inhibitions with group 3 and group 4 were significantly larger than negative control but smaller than group 1 and group 2. There was no significant difference between group 3 and group 4. There was no effect of negative control.

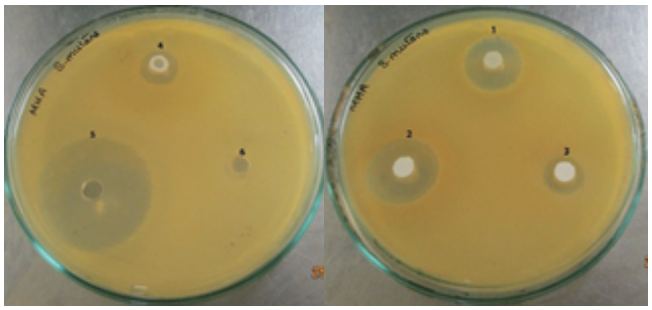


Fig 1: Zone of inhibition of Streptococcus mutans

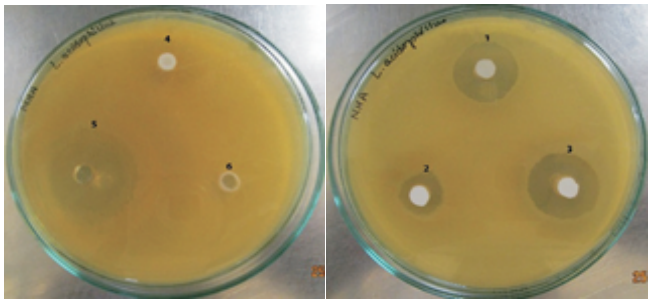
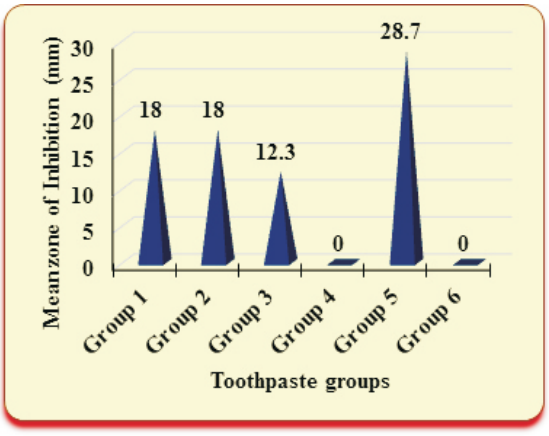
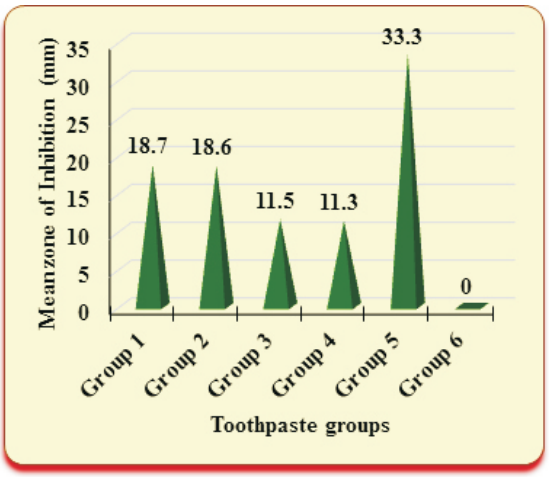


Fig 2: Zone of inhibition of Lactobacillus acidophilus

Graph 1: Antimicrobial activity of different toothpastes and control against Lactobacillus acidophilus



Graph 2: Antimicrobial activity of different toothpastes and control against Streptococcus mutans.



DISCUSSION: Commercially available dentifrice nowadays claims to be effective antimicrobial agents against oral microorganism. Hence, the present study was undertaken to assess the in-vitro antimicrobial efficacy of commercially available dentifrice used in children. The dentifrices tested contained several different constituents with anti-inflammatory and anti-bacterial properties, which could be useful in controlling the oral microbes.2

The microorganisms used in the present study include S. mutans and L.acidophilus. S. mutanshas been strongly associated with the initiation of caries, while there is a correlation between Lactobacilli is involved in the further development of carious lesions.5It has been established that Streptococcus mutansplays a major role in the tooth decay by metabolizing sucrose to lactic acid.2 Thus, it would be beneficial if oral formulations having established antimicrobial properties against common oral micro-organisms.

