Original Article

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Antimicrobial Efficacy of Commercially Available Toothpastes – An *In vitro* Study

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ABSTRACT

Background: The use of antimicrobial chemotherapeutic agent has been proposed as a means of reducing the levels of oral bacteria. Many chemotherapeutic agents claim to have antimicrobial properties. Hence this *in vitro* study had been undertaken. **Methods***:* This *in vitro* study comprised of seven toothpastes which have been tested for their antimicrobial activity against three oral pathogens namely, *Streptococcus mutans, Escherichia coli* and *Candida albicans* by well agar diffusion assay at the dilution of 1:1, 1:2, 1:4, 1:8 and 1:16. **Results:** Study results showed that toothpaste 'five' with sodium fluoride and sylodent as main ingredients showed maximum zone of inhibition against *Streptococcus mutans* where as against *Candida albicans,* toothpaste 'six' with xylitol and sodium fluoride as main ingredients showed maximum zone of inhibition. And against *E. coli,* toothpaste 'one' with Triclosan and Zinc sulphate as main ingredients showed maximum zone of inhibition among all toothpastes. It was observed that tooth paste 'six' with sodium fluoride and Neem, Meswak as main ingredient showed minimum zone of inhibition against Streptococcus mutans at 1:1 dilution among seven toothpastes used in the present study. **Conclusion:** In the present study, it has been demonstrated that triclosan containing toothpastes formulations are more effective in control of oral micro flora.

Key words: Antimicrobial activity, Candida, Dental caries, *E. coli*, Periodontal disease, Streptococcus mutans, Toothpaste.

INTRODUCTION

Oral cavity consists of large number of gram positive and gram negative microorganisms. Several studies have shown that oral cavity acts as a paradise with its warm and moist environment, nutrients and unique anatomical features for the microbial growth, proliferation and multiplications.[1] A bio film which provides ground for the formation and inhabitants of pathogenic bacteria leading to tooth decay, gingivitis and periodontitis.

Streptococcus mutans is one of the main opportunistic pathogens of dental caries, which plays a central role in fermenting carbohydrates resulting in acid production

Access this article online	
Journal Sponsor	Website: www.jyoungpharm.org
www.phcog.net	DOI: 10.5530/jyp.2015.3.8

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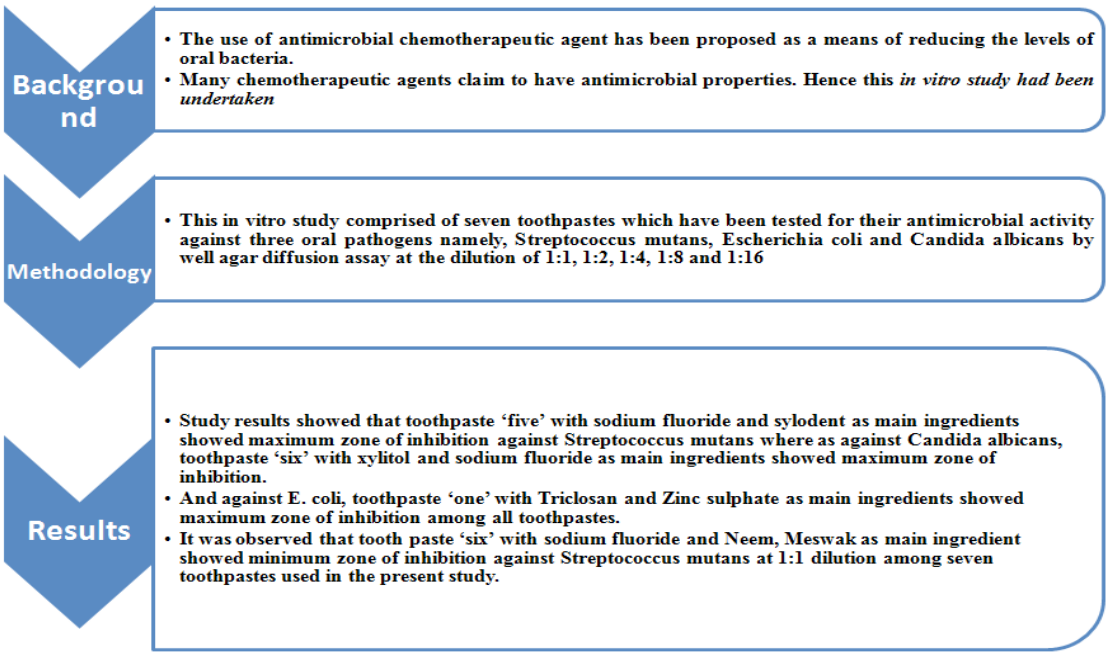


