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Original Article

Microbiology Section

Comparison of Antimicrobial Efficacy of Triclosan- Containing, Herbal and Homeopathy Toothpastes- An Invitro Study

BASU ANUSHREE[1], MOHAMMED ALIMULLAH FAWAZ[2], RAO NARAHARI[3], TANVEER SHAHELA[4], AFROZ SYED[5]

ABSTRACT

Background: Use of antimicrobial agents is one of the important strategies to prevent oral diseases. These agents vary in their abilities to deliver preventive and therapeutic benefits.

Objectives: This invitro study was conducted to assess antimicrobial efficacy of different toothpastes against various oral pathogens.

Materials and Methods: A total of nine toothpastes in three groups were tested for their antimicrobial activity against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 0266P) and *Candida albicans* (Laboratory Strain) by modified agar well diffusion method. Statistical Analysis was performed using Minitab Software. A p-value of less than 0.05 was considered significant.

Results: Triclosan-based dental formulation with combination of fluoride (1000ppm) exhibited higher antimicrobial activity against test organisms than the combination of lower fluoride-concentration or sodium monofluorophosphate. Among herbal dentifrices, formulation containing Neem, Pudina, Long, Babool, Turmeric and Vajradanti showed significant antimicrobial activity against all the four tested microorganisms ($p<0.05$). However, against *Streptococcus mutans*, all three herbal products showed significant antimicrobial activity. Homeo products showed least antimicrobial activity on the tested strains. Formulation with kreosotum, Plantago major and calendula was significantly effective only against *Streptococcus mutans*.

Conclusion: In the present study, antimicrobial activity of the toothpaste containing both triclosan and fluoride (1000ppm) as active ingredients showed a significant difference ($p< 0.05$) against all four tested microflora compared to that of with lower fluoride-concentration or sodium monofluorophosphate. Of herbal groups, the only dentifrice containing several phytochemicals was found to be significantly effective and comparable to triclosan-fluoride (1000ppm) formulation. Thus, this herbal toothpaste can be used as alternative to triclosan-based formulations. However, these results might not be clinically useful unless tested invivo.

Keywords: Antimicrobial activity, Antimicrobial agents, Herbal toothpaste, Homeo toothpaste, Triclosan

INTRODUCTION

Dental diseases like dental plaques, dental caries and periodontal diseases, are primarily caused by the virulence of complex oral micro-communities [1]. Dental plaque has been proved to be a paramount factor in initiation and progression of gingival and periodontal diseases through caries production [2].

Poor oral hygiene is one of the reasons for accumulation of microbes and their harmful activities [1-3]. The customary oral hygiene method of chemical plaque control can be used as an adjunct to mechanical plaque control procedure [2]. Besides cleaning of teeth, the use of chemical agents with antiplaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease by limiting the cariogenic bacteria in the oral cavity [4].

Recently, a number of chemical agents have been advocated which are available in a toothpaste or dentifrices or in the form of a mouthwash [2]. Since its first use in European toothpaste in 1985 triclosan a low-toxicity, non-ionic phenolic derivative with a wide spectrum of antimicrobial activity has been the active ingredient in many oral hygiene formulations [1]. Besides, many herbal and homeo-based dentifrices claim to have antimicrobial properties. While the herbal formulations may be more appealing as they do not require alcohol, artificial preservatives, flavours or colours for their activity [5]; the basic homeopathic principle is that a substance in microdose efficiently cures those similar symptoms that would require larger dose otherwise and homeopathic formulations have effectiveness as non-toxic, anti-inflammatory, antimicrobial [6-9].

These different types of toothpastes claim to have antimicrobial properties, but in fact more research is needed to evaluate this claimed effectiveness. Thus, the present study was designed to investigate antimicrobial efficacy of triclosan, herbal and homeo-based toothpastes by using standard agar well diffusion method.

AIMS AND OBJECTIVES

To investigate antimicrobial efficacy of triclosan, herbal and homeo-based toothpastes by using standard agar well-diffusion method.

MATERIALS AND METHODS

The survey was aimed at knowing the antimicrobial efficacy of the brands of toothpastes that are mostly used. As a result, nine toothpastes in three groups, purchased from different pharmacies in Hyderabad, Andhra Pradesh, India, were selected for assessment of their in vitro antimicrobial activities. This study was conducted in the Department of Microbiology, Sri Sai College of Dental Surgery, Vikarabad, Andhra Pradesh, India. The composition of these dentifrices is given in [Table/Fig-1].

Microorganisms

Pure cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus mutans* (ATCC 0266P) were cultured in Brain heart infusion broth (Hi-Media, India) at 37°C for 24 h while *Candida albicans* (laboratory strain) was cultured in Sabouraud's dextrose broth for 48 hours.

