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In vitro Study of Anti-Bacterial Efficacy of Herbal Toothpastes vs Triclosan and Fluoride Containing Control

Prema Sivakumar, R.V.Geetha
Saveetha Dental College, Poonamallee Highroad, Chennai- 600095

Abstract

Aim:
This In-vitro study has been conducted to test the anti-bacterial action of 6 commercially available herbal toothpastes against a control, containing triclosan and fluoride.

Objective:
To compare the anti-bacterial efficiency of each tooth paste with the control, and see the general efficiency of herbal toothpastes against triclosan and fluoride toothpastes.

Method:
Clinically isolated S.mutans bacteria is the test organism. The procedure involved agar well diffusion method, and subsequent comparative analysis of the various zones of inhibition of the toothpastes of various formulations.

Reason:
This study is being done to see if herbal toothpastes are really as efficient as they claim to be. The main action of toothpastes is the anti-microbial action, which has been tested and documented for comparison.

Results:
Toothpaste D(aloe vera based with xylitol) showed maximum zone of inhibition(33mm) , closely followed by the triclosan and fluoride containing control(A) and toothpaste F(30 mm).

Conclusion:
From this study it can be concluded that few toothpastes with herbal and synthetic anti-microbial components may be better than fluoride and triclosan containing toothpastes and some have nearly the same efficacy of fluoride and triclosan containing toothpastes, but toothpastes with almost only herbal components show less efficacy(E and G)

Keywords: herbal toothpastes, anti-bacterial, triclosan, fluoride, in-vitro study, *Streptococcus mutans.*

INTRODUCTION

Dental caries is a dynamic microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the teeth, which often leads to cavitation. It along with periodontal disease are probably the most common chronic diseases in the world not only causing damage to the teeth but is also responsible for several morbid conditions of the oral cavity and other systems of the body. These diseases affect people from all around the world, in different walks of life. With the current change in eating habits, involving diets containing more sugar content, these diseases are more likely to occur.[1] 60-65% of the Indian population is affected with dental caries[2,3] Poor oral hygiene is the main reason for the development of such conditions. *Streptococcus mutans* is one of the key organisms in initiation and formation of biofilm and subsequent plaque formation due to carbohydrate fermentation.[3]

Periodontal diseases affect the supporting teeth structures (like periodontal ligament, Alveolar bone and cementum) due to the release of the hydrolytic enzymes and the toxic metabolites of bacteria. It may begin as gingivitis and escalate to tooth loss because of severe periodontal disease. The micro-organisms commonly involved are streptococci, spirochaetes and bacterioides. [4]

The toothpaste is a dentifrice which when used with a brush (mechanical aid) help in disrupting plaque and maintaining oral hygiene. The calcium and fluorides in the toothpaste help in maintaining the mineral balance of the tooth and the antibacterial agents disrupt the cell wall of the bacteria. [4]

Recently a number of plant and herbal extracts have been incorporated into oral hygiene agents because of their reportedly good antimicrobial activity. [5-7] but only very limited published data supports this idea [6] And people tend to rely more on natural products these days, owing to their lack of side effects and high efficiency. So this study was carried out to evaluate the claims of these herbal toothpastes.

MATERIALS AND METHODS:

This study was conducted in the microbiology department of Saveetha Dental College. Six commercially available herbal toothpastes and a fluoride and triclosan containing positive control were tested in this *in vitro* study conducted in an agar well diffusion method. (Refer Table 1)

The test organism used was obtained from a culture of clinically obtained *Streptococcus mutans*. Two petri plates with Mueller hinton Agar were used for the study. A lawn culture of the bacterial isolate was made on both the petri plates. Four wells were dug into the both the petri plates with the well borer. Then the tooth pastes were placed in the pattern of a control and the three other herbal pastes in each petri plate (A, B, C, D in one plate and A, E, F, G in the other plate) into the wells that had been dug. These plates were then incubated at 37 degrees Celsius for 24 hours .Then the zones of inhibition were measured with a digital Vernier caliper and noted down. The zones of inhibition indicate the antimicrobial activity and generally, higher diameter of zones of inhibition indicated better anti-bacterial efficacy.