Graphical Abstract

and leading to the demineralization of the tooth enamel. In addition, other micro flora like Escherichia coli and Candida albicans are also associated with active caries lesions. Candida albicans is the most common yeast isolated from the oral cavity. Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities. In India, as in other developing countries, a very significant proportion of dental problems are due to poor oral hygiene.[2]

Table 1: Toothpastes with their ingredients

Toothpaste number	Ingredients
1	Sodium monofluorophosphate, Triclosan, Zinc sulphate, Alum.
2	Amine fluoride, Sorbitol, Propylene glycol, Silica, Cocamidopropylbetain, Titanium dioxide, Hydroxy ethyl cellulose, Sodium saccharin.
3	Triclosan, PVM/MA Copolymer, Silica, Sorbitol, Sodium lauryl sulphate, Titanium dioxide, Sodium fluoride, Sodium saccharin.
4	Hydrated Silica, Sorbitol, Water, Sodium lauryl sulphate, PEG 32, cocamidopropyl betain, Cellulose gum, Sodium saccharin, Sodium fluoride, Zinc sulphate, Sodium hydroxide.
5	Hydrated Silica, Sodium fluoride, Glycerin, Sorbitol, Propylene glycol, Sodium lauryl sulphate, Xylitol, Cellulose gum, PEG 8, Titanium dioxide, Sodium saccharin, Methyl paraben, Propyl paraben.
6	Glycerin, Silica, Xylitol, Potassium chloride, Cocamidopropylbetain, PEG 400, Titanium dioxide, sodium carboxy methyl cellulose, Sodium saccharin Sodium lauryl sarcosinate, Menthol, Tetra sodium pyrophosphate, Sodium fluoride, Neem, Meswak, Papain.
7	Calcium carbonate, hydrated silica, Sodium silicate, Benzyl alcohol, Triclosan, Potassium Nitrate, Titanium Dioxide.

Today, to maintain good oral hygiene many chemical and mechanical measures are coming up. Only mechanical that is using toothbrush and or interdental dental aids will be insufficient to maintain plaque control for long period of time. So as an adjutant chemical measure such as toothpaste, mouth-rinses are used. So these chemical measures will be having active in gradients against micro-organisms. Studies have shown that use of antimicrobial chemotherapeutic agent as a means of reducing the levels of oral bacteria, specifically Streptococcus mutans.[3] Many chemotherapeutic agents claim to have antimicrobial properties but very little research has been conducted. Hence present study was undertaken to investigate antimicrobial efficacy of different toothpastes by using standard agar well diffusion method.

MATERIALS AND METHODS

The present in vitro study conducted to assess the antimicrobial efficacy of seven commercially available toothpastes on three common microorganisms involved in dental diseases namely, Streptococcus mutans, *E. coli*, Candida albicans. Commonly available seven toothpaste were selected and (Table 1) shows composition of each tooth paste.

Figure 1: ***Strains of S. mutans, E coli, and Candida albicans***

Selection of the toothpastes

Prior to the start of the study, various brands of commercially available toothpastes were obtained. Seven toothpastes with different ingredients were selected. Owing to the constraints pertaining to the available time and expenditure, only seven toothpastes were selected for the present study. Only those test products with a date of manufacture within the last six months were selected for the present study for the purpose of standardization. The serial dilutions of selected toothpastes was made by mixing the calculated amount of toothpaste (2.0 gm) in measured volume (2 ml) of sterile pyrogen free distilled water to obtain 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.

Preparation of subcultures of microorganisms

Pure cultures of Candida albicans (ATCC 10231), Escherichia coli (ATCC 25922) were obtained from the Department of Microbiology, Sri Siddhartha Medical College and Hospital, Tumkur. Pure cultures of Streptococcus mutans (MTCC 890) were obtained from the Institute of Microbial Technology, Chandigarh, India. These test microorganisms were sub cultured on specific media, such as Brain heart Infusion for Streptococcus mutans and nutrient agar for Candida albicans and Escherichia coli. (Figure 1) shows the strains.