Toothpastes	Ingredients as listed on packages
I	Triclosan, Calcium Carbonate, Sorbitol, Sodium lauryl sulfate, Sodium silicate, Sodium monofluorophosphate, Sodium bicarbonate, Sodium saccharin, Carrageenan, Titanium dioxide, 1000 ppm fluoride and Flavour in aqueous base.
II	Triclosan, Sodium monofluorophosphate, Potassium nitrate, 917 ppm fluoride and Flavour.
III	Triclosan, Calcium Carbonate, Sorbitol, Sodium monofluorophosphate, Hydrated silica, Sodium lauryl Sulfate, Cellulose gum, Sodium silicate, Sodium saccharin, Potassium nitrate, Benzyl alcohol, Titanium dioxide, Water and Flavour.
IV	Neem, Pudina, Long, Babool, Turmeric, Vajradanti.
V	Miswak extract, Neem Extract, Sorbitol, Sodium lauryl sulphate, Silica, Polyethylene glycol 1500, Sodium Carboxy Methyl Cellulose, Sodium Saccharine, Sodium Benzoate, Brown Agglomerate, Treated water and Flavour.
VI	Miswak extract, Calcium carbonate, Sorbitol, Silica, Sodium lauryl sulphate, Sodium silicate, Sodium saccharin, Carrageenan, Formaldehyde, Cellulose gum, Water and Flavour.
VII	Plantago, Calendula, Hamamelis, Eucalyptus, Calcium carbonate, Sorbitol, Glycerine, Sodium lauryl sulphate, Hydrated silica, Sodium fluoride, Sodium saccharin, Cellulose gum, Water and Flavour.
VIII	Plantago major, Kreosotum, Calendula, Borax, Gel base.
IX	Calendula officinalis, Hamamelis virginica, Plantago major, Aniseed oil, Calcium carbonate, Sodium fluoride and Sorbitol.
Negative Control (Toothpaste base)	Dicalcium phosphate, Carboxymethyl cellulose, Liquid paraffin, Distilled water.

[Table/Fig-1]: Ingredients of various toothpastes tested for antimicrobial potential

Evaluation of Dentifrices

Dentifrices solutions were made by mixing the calculated amount of selected toothpastes (2.0 gm) in measured volume (2 ml) of sterile pyrogen-free distilled water to attain 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.

Antimicrobial Assay

The antimicrobial activity of different concentrations of the dentifrices was determined by modified agar well diffusion method [1]. In this method, Mueller hinton agar plates were inoculated with 0.5 mL of 24 h broth cultures of each isolate (Wilkins chalgren agar was used for *Streptococcus mutans* strain and Sabouraud's dextrose agar was for yeast). After the plates were dry, five wells at equidistance were punched in each of the plates using sterile corkborer (8 mm). Then each well was filled with 0.2 mL of the dentifrice dilutions. The same amount of control was also introduced into the wells meant for it. The plates were incubated at 37°C for 24 h (48 h for *Candida albicans*). The antimicrobial activity was interpreted as the diameter of zones of inhibition (in mm). All the plates were made in triplicates.

STATISTICAL ANALYSIS

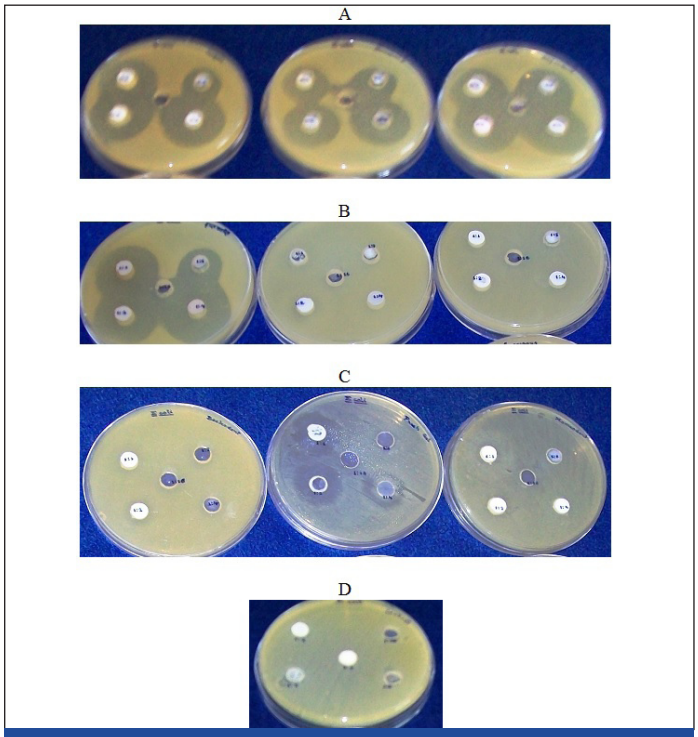
Statistical Analysis was performed using Minitab Software by regression method. Level of statistical significance was assumed at $p < 0.05$.

