Original Article

Antimicrobial Efficacy of Different Toothpastes and Mouthrinses: An In Vitro Study

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ABSTRACT

Background: Anti-microbial agents have been used as a chemotherapeutic agent to improve oral health. This *in vitro* study was carried out to determine antimicrobial efficacy of different toothpastes and mouthrinses against the oral pathogens.

Methods: A total of five toothpastes and five mouthrinses were tested for their antimicrobial activity against three oral pathogens namely, *Streptococcus mutans* (MTCC 890), *Escherichia coli* (MTCC 579) and *Candida albicans* (MTCC 854) by well agar diffusion assay. Statistical Analysis was performed using a statistical package, SPSS windows version 15, by applying mean values using analysis of variance (ANOVA) with post-hoc least square differences (LSD) method($\alpha = 0.05$).

Results: Toothpaste formulation A showed maximum zones of inhibition against the test organism, *Escherichia coli* ($P<0.001$) compared to all other toothpastes formulations. Against *Streptococcus mutans* and *Candida albicans*, the zones of inhibition were less in comparison to *E.coli* but were significantly different at higher dilutions (1:8, 1:16 $P<0.05$) for toothpaste formulation A.

Mouthrinses formulation H showed maximum efficacy against the test organism, *Escherichia coli* ($P<0.001$) compared to all other mouthrinse formulations. Against *Streptococcus mutans*, mouthrinses formulations F, G and J showed significant antimicrobial activity ($P<0.05$) compared to formulation H and I.

Conclusion: In the present study, it has been demonstrated that triclosan containing toothpastes formulations are more effective in control of oral microflora compared to non-triclosan containing synthetic toothpastes. Among mouthrinses formulations, chlorhexidine was found to be more effective than or as effective as triclosan against the organisms tested.

***Keywords*:** Antimicrobial activity**,** Antimicrobial agents, Chlorhexidine gluconate, Mouthrinse, Toothpaste, Triclosan.

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Introduction

In India, as in other developing countries, a very significant proportion of dental problems are due to microbial infections. Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases.[1]

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. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries,[2] which plays a central role in fermenting carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel.

In addition, other microflora like *Escherichia coli* and *Candida* are also associated with active caries lesions. *C. albicans* is th-e most common yeast isolated from the oral cavity. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intercanal medication.[3] Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities.

Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar

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bone). The endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, an inflammatory condition of gum, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may results in tooth loss. *Streptococci*, *spirochetes* and *bacteroides* are found to be the possible pathogens responsible for the disease.

In many individuals, the customary oral hygiene method of tooth brushing is, by itself, usually insufficient over a long period to provide a level of plaque control consistent with oral health. Consequently, the incorporation of chemical agents with antiplaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease.[4] The use of antimicrobial chemotherapeutic agent has been proposed as a means of reducing the levels of oral bacteria, specifically *Streptococcus mutans.*[5]

Recently, Triclosan, a low-toxicity, non-ionic phenolic derivative with a wide spectrum of antimicrobial activity has been successfully incorporated into toothpastes and mouthrinses, resulting in moderate but distinct positive effects on both dental biofilm and marginal inflammation or gingivitis.[6] There is evidence indicating that the ingredients in the formula of triclosan-containing mouthwashes, including vehicle and other active substances, may influence its antimicrobial activity, and consequently its clinical efficiency.[7]

Triclosan has been used for over 30 years in skincare products, such as soaps, deodorants, and creams. In dentistry, it was first used in a European toothpaste in 1985.[8] Today triclosan is the active ingredient in many oral hygiene formulations. McMurry et al.[9] demonstrated in a study with *Escherichia coli* that the antiseptic activity of triclosan is due to its ability to block the synthesis of fatty acids by inhibiting the enoyl-acyl carrier protein reductase enzyme.

Dentifrices need to contain various antimicrobial agents in order to reduce, control and prevent different kinds of dental diseases. Many dentifrices claim to have antimicrobial properties but very little research has been conducted to investigate these claims. Based on this scanty information, the present study was designed to investigate antimicrobial efficacy of different toothpastes and mouthrinses by using standard agar well diffusion method.

