Original Article

Antimicrobial Efficacy of Few Commercially Available Herbal and Non Herbal Tooth Pastes Against Clinically Isolated Human Cariogenic Pathogens

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Abstract

Objective: *The aim of this study was to compare the antimicrobial efficacy of ten commercially available herbal and non herbal toothpastes.*

Methods: *Ten toothpaste brands (A-J) were collected. A, C, D, E contained Herbal components only, toothpastes B, F, G contained herbal components and fluoride as main anticariogenic agent, toothpastes H and I contained fluoride and triclosan combination and toothpaste J contained fluoride only as main anticariogenic component. Dental caries samples were collected from 6-14 years old children having occlusal caries in permanent fully erupted molar teeth involving enamel and dentin but caries not involving dental pulp and Streptococcus mutans bacteria were isolated by laboratory method. The antimicrobial activity was assessed by measuring the inhibition zones by agar well diffusion method.*

Results: *Fluoridated toothpaste with triclosan had maximum antimicrobial activity at all concentrations when compared to Herbal toothpaste.*

Conclusion: *Toothpastes containing fluoride and triclosan are most effective but herbal tooth pastes have the prospect of becoming safer and effective altenative and can provide an ideal home care anticariogenic regimen.*

Keywords: *Toothpaste, Fluoride, Antimicrobial, Herbal.*

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.[1]

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It along with periodontal diseases 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. Findings by the Centre for Disease Control and Prevention (CDC) released in August 2005 revealed high increasing prevalence of dental caries in children, with 27% of preschoolers, 42% of school-age children, and 91% of dentate adults having caries experience once in lifetime.[2] Although dental caries is a multifactorial disease it cannot occur without significant role of microorganisms, chiefly a variety of bacteria present in dental plaque. Dental plaque is an oral biofilm comprised of a diverse and complex microbial community. Traditional culture techniques have estimated that around 700 bacterial types exist in the human oral microbiome. Recent advances in molecular sequencing methods put this figure at closer to 25,000 phylotypes in the global oral human microbiome.[3] Among them mutans streptococci namely *Streptococcus mutans* are chiefly responsible for initiation and development of dental caries. It is thus obvious that to maintain ideal oral environment regular plaque removal is very important.[4]

Both chemical and mechanical oral hygiene aids are used for removal and prevention of plaque. Among all home oral hygiene measures tooth brushing is the most common and effective.

Yet, in most people, brushing alone is inadequate to remove oral biofilm to an extent that the development of periodontal diseases and caries is prevented. Thus several active antimicrobial agents are incorporated in the tooth pastes for reducing the microbial load and to prevent microbial diseases like dental caries and periodontitis.[5]

Several toothpastes are available in the market which are claimed to be anticariogenic due to presence of several active constituents. Some tooth pastes contain non herbal components like triclosan and fluoride whereas some toothpastes contain several herbal components and there are a few which contain both. Therefore, the aim of this study was to evaluate and compare the activity of these three groups of dentifrices against clinically isolated human Streptococcus mutans.

Materials and Methods

Toothpastes sampling

Ten toothpaste brands (A-J) were brought from local market of Kolkata, West Bengal, India. Toothpastes A, C, D, E contained Herbal components only, toothpastes B, F, G contained herbal components and fluoride as main anticariogenic agent, toothpastes H and I contained fluoride and triclosan combination and toothpaste J contained fluoride only as main anti-cariogenic component.

Criteria for selection of patients

In the present study, 6-14 years old children having occlusal caries in permanent fully erupted molar teeth involving enamel and dentin but caries not involving dental pulp were included. Those patients with systemic diseases or taking antibiotics or chemical anti-plaque agents prior to six months of study initiation were excluded from the study. On the basis of inclusion and exclusion criteria three patients were selected.