Dental plaque plays a key role in initiation of dental caries. The plaque bacteria produce acidic environment and series of microenvironment alterations progress to cavitation. Hence, antibacterial efficacy of dentifrices is one of the key factors in selection. The ingredients present having antibacterial properties kill the microbes and reduce their growth and colonization on tooth surface. Results of present study suggest that products evaluated exhibited wide variations in their effectiveness against the test microorganisms, due to their antimicrobial content they were effective against both the bacteria.6

Nowadays, newerremineralizing agents are available like Anticay which is a mixture of calcium sucrose phosphate with inorganic amorphous calcium phosphate.7In this study, dentifrice containing calcium sucrose phosphate showed mean zone of inhibition of 18mm and 18.7mm against S. mutans and L. acidophilus respectively.

Fennel (Foeniculum vulgare) containing dentifrice showed mean zone of inhibition of 11.3mm against S.mutans and had no antimicrobial activity against L. acidophilus. It is an annual erbaceous plant of family Apiaceae andis widely cultivated throughout the temperate and tropical regions of the world.8The results of this study were in concurrence with Agrawal D et al. (2017).

Sugar substitutes proposed to have anticariogenic properties. These include lactitol, maltitol, mannitol, sorbitol, isomalt, and xylitol and are commonly used in foods to replace sugars. Dentifrices usually contain sorbitol and very few contain maltitol as sweeteners.9The dentifrice containing sorbitol showed mean zone of inhibition of 18.6 mm and 18mm against S. mutans and L. acidophilus respectively whereas dentifrice containing maltitol showed mean zone of inhibition of 11.5 mm and 12.3 mm against S. mutans and L. acidophilus respectively. The results were in accordance with Prasanth (2011) where they have evaluated zone of inhibition of various toothpaste and mouthrinse against S. mutans, E. coli and C. albicans.

The dentifrice containing natural antimicrobial agents were found equally effective in controlling the oral microflora compared to toothpastes containing synthetic antimicrobial agents. Amongst different formulations CaSP containing was found to be the most effective because of high remineralization potential.

CONCLUSION: The results of this study have revealed the differences in antimicrobial efficacy of different dentifrices. It has been demonstrated that Calcium sucrose phosphate containing dentifrice formulations are more effective in

control of oral microflora.

REFERENCES:

1. Prasanth M. Antimicrobial Efficacy of Different Toothpastes and Mouthrinses: An In Vitro Study. Dent Res J; 2011; 8(2): 85-94.
2. Kallahalli MR, Gopal SV, Govindaraju P, Bennadi P, Bhadravathi VR, Antharasanahalli SM. Antimicrobial Efficacy of Commercially Available Toothpastes - An In vitro Study; J Young Pharma; 2015; 7(3): 187-193.
3. Brambilla E,Ionescu A, Cazzaniga G, Edefonti V, Gagliani M. The influence of antibacterial toothpastes on in vitro Streptococcus mutansbiofilm formation: A continuous culture study; Amer J Dent; 2014; 27(3):160-6.
4. Malhotra R, Singla S, Shashikiran ND. Comparison of Antimicrobial Activity of Child Formula Dentifrices at different Concentrations: An in vitro Study; Int J Clin Pediatr Dent; 2017; 10(2): 131-5.
5. George D, Bhat SS, Antony B. Comparative evaluation of the antimicrobial efficacy of aloe vera tooth gel and two popular commercial toothpastes: An in vitro study; Gen Dent; 2009; 238-41.
6. Mohan KKP, Priya NK, Madhushankari GS. Anti-Cariogenic Efficacy of Herbal and Conventional Tooth Pastes - A Comparative In-Vitro Study. J Int Oral Health; 2013; 5(2): 8-13.
7. Titty TM, Shrikrishna SB, Rao A, Shenoy R, Natarajan S. Remineralizing effectiveness of calcium sucrose phosphate and fluoride dentifrices: An In vitro study; Contemp Clin Dent 2018; 9: 276-82.
8. Agarwal D, Sharma LK, Saxena SN. Anti-microbial properties of fennel (Foeniculum vulgare Mill.) seed extract; J Pharma Phyto; 2017; 6(4): 479-82.
9. Shwetha R, Vivek S. Effect of dentifrices containing sorbitol, combination of xylitol and sorbitol on salivary Streptococcus mutansand Lactobacillus counts in 14-15 years old children: a randomized trial; Int J Clin Trials; 2017; 4(4): 184-90.

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