Materials and Methods

Microorganisms

Pure cultures of *Candida albicans* (MTCC 854), *Escherichia coli* (MTCC 579) and *Streptococcus mutans* (MTCC 890) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures of *Candida albicans* (MTCC 854), *Escherichia coli* (MTCC 579) were cultured in nutrient broth (Hi-Media) at 37°C for 24 h while *Candida albicans* was cultured for 48 hours. *Streptococcus mutans* (MTCC 890) was cultured in brain heart infusion broth (Hi-Media) at 37°C for 24 h.

Evaluation of Dentifrices

The survey was aimed at knowing the brands of toothpastes and mouthrinses that are mostly used. As a result, five toothpastes and five mouthrinses were selected for assessment of their *in vitro* antimicrobial activities. They were purchased from local markets in Hyderabad, Andhra Pradesh, India. The composition of these dentifrices is given in Tables 1 and 2. The selected dentifrices solutions were made by mixing the calculated amount of toothpastes (2.0 gm) in measured volume (2 ml) of sterile pyrogen-free distilled water to give 1:1 dilution; they were further diluted in sterile distilled water and four different dilutions of 1:2, 1:4, 1:8 and 1:16 were made. Similarly, each mouthrinse (2 ml) was mixed with 2 ml of sterile distilled water and serial dilutions were made as above. Nutrient agar and brain heart infusion agar plates were prepared to assess the antimicrobial activity of dentifrices against the pathogens. All other chemicals and reagents used were of analytical grade.

Antimicrobial assay

The antimicrobial activity of different concentrations of the dentifrices was determined by modified agar well diffusion method.[10,11] In this method, nutrient agar plates were seeded with 0.5 mL of 24 h broth cultures of each isolate (brain heart infusion agar was used for *Streptococcus mutans* strain). The plates were allowed to dry for 1 h. A sterile 8 mm cork-borer was used to cut one central and five wells at equidistance in each of the plates. 0.2 mL of the dentifrice dilutions was introduced into each of the five wells while the same amount of sterile distilled water was introduced into the first well as control. The plates were incubated at 37°C for 24 h (48 h for yeast species). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (in mm) (Figure 1). All the plates were made in triplicates and the experiments repeated thrice.

Table 1. Ingredients of various toothpastes tested for antimicrobial potential

Toothpastes	Ingredients as listed on packages
A	Triclosan, Sodium monofluoro phosphate, Sorbitol and Flavor.
B	Sodium monofluorophosphate, Calcium Carbonate, Sorbitol.
C	Sorbitol, Water, Hydrated silica, Sodium lauryl Sulfate, PEG-32, Flavor, Cellulose gum, Sodium fluoride, Sodium saccharin, Cl -16255, Cl –17200.
D	Babhul, Jambhul, Lavang, Manjishtha, Dalchini, Bor, Vajradanti, Acrod, Khair,Patang,Akkal kadha, Bakula, Jesthamadh, Kabab chini (Chirfal), Anant mul,Maifal, Trifala (Amal,Harda,Behada), Ajwan, Calcium- Carbonate, Tragacanth gum, Sorbitol, Methyl paraben sodium, propyl paraben sodium, Sodium Lauryl Sulfate, Sodium hydroxide, Flavor, water.
E	Dadima (punicagranatum), Sodium benzoate, Bonopol, Tumburu (Xanthoxylum alatum), Babbula (Acacia arabica), Triphala, Vidanga (Embelia ribes), Nirgundi (Vitex negundo), Vaikranta bhasma, Nimba (Azadirachta indica), Ajamoda satva, Pilu (Salvadora persica), Irimeda (Acacia farnesiana), Khadira (Acacia catechu), Bakula (Mimosops elengi), Sweetener, Saccharine

Table 2. Ingredients of various Mouthrinses tested for antimicrobial potential

Mouthrinses	Ingredients as listed on packages
F	Triclosan, Sodium fluoride, Ethyl Alcohol.
G	Chlorhexidine Gluconate.
H	Chlorhexidine Gluconate, Sodium fluoride, Zinc Chloride.
I	Potassium Nitrate, Sodium Fluoride
J	Triclosan, Sodium fluoride, Alcohol