A = Aloe Barbadensis gel

B = Neem extract + Sodium monofluro phosphate

C = Acacia arabica + Clove oil

D = Meswak extract

E = (Piper longum + Zingiber officinale + Terminalia chebula + Zanthoxylum alatum + Syzgium aromaticum + Mentha species) extracts

F= (Meswak+ Peucedanum Graveolens + Foeniculum vulguris fruit + Salvadora Persica stem + Eugenia cayophyllus bud + Elettaria cardamomum fruit) extract + Sodium monofluro phosphate

G = (Eucalyptus +Chamomile + Myrrh + Sage) extracts + Sodium monofluro phosphate

H = Sodium monofluro phosphate + TiO_2 + Triclosan

I = Sodium monofluro phosphate + Triclosan

J = Sodium monofluro phosphate

Chart showing main antimicrobial components of all toothpastes

Collection and isolation of bacteria from Dental caries

Dental caries status of the selected tooth (as per study criteria) was checked clinically with a mirror and a blunt explorer and radiographically with Intra Oral Periapical Radiograph. The patient's were asked not to brush his or her teeth with tooth paste on the morning of the procedure. On day of caries excavation prior to collection of dental caries sample patients were asked to rinse the tooth with sterile water and the teeth were isolated with sterile rubber dam. The food debris on the chewing surface was removed 1st using normal saline irrigated with a syringe and then using a dental excavating instrument (Maillefer, Dentsply, India). The dental caries sample was collected from the patient using another sterile excavator (Maillefer, Dentsply, India) under aseptic conditions and was transferred to the tip of a sterile swab stick. The sample was inoculated immediately streaked onto selective medium MSB agar at 37°C for 24 hours. After growth of the bacteria the bacteria was identified using several biochemical tests. Further identification was performed with the help of a specialized identification kit (HiStrep™ Identification Kit, Hi Media, India).

Biochemical tests to identify Streptococcus mutans

Tests	Results
Colony morphology in Blood agar	Greenish discoloration with 1-2mm wide small pin point lytic zones
Gram reaction	Gram positive
Catalase test	Negative
Oxidase test	Negative
Voges Proskauer's	Positive
Esculin hydrolysis	Positive
PYR	Negative
ONPG (b-galactosidase)	Negative
Arginine Utilization	Negative
Glucose	Positive
Lactose	Positive
Arabinose	Negative
Sucrose	Negative
Sorbitol	Positive
Mannitol	Positive
Raffinose	Positive

Antimicrobial assay

Initial concentration of the tooth pastes was prepared by mixing 2 grams of the tooth paste with 2 ml of sterile distilled water (1:1). Further dilutions were made with distilled water 1:2, 1:4, 1:8 and 1:16. Distilled water was taken as negative control and 0.2% Chlorhexidine was taken as positive control.

The cup plate method or well diffusion method was used to evaluate the antibacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. 100 micro litres of diluted inoculums of McFarland standard 1.5×10^8 CFU/ml of 24 hours old cultures of test organisms were mixed in Muller Hinton agar medium and shaken. Then media was poured in ten sterilized Petri dishes and allowed to solidify for 20 minutes.

Wells of 5 mm diameter were punched into the agar medium with the help of a sterile cork borer. 7 wells were made on each petridish, 5 for tooth pastes dilutions, 1 for positive control and 1 for negative control. 50µl of each solvent was poured on the wells designated for each of them. The plates were then kept in refrigerator for 20 minutes for diffusion of plant solutions. After diffusion of extract the plates were incubated at 37ºC for 24 hours in an incubator. After the incubation, zones of inhibition appeared as a clear, circular halo surrounding the wells. The inhibition zones were measured in mm with the help of an inhibition zone diameter scale (HiMedia). The tests were repeated 3 times to overcome any technical errors that might occur during a single attempt.

Figure 01: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste A.

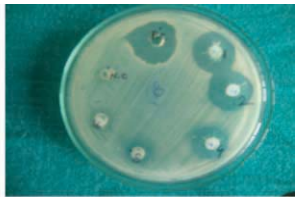


Figure 02: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste B.

Figure 03: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste C.