Determination of antimicrobial Assay

The antimicrobial activity of different concentrations of the dentifrices was determined by modified agar well diffusion method. In this method nutrient agar plates were seeded with 0.5 ml of 24 hour broth cultures of each isolate. Brain heart infusion agar was used for streptococcus mutans strain. The plates were allowed to dry for 1 hour. 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 distilled water was introduced into the first study design. The plates were incubated at 37°C for 24 hours (48 hours for yeast species). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (in mm). All the plates were in triplicates and the experiments were repeated thrice.

STATISTICAL ANALYSIS

Collected data was subjected for statistical analysis using SPSS software Version 16. The statistical test used was Kruskal-Wallis test. The level of significance used is α=0.05.

RESULTS

Comparison of S. mutans among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8, and 1:16

Our study results showed that toothpaste five (5) with the calcium carbonate as a main ingredient shows maximum zone of inhibition which was statistically significant (p=0.003). Toothpaste 6 with amine fluoride as a main ingredient shows minimum zone of inhibition. It is also observed that zone of inhibition decreases with the increase in dilution (Table 2).

Comparison of E. coli among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8, and 1:16

Toothpaste-1 with the triclosan as a main ingredient shows maximum zone of inhibition and statistically significant (p=0.003). Toothpaste–4 with Sodium monofluorophosphate and Zinc sulphate as a main

Table 2: Comparison of *S. mutans* among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16

Toothpastes	1:1Dilution	1:2 Dilution	1:4 Dilution	1:8 Dilution	1:16 Dilutio
	Mean value ± SD	Mean value ± SD	Mean value ± SD	Mean value ± SD	Mean value ± SD
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00 ±0.00
2	25.17±0.15	24.17±0.15	23.10±0.10	22.10±0.10	21.10±0.10
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	27.37±0.32	26.67±0.15	26.20±0.20	25.17±0.15	24.20±0.20
5	28.17±0.15	27.23±0.21	26.10±0.10	25.10±0.10	24.17±0.15*
6	21.10±0.10	20.37±0.56	19.90±0.10	18.70±0.26	17.13±0.12
7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

SD= Standard Deviation; 1-7 Toothpaste numbers * statistically significant p<0.05

Table 3: Comparison of *E. coli* among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16

Toothpastes	1:1Dilution	1:2 Dilution	1:4 Dilution	1:8 Dilution	1:16 Dilution
	Mean value ± SD	Mean value ± SD	Mean value ± SD	Mean value ± SD	Mean value ± SD
1	37.60±0.10	37.17±0.15	36.37±0.32	35.17±0.15	34.17±0.15*
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	26.23±0.21	25.60±0.10	25.30±0.26	24.87±0.32	24.17±0.15
5	32.17±0.15	31.33±0.31	30.17±0.15	29.17±0.15	28.73±0.21
6	31.17±0.15	30.23±0.21	29.10±0.10	28.10±0.10	27.37±0.32
7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

SD= Standard Deviation; 1-7 Toothpaste numbers * statistically significant p<0.05

Table 4: Comparison of *Candida* among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16

Toothpastes	1:1Dilution Mean value ± SD	1:2 DilutionMean value ± SD	1:4 Dilution Mean value ± SD	1:8 Dilution Mean value ± SD	1:16 Dilution Mean value ± SD
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	22.17±0.15	21.17±0.15	20.43±0.38	19.17±0.15	18.10±0.10
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	25.17±0.15	24.60±0.10	24.10±0.10	23.17±0.15	22.37±0.32
5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
6	29.10±0.10	28.17±0.15	27.10±0.10	26.10±0.10	25.10±0.10*
7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

SD= Standard Deviation; 1-7 Toothpaste numbers * statistically significant p<0.05

ingredient shows minimum zone of inhibition. It is also observed that zone of inhibition decreases with the increase in dilution (Table 3).

Comparison of Candida among the different toothpastes in concentration of 1:1, 1:2, 1:4, 1:8, and 1:16