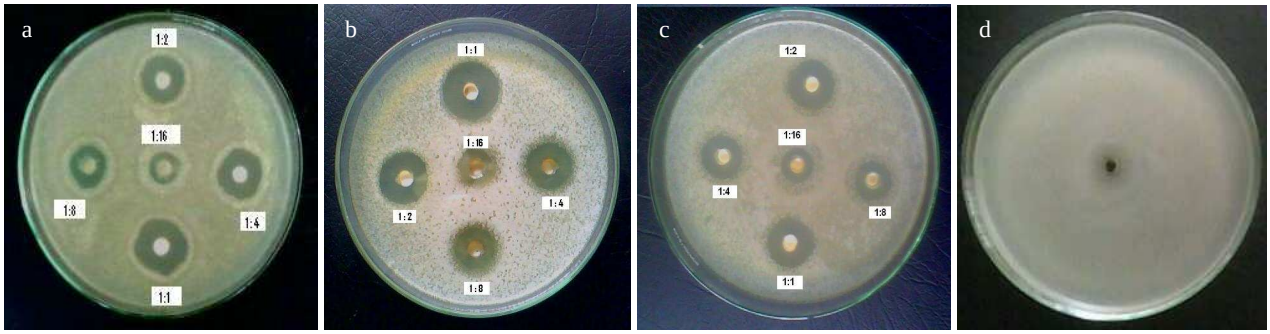

Figure 1. Zones of inhibition produced by toothpaste formulation A at 24 h against the three tested microorganisms at five different dilutions. (a) *Escherichia coli*, (b) *Streptococcus mutans*, (c) *Candida albicans*, (d) Control.

Statistical Analysis

Statistical Analysis was performed using a statistical package, SPSS windows version 15 by applying mean values using analysis of variance (ANOVA) with post-hoc least square differences (LSD) method.

Results

The results of this investigation showed that toothpaste formulation A had maximum zones of inhibition against the test organism, *Escherichia coli* ($p<0.001$, Table 3) compared to all other toothpaste formulations. In *Streptococcus mutans* and *Candida*

albicans, the zones of inhibition were less in comparison to *E. coli* but were significantly different at higher dilutions (1:8, 1:16, $p<0.05$, Tables 4 and 5) for toothpaste formulation A.

Table 3. Anti-microbial activity of dentifrice formulations against *Escherichia coli*

	1:1Dilution Mean value ± Std. Deviation	1:2 Dilution Mean value ± Std. Deviation	1:4 Dilution Mean value ± Std. Deviation	1:8 Dilution Mean value ± Std. Deviation	1:16 Dilution Mean value ± Std. Deviation
A 0 (Zone of inhibition in mm)	28.33 ± 3.512*	25.67 ± 4.041*	23.33 ± 4.263*	21.33 ± 4.163**	18.33 ± 4.163**
B (Zone of inhibition in mm)	21.33 ± 1.528	18.67 ± 1.263	16.33 ± 1.528	13.67 ± 1.528	11.00 ± 1.000
C (Zone of inhibition in mm)	21.33 ± 0.600	19.33 ± 0.600	17.33 ± 1.528	15.33 ± 1.528	12.67 ± 1.528
D (Zone of inhibition in mm)	20.67 ± 1.200	18.33 ± 0.600	15.33 ± 1.528	13.67 ± 1.528	11.00 ± 1.732
E (Zone of inhibition in mm)	20.33 ± 0.600	19.00 ± 1.000	16.67 ± 0.600	14.67 ± 0.600	12.33 ± 0.600
F (Zone of inhibition in mm)	19.70 ± 2.082**	17.40 ± 1.528	15.00 ± 1.000	13.00 ± 1.000	11.33 ± 0.577
G (Zone of inhibition in mm)	19.33 ± 2.082**	16.67 ± 1.528	14.00 ± 1.732	11.67 ± 1.155	10.00 ± 1.000
H (Zone of inhibition in mm)	33.33 ± 3.215*	27.00 ± 1.000	14.67 ± 4.726	10.00 ± 1.000	7.67 ± 1.155
I (Zone of inhibition in mm)	20.67 ± .577**	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
J (Zone of inhibition in mm)	20.33 ± 1.155**	17.67 ± 1.528	16.00 ± 1.000	14.00±1.000	12.33±.577

A-E are toothpaste samples while F-J are mouthrinses.

n=3, *P<0.001, **P<0.05

Table 4. Anti-microbial activity of dentifrice formulations against *Streptococcus mutans*