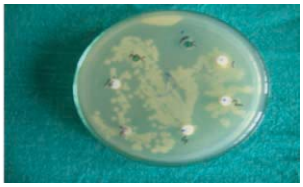


Figure 04: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste D.

Figure 05: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste E.

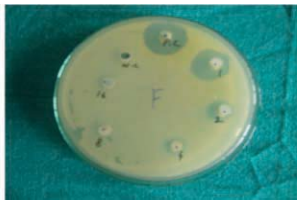


Figure 06: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste F.

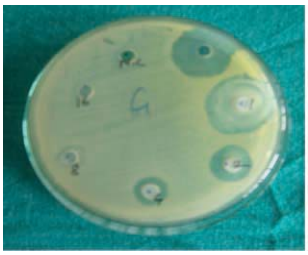


Figure 07: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste G.

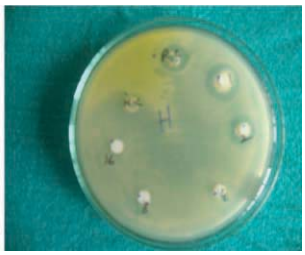


Figure 08: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste H.

Figure 09: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste I.

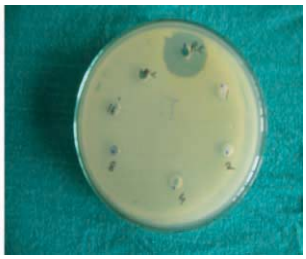


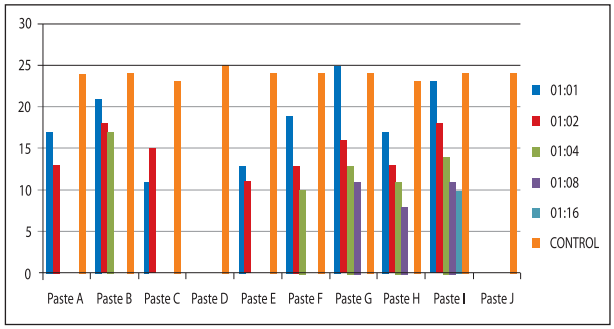
Figure 10: Antimicrobial Activity (In terms of Inhibition Zone in mm) of Toothpaste J.

Results

Tooth paste I and G showed maximum antimicrobial activity. They showed antimicrobial effects (in terms of inhibition zones in mm) in all its dilutions. Toothpaste H was effective up to 01:08 dilution. Toothpastes B and F were effective up to 01:04 dilutions followed by toothpastes A and E that were effective up to 01:02 dilutions. No antimicrobial activity was shown by Toothpastes D and J.

Table 01 shows inhibition zones (in mm) by all toothpaste dilutions

	01:01	01:02	01:04	01:08	01:16	CONTROL
Paste A	17.62±0.47	13.2±0.44	NIL	NIL	NIL	24±0
Paste B	20.8±0.44	17.8±0.27	17.3±0.44	NIL	NIL	24.2±0.22
Paste C	11±0	15.5±0.5	NIL	NIL	NIL	23.6±0.52
Paste D	NIL	NIL	NIL	NIL	NIL	24.2±0.27
Paste E	13.2±0.44	11.3±0.44	NIL	NIL	NIL	24.6±0.28
Paste F	19.3±0.44	13.40.41	10.5±0.5	NIL	NIL	24.6±0.28
Paste G	25.5±0.5	16±0.57	13.6±0.41	11±0	10.5±0.5	24.4±0.28
Paste H	17.4±0.57	13.5±0.5	11.2±0.27	8±0.5	NIL	23±0.57
Paste I	23.5±0.57	18.4±0.57	14±0.5	11.6±0.57	10±0.5	24.2±0.27
Paste J	NIL	NIL	NIL	NIL	NIL	24.5±0.5