RESULTS AND OBSERVATIONS

Triclosan- containing Toothpastes: The results of this investigation showed that the zones of inhibition were statistically significant ($p<0.05$) for triclosan-formulation I against all the four test organisms [Table/Fig-2-6]. Formulation II did not possess significant antibacterial activity against *Escherichia coli*. Against *Streptococcus mutans*, toothpaste formulations III failed to demonstrate any significant activity.

Herbal Dentifrices: Ayurvedic products such as formulations IV, V and VI were found to have antimicrobial activities, although these were not statistically significant except for that of IV. However, against *Streptococcus mutans*, all three herbal products showed significant antimicrobial activity.

Homeo Dentifrices: Formulations VII, VIII and IX are homeo- based products and exhibited least effectiveness compared to the other test formulations. Among all the investigated homeo toothpastes, formulation VIII emerged as the significantly effective, against the tested microorganism, *Streptococcus mutans*. No zone of inhibition was observed for the control.



[Table/Fig-2]: Zone of inhibition produced by different toothpaste formulations at 24hrs against *Escherichia coli* at five different dilutions; zone of inhibitions by A) triclosan based toothpastes, B) herbal toothpastes, C) homeo toothpastes, D: by control)

Toothpastes	1:01	1:02	1:04	1:08	1:16	p-value
I	33.67	33	31.83	22.17	0	0.002
II	29.17	28.67	25.67	21.5	0	0
III	32.83	31.67	31	30.17	0	0.015
IV	33.33	31.17	30.33	22.33	0	0.001
V	19.67	18	0	0	0	0.183
VI	16.33	0	0	0	0	0.417
VII	26	23	0	0	0	0.185
VIII	24.67	20.17	17.67	0	0	0.061
IX	13.5	0	0	0	0	0.417

[Table/Fig-3]: Antimicrobial activity of detifrice formulations against *Escherichia coli*

Toothpastes	1:01	1:02	1:04	1:08	1:16	p-value
I	22.67	22	19.67	0	0	0.046
II	29.3	24.17	23.67	14.5	0	0.016
III	24	23.5	22.67	22.67	0	0.021
IV	29.67	25.67	23.5	14.17	0	0.014
V	15.17	13.67	0	0	0	0.184
VI	13.33	13.33	0	0	0	0.185
VII	15.67	14.5	0	0	0	0.183
VIII	16.67	16	6.67	0	0	0.128
IX	16.17	14.33	0	0	0	0.184

[Table/Fig-4]: Antimicrobial activity of detifrice formulations against *Staphylococcus aureus*

Toothpastes	1:01	1:02	1:04	1:08	1:16	p-value
I	28.17	27	21.83	0	0	0.049
II	33.83	29.67	28.83	15.67	0	0.014
III	31	30.5	29.67	20.17	0	0.002
IV	34	33.5	31.67	10.83	0	0.031
V	27	25	17.83	0	0	0.064
VI	19.83	19.33	19.17	0	0	0.052
VII	21.83	14.67	0	0	0	0.228
VIII	26.67	26	15.5	0	0	0.084
IX	16.33	15	0	0	0	0.183

[Table/Fig-5]: Antimicrobial activity of detifrice formulations against *Candida albicans*

Toothpastes	1:01	1:02	1:04	1:08	1:16	p-value
I	33.67	33.67	34	30	0	0.010
II	37.33	36.67	31.67	29.67	0	0.002
III	40.67	40	37.33	31.67	0	0
IV	36.63	34	36.67	30.33	0	0.009
V	20	19.33	16.33	0	0	0.047
VI	19.33	18	17.67	0	0	0.049
VII	18	0	0	0	0	0.417
VIII	28.33	28	26.33	18	0	0.002
IX	17.67	16.67	0	0	0	0.183

[Table/Fig-6]: Antimicrobial activity of detifrice formulations against *Streptococcus mutans*

DISCUSSION

The biofilms by the oral microflora being the centre of caries and periodontal disease, it is of utmost importance to control these biofilms by mechanical debridement and use of adjunctive antimicrobials in toothpastes in prevention of plaque-mediated diseases [10]. Several clinical studies have demonstrated the inhibitory effects of antimicrobial dentifrice on oral bacteria and gingival [11]. Concerns regarding the increase in antibiotic resistance in microorganisms against triclosan [12-14] has promoted interest in the therapeutic use of non-conventional or alternative dentifrices and thus this study.