	1:1Dilution Mean value ± Std. Deviation	1:2 Dilution Mean value ± Std. Deviation	1:4 Dilution Mean value ± Std. Deviation	1:8 Dilution Mean value ± Std. Deviation	1:16 Dilution Mean value ± Std. Deviation
A (Zone of inhibition in mm)	22.33 ± 1.200	20.67 ± 1.528	18.67 ± 1.528	16.33 ± 1.528**	14.00 ± 2.000**
B (Zone of inhibition in mm)	20.00 ± 1.732	17.67 ± 1.528	15.00 ± 1.000	12.33 ± 1.528	3.67 ± 2.0
C (Zone of inhibition in mm)	19.33 ± 2.082	17.33 ± 1.200	15.33 ± 1.200	13.33 ± 1.200	9.00 ± 2.000
D (Zone of inhibition in mm)	18.33 ± 1.200	17.00 ± 1.000	14.67 ± 0.600	12.00 ± 0.000	2.00 ± 1.200
E (Zone of inhibition in mm)	17.33 ± 1.200	15.00 ± 1.000	12.67 ± 1.528	10.33 ± 1.155	.00 ± .000
F (Zone of inhibition in mm)	27.67 ± 1.155**	25.33 ± 1.528	23.33 ± 1.528	21.67 ± .577	19.67 ± .577
G (Zone of inhibition in mm)	22.67 ± 1.155**	20.33 ± .577	17.00 ± 1.000	14.33 ± 1.155	11.67 ± .577
H (Zone of inhibition in mm)	17.00 ± 1.000	14.33 ± 1.528	13.00 ± 1.000	11.00 ± 1.000	9.00 ±1.000
I (Zone of inhibition in mm)	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
J (Zone of inhibition in mm)	22.67 ± 1.528**	21.00 ± 2.000	18.67 ± 2.517	16.67 ± 2.517	15.00 ± 2.646

A-E are toothpaste samples while F-J are mouthrinses

N = 3, *P < 0.001, **P < 0.05

Table 5. Anti-microbial activity of dentifrice formulations against *Candida albicans*

	1:1Dilution Mean value ± Std. Deviation	1:2 Dilution Mean value ± Std. Deviation	1:4 Dilution Mean value ± Std. Deviation	1:8 Dilution Mean value ± Std. Deviation	1:16 Dilution Mean value ± Std. Deviation
A (Zone of inhibition in mm)	28.33 ± 0.600	25.67 ± 0.600	22.67 ± 1.155	20.33 ± 2.082**	18.00 ± 1.732 **
B (Zone of inhibition in mm)	26.00 ± 1.000	24.00 ± 1.000	22.00 ± 1.000	17.67 ± 1.528	14.33 ± 1.200
C (Zone of inhibition in mm)	23.00 ± 2.000	20.33 ± 1.528	17.67 ± 1.528	15.33 ± 1.528	12.67 ± 1.200
D (Zone of inhibition in mm)	24.33 ± 0.600	21.00 ± 2.000	17.67 ± 2.400	13.67 ± 1.528	10.33 ± 1.528
E (Zone of inhibition in mm)	18.33 ± 1.200	16.67 ± 1.600	15.00 ± 1.000	12.67 ± 0.600	10.33 ± 0.600
F (Zone of inhibition in mm)	26.67 ± .577**	25.33 ± 0.577	24.00 ± 0.000	22.33 ± .577	20.00 ± 0.000
G (Zone of inhibition in mm)	16.33 ± 0.577	14.33 ± 0.577	12.00 ± 0.000	10.00 ± 1.000	8.67 ± 0.577
H (Zone of inhibition in mm)	14.33 ± 3.215	12.33 ± 3.215	10.67 ± 3.055	6.67 ± 5.859	0.00 ± 0.000
I (Zone of inhibition in mm)	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
J (Zone of inhibition in mm)	22.33 ± .577*	20.67 ± .577	19.33 ± 1.155	17.00 ± 1.732	15.00 ± 2.646

A-E are toothpaste samples while F-J are different mouthrinse formulations.

n = 3, *P < 0.001, **P < 0.05

Mouthrinse formulation H showed maximum efficacy against the test organism, Escherichia coli ($p<0.001$, Table 3) compared to all other mouthrinse formulations. However, mouthrinse formulations F, G, I, J also showed significant difference ($p<0.05$, Table 3). Against Streptococcus mutans, mouthrinse formulations F, G and J showed significant antimicrobial activity ($p<0.05$) compared to formulations H and I (Table 4), while against Candida albicans, the zones of inhibition were statistically significant ($p<0.05$) for formulation F (Table 5). The mean values ± standard deviation of zones of inhibition are

given for all the test organisms. Each experiment was repeated thrice (n = 3).