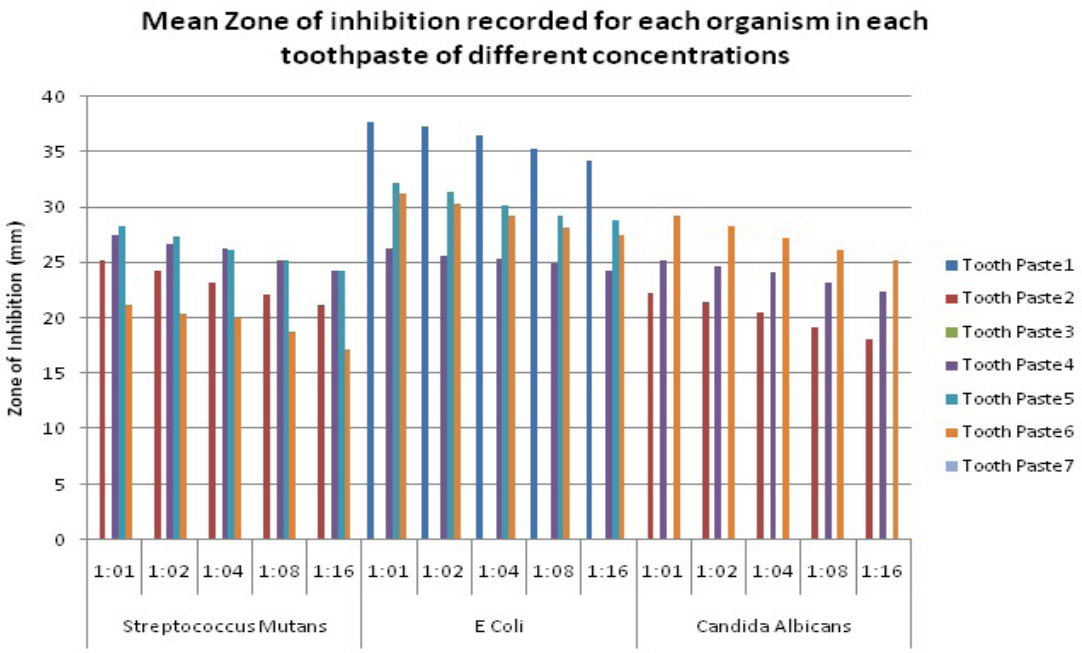
It can be observed that toothpaste six with Sodium fluoride and Neem as main ingredients shows maximum zone of inhibition and statistically significant (p=0.003). Toothpaste two with Amine fluoride as a main ingredient shows minimum zone of inhibition against *Candida*. It is also observed that zone of inhibition decreases with the increase in dilution (Table 4)

Anti-microbial efficacy of seven toothpastes at different dilutions against three oral microorganisms

It has been observed that Toothpaste-1 with Triclosan as main ingredient showed maximum zone of inhibition against *E.coli* organism at 1:1 dilution. And also it was observed that Toothpaste-6 with Sodium fluoride and Neem, Meswak as main ingredient showed minimum zone of inhibition against *Streptococcus mutans* at 1:1 dilution. (Graph 1)

DISCUSSION

Now a days, most of the people use tooth brush and paste to maintain their oral hygiene. So mechanical and chemical form of oral hygiene aids are one of the common tool against dental diseases like dental caries and periodontal diseases. But how effectively these are working is a big question. Dental marketing is wide spread. Many of the dental tooth pastes are well marketed. Consumers are coming across many toothpaste advertisements with their highlighting benefits. Many of these products claim to be effective antimicrobial agents. As a result consumers are in dilemma about the use of these products. Hence



Graph 1: Antimicrobial efficacy of seven toothpastes at different dilutions against the three oral microorganisms

the present study was undertaken to assess the in-vitro antimicrobial efficacy of commercially available toothpastes. The toothpastes tested in this study contained several different constituents with anti-inflammatory and anti-bacterial properties, which theoretically could be useful in controlling the oral microbial population.

It has been established that *Streptococcus mutans* plays a major role in the tooth decay by metabolizing sucrose to lactic acid. *Candida albicans,* yeast like fungus, relatively common inhabitant of the oral cavity, the chief causative factor of candidiasis an opportunistic infection. *E coli* is a gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia*. LPS-induced cytokine expression is via a pathogen ligand called toll-like receptors (TLRs). Studies have shown the growth of *E. coli* in sub gingival flora of aggressive and chronic periodontitis.[5,6] Thus it would be beneficial if oral formulations having established antimicrobial properties against common oral micro-organisms.[4]

Maintenance of good oral hygiene is the key to the prevention of dental diseases. The Dental plaque is one of primary etiological factor for dental diseases. 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 micro community that ends up in the destruction of hard enamel tissue.[7] Halitosis is due to the activities of oral micro flora. So antimicrobial agents are necessary to be added to toothpaste and mouthrinses. 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.[8] 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). Among all the investigated toothpastes, Toothpaste 1 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 *E coli*. Toothpaste 1 contains triclosan and zinc sulfate in it. Our study results were in agreement with other studies.[2]