Table 1: Ingredients of the Toothpastes

Tooth paste	Ingredients as on the package
A (Control)	Calcium Carbonate, Sorbitol, Solium Lauryl sulphate, silica, Titanium dioxide, Sodium Silicate, Flavor, Carrageenan, Sodium Monofluorophosphate, Sodium Bicarbonate, Benzyl Alcohol, Sodium saccharin, Triclosan , in aqueous base
B	Sorbitol, Water, Hydrated Silica, Glycerin, Silica, Sodium Lauryl Sulphate, Flavour, Xanthan Gum, Titanium Dioxide, Sodium Saccharin, Menthol, Sodium Benzoate, Punica Granatum Pericarp Extract, Potassium Sorbate, Calcium Fluoride, Zanthoxylum Alatum Fruit Extract, Acacia Arabica Stem Bark, Terminalia Chebula Fruit Extract, Terminalia Bellerica Fruit Extract, Emblica Officinalis Fruit Extract, Embelia Ribes Fruit Extract, Azadirachta Indica Bark Extract, Vitex Negundo Extract, Thymol, Citric Acid, Salvodora Persica Stem Extract, Acacia Farmesiana Flower/Stem Extract, Acacia Cetechu Bark Powder, mimosops Elengi Flower Extract. Contains Fluoride (1450ppm F)
C	Calcium Carbonate, Sorbitol, Sodium Lauryl Sulphate, Polyethylene Glycol, Flavor, Sodium Carboxymethyl Cellulose, Sodium Monofluorophosphate, Sodium Saccharin, Benzyl Alcohol, Sodium Silicate, Xanthan, CI 74260, Myrrh extract, Chamomile extract, Tea tree oil, Sage oil, Eucalyptus oil, Eugenol, in Aqueous base.
D	Glycerin, Sorbitol, Hydrated Silica, Aloe Barbadensis Gel, Aqua, Xylitol, Sodium Lauroyl Sarcosinate, Mentha Piperita Oil, PVP, Hydroxyethylcellulose, Menthol, Melaleuca Alternifolia(tea tree) Oil, Sodium Hydroxymethylglycinate, Citric Acid, CI75810, limonene
E	Sunthi (Zingiber officinale) rhizome ,Maricha (Piper nigrum) fruits , Pippali (Piper longum) inflorescence , Karpoora (Cinnamomum camphora) ,Tomar seeds (Zanthoxylum alatum) fruits Lavanga (Syzygium aromaticum) floral buds ,Pudina (Mentha piperita) ,Kashni (Cichorium intybus) roots, Hareetaki (Terminalia chebula) fruits, sodium benzoate, Methyl paraben,Propyl paraben, Excipient sufficient
F	Calcium carbonate, Sorbitol, Water, Silica, SLS, flavour, Miswak extract, Cellulose gum, Carrageenan , Sodium silicate, PVM/MA Copolymer, Sodium Saccharin ,Sodium Benzoate
G	Purified Chalk, Water, Sorbitol, Sodium Lauryl Sulphate, Gum Tragacanth. Extracts of Indian Licorice root, Indian Almond, Common Jujube, Currant, Sarsaparilla, Cinnamon, Sappan Wood, Persian Walnut, Rose Apple, Medlar, Barleria Prinoitis, Prickly Ash, Asian Holly Oak, Bedda Nut, Bengal Madder, Bishop's Weed, Catechu, Mayweed and Geranium. Menthol, Thymol, Oils of Clove, Eucalyptus and Peppermint.

RESULTS

After the 24 hours of incubation, all the 7 tooth pastes showed appreciable zones of inhibition (Refer Figure 1, 2 and 3). Tooth paste D showed a maximum of 33 mm as its zone of inhibition and toothpastes E and G showed a minimum of 16 mm. toothpaste A which is the control showed a zone of inhibition of 31 mm. (Refer table 2 and figure 4)

Table 2: Diameter of Zones of Inhibition as exhibited by the toothpastes

Toothpaste	Diameter zone of inhibition (In mm)
A(Control)	31
B	25
C	20
D	33
E	16
F	30
G	16

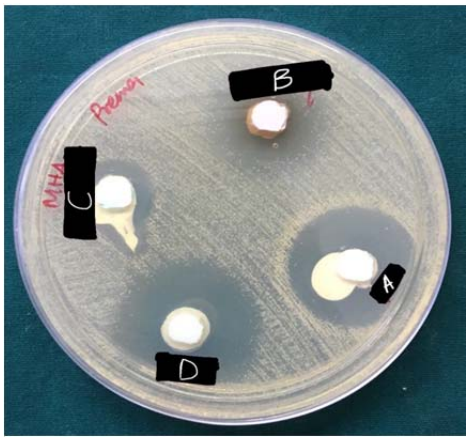

Figure 1: Zones of inhibition as exhibited by toothpastes A (control), B, C and D

Figure 2: Zones of inhibition as exhibited by toothpastes A (Control), E, F and G.