Discussion

Maintenance of good oral hygiene is the key to the prevention of dental diseases. The primary etiological factor for dental diseases is dental plaque. The formation of plaque on the tooth surface is characterized by the progression from a limited number of pioneer microbial species to the complex flora of mature dental plaque. This progression involves initial adherence of bacteria to the salivary pellicle and subsequent accumulation by growth and interbacterial adherence. Ultimately, the tooth surface gets coated with a dense, complex microcommunity that ends up in the destruction of hard enamel tissue.[2]

The activities of oral microflora being responsible for mouth odor and most oral diseases are not in doubt. The need to keep these oral organisms to a level consistent with oral health by antimicrobial agent inclusion in dentifrice has been stressed.[12] When these substances are added to oral products, they kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. They also prevent bacterial aggregation, slow multiplication and release endotoxins.[13] Several clinical studies have demonstrated the inhibitory effects of antimicrobial dentifrice on oral bacteria and gingiva.[14]

Data from the present study is in support of this assertion as all the investigated dental care products exhibited wide variations in their effectiveness against the three test microorganisms, a feature that may have been largely due to their antimicrobial active ingredients (Table 1 and Table 2). Among all the investigated toothpastes, formulation A emerged as the most effective, based on the mean diameter of the zone of microbial inhibition produced by the toothpastes in agar well diffusion method, against all the three microorganisms tested. The exceptional ability of formulation A to retain its *in vitro* antimicrobial activity against all the three tested pathogens even at higher dilution of 1:16 is notable. This might be due to the presence of triclosan in its formulation. This become more plausible as the utility and effectiveness of a 1% triclosan formulation in health care industry has been reviewed by Jones *et al.*[8]

Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] has been used for more than 30 years as a general antibacterial and antifungal agent, which is found in formulations such as toothpastes and mouthrinses. It has recently been suggested that triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR).[9] Systematic reviews of six-month clinical studies have concluded that formulations containing triclosan and copolymer significantly improve plaque control and periodontal health.[15,16] In a previous study, Sullivan *et al*.[17] investigated toothpaste containing triclosan on resistant oral *Streptococci* and measured the *in vitro* sensitivity of *Streptococci* strains against triclosan.

Next to triclosan, fluorinated products such as formulations B and C were found to have antimicrobial activities, although these were not statistically significant; this may be due to the ingredients present in their formulations. These dentifrices contained sodium monofluorophosphate and sodium fluoride as active ingredients. Fluorides are abundantly used in many oral health products including toothpastes and mouthrinses as they help in caries prevention.[18] When formulated correctly and used as directed, fluoride toothpaste will help to safely and effectively prevent tooth decay. It is well documented the ability of fluoride to inhibit or even reverse the initiation and progression of dental caries.[19] However, if the bacterial challenge is too high, it is not possible for fluoride to overcome the challenge completely.[20] In a previous study, Jenkins[21] stated that fluoride products such as toothpaste and mouthrinse formulations have shown to reduce caries between 30 and 70% compared with no fluoride therapy. A systematic review indicated that a toothpaste containing triclosan/copolymer provides a more effective level on plaque control and periodontal health than conventional fluoride toothpaste.[15] The effectiveness of fluoride toothpastes are concentration dependent.[22]

Formulations D and E are herbal based products and exhibited least effectiveness compared to the other test formulations. This may be due to the ingredients present. Using natural medicines to cure various diseases has become an increasing trend. Herbal medicine has made significant contribution to modern medical practice.[23]

Though studies in animals and *in vitro* have shown the antimicrobial properties of several of these herbs, there is no other way of knowing their real clinical effects without a randomized clinical trial. In the present study, the herbal formulations studied appeared to be equally effective as the fluo-

ride formulations, but not superior to them.[24] The antimicrobial activity of the herbs is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins.[25] Synergistic interactions between the principal components of these herbs are considered to be a vital part of their efficacy. This synergistic activity, however, needs to be established. Many studies on herbal base toothpaste in control of plaque and gingivitis are reported.[24,26] A systematic review concluded that herbal toothpastes have rarely been shown to have significantly greater anti-plaque activity than conventional pastes.[27] Our data are in accordance with the literature cited above.