Among all the investigated triclosan-toothpastes, formulations I emerged as the most effective, based on the significant zone of microbial inhibition produced by it in agar well diffusion method, against all the four tested microorganisms. This might be due to the presence of triclosan and fluoride in its formulation. The results of this study were consistent with the previous reports by Yigit et al., and Deng et al., that fluoride combination is more effective than that of sodium monofluorophosphate [15,16]. Triclosan (5-Chloro-2(2, 4-dichlorophenoxy) phenol), a halogenated phenol has been used for more than 30 years as a non-ionic broad spectrum antimicrobial [1]. It is an active component in detergents, clothing, toothpastes and mouthwashes [17]. Many studies using triclosan as an anti-plaque agent were carried out [18] and have given good results. Study carried out by Jenkins et al., reported a significant reduction in total microbial count in saliva by 0.2% triclosan [19]. Systematic reviews by Davies et al., and Gunsolley et al., have concluded that formulations containing triclosan and copolymer significantly improve plaque control and periodontal health [20,21]. In another invitro study by Manupati Prasanth, triclosan showed a significant reduction in *Candida albicans* and *Streptococcus mutans* counts with antimicrobial activity even at higher dilution of 1:16 [1].

Next to triclosan, are herbal products. "Ayurveda" system has been used successfully for treating various systemic ailments in Indian medicine [2]. Using natural medicines to cure various diseases has become an increasing trend [1]. In recent years, a number of toothpaste preparations containing herbal ingredients have made significant contribution to dental prophylaxis in improving oral health. The popularity of herbs is due the antimicrobial and anti-inflammatory effects of their ingredients known as Phytochemicals [5].

In the present study, herbal formulations such as formulations IV, V and VI were found to have antimicrobial activities, although these were statistically significant only for formulation IV. This may be due to the synergistic interactions between the ingredients present in their formulations, which, however, need to be established [1]. The principle components of this toothpaste include Neem, Pudina, Long, Babool, Turmeric and Vajradanti. The presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins in these ingredients are considered to be the sole reason of their antimicrobial efficacy [22]. Some of these ingredients were previously demonstrated and known to have antimicrobial activity [2,5]. Against *Streptococcus mutans*, all three herbal formulations showed significant antimicrobial activity ($p<0.05$). Many studies on anti-plaque activity of herbal base toothpaste have been reported [23,24]. A systematic review by Moran et al., concluded that herbal toothpastes had shown less anti-plaque activity than conventional pastes [25]. Our data are in consistent with a recent report by Manupati Prasanth which found herbal based products to be less effective compared to triclosan formulations [1].

With respect to homeo-based toothpastes, only formulation VIII has shown significant activity only against *Streptococcus mutans*. This may be due to the presence of kreosotum, Plantago major and calendula as major ingredients in its formulation. This observation contradicts the earlier information claiming kreosotum and plantago as efficient anticandidal ingredients but coincides the earlier experiments done by Stanisavljevic et al., Sharifa et al., and Kemal et al., Hoeletz, Kahyaolu et al., that demonstrated Plantago major extract to possess light antimicrobial activity against various bacteria including *Escherichia coli* [7-9,26,27].

CONCLUSION

This study have shown that antimicrobial activity of the toothpaste containing both triclosan and fluoride (1000ppm) as key ingredients showed a significant difference ($p< 0.05$) against all four tested microflora compared to that of with lower fluoride-concentration or sodium monofluorophosphate. Also, both the herbal toothpaste (having several Phytochemicals) and triclosan-fluoride (1000ppm) formulation have comparable antimicrobial properties. Therefore, this herbal dentifrice can be used as alternative to triclosan-based formulations. But, the results of this study might not be clinically applicable. Firstly, the agar diffusion test solely depends on the diffusing ability of the testing agents through the agar matrix. Secondly, formulations for topical dentifrices might not be equally active within limited exposure times under actual conditions in vivo. Nevertheless, invitro method is commonly used in screening the antimicrobial agents before invivo testing. Thus, dental professionals may recommend a dentifrice based on patients clinical conditions and possible susceptibilities.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Sri Sai College of Dental Surgery, Vikarabad, India.
2. Professor and Head, Department of Microbiology, Sri Sai College of Dental Surgery, Vikarabad, India.
3. Professor and Head, Department of Pharmacology, Sri Sai College of Dental Surgery, Vikarabad, India.
4. Assistant Professor, Department of Oral Pathology, Sri Sai College of Dental Surgery, Vikarabad, India.
5. Professor and Head, Department of Oral Pathology, Sri Sai College of Dental Surgery, Vikarabad, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Basu Anushree,
Sri Sai College of Dental Surgery, Vikarabad-501101, India.
E-mail : anushreejob@gmail.com

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