Triclosan (2, 4, 4'-trichloro-2'-hydroxydiphenyl ether) is a broad spectrum antibacterial, anti-inflammatory, agent that has been shown in, *in vitro* studies to be active against many of the organisms associated with plaque and plaque associated gingivitis.[1] It has recently been suggested that triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR). Systematic reviews of six-month clinical studies have concluded that formulations containing 0.3% triclosan and copolymer significantly improve plaque control and

periodontal health.[9] Recent *in vitro* studies demonstrated the effect of the Triclosan/copolymer formulations in reducing oral malodour and the bacteria associated with oral malodor.[10] One of the study observed that triclosan induced suppression of molecules related to microbial pathogen recognition pathways and of acute and chronic mediators of inflammation, such as Toll-like receptor-signalling molecules and other multiple inflammatory molecules, including interleukin-1 and -6.[11]

Recent evidence suggests that triclosan exposure may alter cancer risk, although human studies are lacking in both number and scope.[12]

Next to triclosan, Toothpaste 5 with sodium fluoride and sylodent as main ingredients showed maximum zone of inhibition against *E. coli*. Sylodent acts as a polishing agent which safely cleans and helps in eliminating stains to whiten teeth. It is followed by fluorinated products such as Toothpaste 6 against *E coli* and *Candida*. This toothpaste contains Xylitol as a main ingredient. Fluorides are abundantly used in many oral health products including toothpastes and mouth rinses as they help in caries prevention.[13] fluoride products such as toothpaste and mouth rinse formulations have shown to reduce caries between 30 and 70% compared with no fluoride therapy. The effectiveness of fluoride toothpastes are concentration dependent.[14] Excess of fluoride concentration can lead to dental fluorosis during developing stage of tooth. When formulated correctly and used as directed, fluoride toothpaste will help to prevent tooth decay more effectively. It is well documented that fluoride can inhibit or even reverse the initiation and progression of dental caries. However, if the bacterial challenge is too high, it is not possible for fluoride to overcome the challenge completely.[2]

It is followed by Toothpaste 4 containing hydrated silica and sodium fluoride as ingredients against *streptococcus mutans* and *E coli*. Least efficacy was shown by Toothpaste 2 containing Amine fluoride as a main ingredient against Candida compared to other test formulations this may be due to the ingredients present.

Healthy mouth means a balance exists between oral immunity and microorganisms and other factors like maintaining hygiene, intrinsic and extrinsic factors of saliva, tooth position and composition etc. If this harmony breaks then proliferation of micro organisms takes place initiating the disease process.

CONCLUSION

A product to maintain oral health should be viewed in all dimensions. Some in gradients are very helpful but its excess/lower levels might lead to disease. Example fluoride, triclosan. 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.[2] In our study triclosan containing toothpastes formulations are more effective in control of oral microflora. But other study[12] has shown triclosan is carcinogenic. Products have to be viewed in all dimension.

Further studies are needed to know proper concentration of ingredients. *In vivo* studies required to know the role of saliva, plaque and mechanism of action of these active ingredients. Nevertheless, the *in vitro* method is a well-established technique that is commonly used in screening the antimicrobial efficacy of chemicals before *in vivo* testing.[2] Hence further studies have to be conducted *in vivo* to know the efficacy of oral care products.

CONFLICT OF INTEREST

Authors declared there is no conflict of interest.

ACKNOWLEDGEMENTS

I would like to thank and acknowledge Dr. Nagaraj, Prof & HOD, Dept. of Microbiology, Sri Siddhartha Medical College and Hospital, Tumkur, India and Dr. Kishore Bhat, Prof & HOD, Dept. of Microbiology, Marata Mandal Dental College, Belagaum, India for helping me out with the micro organism strains.

Highlights of Paper

- Study showed that toothpaste 'five' with sodium fluoride and sylodent as main ingredients showed maximum zone of inhibition against *Streptococcus mutans*.
- Toothpaste 'six' with xylitol and sodium fluoride as main ingredients showed maximum zone of inhibition against *Candida albicans*.
- And against *E. coli*, toothpaste 'one' with Triclosan and Zinc sulphate as main ingredients showed maximum zone of inhibition among all toothpastes.
- It was observed that tooth paste 'six' with sodium fluoride and Neem, Meswak as main ingredient showed minimum zone of inhibition against *Streptococcus mutans* at 1:1 dilution among seven toothpastes used in the present study

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