With respect to mouthrinses, formulation H has shown highly significant reduction in *Escherichia coli* and *Streptococcus mutans* count. This may be due to the presence of chlorhexidine gluconate and sodium fluoride as major ingredients in their formulations; this observation adds information to the earlier studies carried out by Spets-Happonen *et al.*[28] and Hefti *et al.*[29]

Chlorhexidine gluconate is a cationic biguanide with broad-spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental biofilm (plaque) and gingivitis have been demonstrated in several clinical studies.[30] Its mechanism of action is that the cationic molecule binds to the negatively charged cell walls of the microbes, destabilizing their osmotic balance.[31,32] Chlorhexidine formulations are considered to be the "gold standard" antiplaque mouthrinses due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential.[31,33] The high efficacy could be explained by its immediate bactericidal action during the time of application followed by a prolonged bacteriostatic action due to adsorption at the tooth surface.[34] Studies involving rinsing with 0.2% chlorhexidine gluconate twice daily for 60 seconds as supplement for normal mechanical oral hygiene procedures resulted in less plaque formation and gingivitis than rinsing with a placebo.[35] Clinical isolates of gram-negative bacteria were found to be highly susceptible to chlorhexidine gluconate.[36]

Gehlen[37] studied the influence of 0.2% chlorhexidine mouth rinse on plaque re-growth. In spite of its better efficacy against the oral infections, local delivery of the drug at the intended site was not successful by conventional method. Conventionally 0.2% chlorhexidine gluconate mouthwash is used for treatment of oral infections. Despite being discovered in the 1950s, it is still considered one of the most effective anti-plaque agents in dentistry. Its long-term use is limited by its disagreeable taste, and propensity to stain teeth brown.[38] Oral administration of antimicrobials for a prolonged period may alter natural microflora of the gastrointestinal tract.

Next to chlorhexidine, triclosan and sodium fluoride products such as formulations F and J were found to have antimicrobial activities and these were statistically significant but less effective when compared to chlorhexidine formulation. Triclosan is a broad spectrum antimicrobial[39] which has antiplaque activity. But, it is equally effective in reducing the *S. mutans* count but shows less effectiveness against *E. coli* when compared to chlorhexidine. Many studies using triclosan as an anti plaque agent were carried out[40] and have given good results. Study carried out by Jenkins *et al.*[41] using 0.2% triclosan reported significant reduction in total microbial count in saliva. In the present study, triclosan showed a significant reduction in *Candida albicans* and *Streptococci mutans* counts. Although data from toothpaste trails evaluating triclosan have been encouraging, the data on triclosan used as a mouthwash is limited.

Formulation I was least effective compared to other test formulations, which may be due to the presence of potassium nitrate and sodium fluoride as active ingredients in the formulation. They lack antimicrobial activity.[42]

It is known that a balance exists in a person's oral microbial population. If this balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes. Therefore, the formulation identified as having the largest microbial inhibition zone and thus, probably the strongest antimicrobial properties may not be necessarily superior to those with smaller diameter inhibition zones. Because the formulation used *in vivo* is likely to be diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution *in vitro* of interest.[11]

This testing method also functioned as a screening method, and may not have been able to detect the effects of a chemical agent that does not diffuse through the agar matrix. More importantly, the test was conducted *in vitro*, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness. Studies have demonstrated that the bacteria in biofilm forms such as

plaque have decreased sensitivity to antibacterial agents. Moreover, formulations for topical antimicrobial oral use, such as mouthrinses and dentifrices, must be able to penetrate the biofilm matrix and deliver the active agents quickly because exposure times are limited under actual conditions. Nevertheless, the *in vitro* method is a well-established technique that commonly is used in screening the antimicrobial efficacy of chemicals before *in vivo* testing.

Conclusion

Results from this study have shown that triclosan containing toothpaste formulations were more effective in controlling the oral microflora compared to non-triclosan containing synthetic toothpastes. Among mouthrinse formulations, chlorhexidine was found to be more effective than or as effective as triclosan against the organisms tested.

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