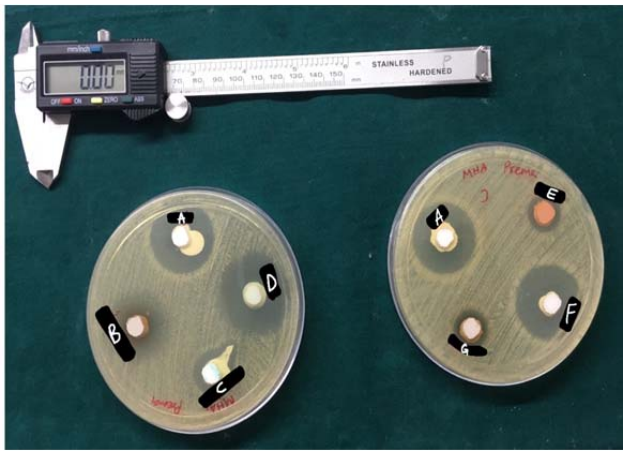

Figure 3: Zones of inhibition of the 7 toothpastes with the aid of a Vernier Caliper

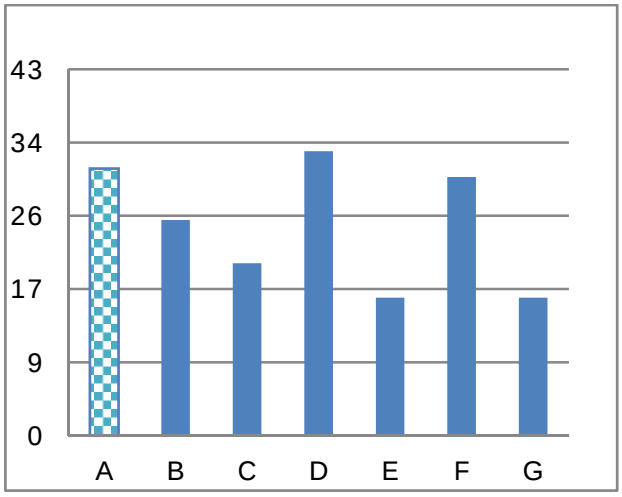

Figure 4: Graph representing Diameter of Zone of inhibition of the seven toothpastes in mm.

DISCUSSION

The substances added to the dental products kill the microbes by disrupting their cell wall and inhibiting their enzymatic activity. They prevent the aggregation of these bacteria over the salivary pellicle which would eventually produce plaque and lead to the destruction of dental hard tissues, slow down the multiplication and the release of endotoxins [8]. The antimicrobials also keep the growth of microflora in check. Triclosan containing toothpastes have been shown to have a higher efficacy in controlling oral micro-flora(blocks lipid biosynthesis by specifically inhibiting the enoyl-acyl carrier protein reductase(ENR), when compared to non-triclosan containing synthetic toothpastes[9] Clinical investigations suggest that the use of a dentifrice with triclosan (0.3%) may reduce halitosis, [10] biofilm formation[10,11] supragingival calculus formation, reduced clinical signs of inflammation and gingivitis[11]

In the study that has been conducted:

- Toothpaste D showed the highest zone of inhibition of 33 mm. It was SLS and Fluoride free, and its antibacterial activity is due to the presence of xylitol, aloe barbadensis gel, tea tree oil, menthol and citric acid.
- Toothpaste A (the control) exhibited 31 mm of zone of inhibition because of the presence of triclosan and fluoride.
- Toothpaste F showed 30 mm of zone of inhibition, which may be attributed to the presence of Miswak extract and SLS. This toothpaste was fluoride free
- The next one on the line was Toothpaste B with a 25 mm zone of inhibition. This particular toothpaste contains herbal products, Fluoride and SLS
- Toothpaste C contains herbal extracts like myrrh, tea tree oil, Chamomile, eucalyptus oil, Eugenol and also fluoride and SLS.
- Toothpaste E and G came with the lowest zone of inhibition of 16 mm , both these toothpastes contained all herbal components except for the excipient and preservatives like methyl and propyl paraben.

This study has been conducted *in vitro* with *Streptococcus mutans* alone, and the toothpaste may not necessarily produce the same effect in the mouth of a person *(in vivo)* i.e., its efficacy in controlling the varied microflora. Also, the concentrations of the substances are not specified, and the results of the study thus conducted are seen only as the collective anti-bacterial action of the various components in the dentifrice. Their individual actions can be seen only if they are separated out in a HPLC for the pure molecular form of its components. Due to practical limitations, only a simple one time experiment was carried out to test the zones of inhibition without any dilutions. Also, there are concerns regarding the fact that the rate of diffusion of the different components may have varied because of their different particle sizes which would have affected their respective zones of inhibition. But it can't be denied that *in vitro* studies must be conducted before *in vivo* studies and clinical trials.

CONCLUSION

Results from this study have shown that Tooth paste D was the most effective toothpaste, owing to its aloe vera, xylitol and tea tree oil content. Triclosan and fluoride containing toothpastes are also highly effective against the gram positive bacteria *Streptococcus mutans*. Toothpastes with a combination of herbal products and fluorides or SLS followed closely after, and toothpastes with only herbal products showed the least effectiveness.

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