Graph 01 shows inhibition zones (in mm) by all toothpaste dilutions

Discussion

Dental plaque is a significant risk factor for the development of dental caries. Dental plaque is a complex biofilm consisting of a variety of bacteria embedded in a polysaccharide matrix. Once colonized the pioneer bacteria, adhere and produce acidic environment which in due course along with series of microenvironment alterations progress to cavitations. Hence antibacterial efficacy of dentifrices is one of the key factors in selection of the tooth paste. The ingredients of tooth paste with antibacterial properties kill the microbes and reduce their growth and colonization on tooth surface.[6]

The present study was aimed to evaluate the anti-cariogenic efficacy of various herbal and non herbal tooth pastes of known antibacterial effect. Of various microorganisms, *Streptococcus mutans* was chosen as test microorganisms as it is a well established fact that *Streptococcus mutans* is chiefly responsible for initiation of carious process through a complex mechanism.[4] The methodology included agar diffusion technique which is the standard method of checking the antibacterial sensitivity.

Results obtained in the present study showed that tooth pastes containing Fluoride and Triclosan (H and I) are very potent against *Streptococcus mutans* followed by tooth pastes containing Herbal component and fluoride (B, F, G). Toothpastes that contain herbal components only also showed moderate antimicrobial efficacy (A, C, E) in the present study. Toothpaste D that also contained herbal components did not show antimicrobial effects. Tooth Paste J containing fluoride only as main anticariogenic component was also ineffective.

Toothpastes containing triclosan and fluoride copolymer provide a more effective antimicrobial efficacy than tooth paste containing fluoride only. Similar results were obtained by Davies, Ellwood and Davies (2004)[7] and Okpalugo, Ibrahim, Inyang (2009)[8].

It has been suggested that triclosan blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR). This feature of fluoride toothpaste containing triclosan can be attributed to the antimicrobial efficiency.[9]

Toothpastes containing herbal component as well as fluoride were more effective than toothpastes containing herbal component only. It can be concluded that presence of Fluoride and active Herbal component act synergistically. Toothpastes containing herbal components only showed similar efficacy except toothpaste D which was ineffective. The antimicrobial activities of the herbal pastes are due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins. There are many publications showing antimicrobial efficacy of herbal tooth pastes which are similar to those of conventional fluoridated tooth pastes and there are other literature that show herbal toothpastes are less effective than conventional toothpastes.[8,10] The results of this present study are not comparable to previous published studies as the constituent herbs of the dentifrices used in the present study are different from those of the other published data. For toothpaste D the study was repeated three times and every time the results were same. The pH of the toothpaste was measured and found to be highly alkaline (8.5). It can be concluded that either Muller Hinton media became ineffective in high alkaline environment or the active component in toothpaste D did not have antimicrobial efficacy against *S. mutans.*

The relative ineffectivity of toothpaste J (containing fluoride only) can be explained as the action of fluoride is mainly preventive by formation of resistant fluroappatite crystal rather than anti microbial. Most of the Dental surgeons advocate fluoridated and triclosan containing toothpastes. But the excess use of the fluoride can cause the dental fluorisis as recently many studies showed adverse effects showed by chronic use of triclosan.[9] So herbal toothpastes may provide an effective and safer alternative.

The present study had several limitations like the concentration of herbs in the toothpastes was not specified thus altering the concentration and calculating the minimum inhibitory concentration of these ingredients can improve the outcome of the dentifrice. Our study could not control other factors like the diffusion of the herbal extracts & the effect of their particle size in diffusion in the agar, data of which were not available. Detergents and abrasives may alter the substantivity or the antimicrobial activity of active ingredients during tooth brushing, which could not be controlled in the study.

The pure extracts of the herbs have to be assessed in the same way to evaluate the antimicrobial efficacy. Even though studies in animals and in-vitro may show the antimicrobial properties of several of these products; randomized clinical trials can provide insight of the overall effect of the dentifrice. The combination of ingredients of the herbal and natural components with the components of the conventional dentifrices can improve the holistic effect of the dentifrice and thus providing better oral health care product.

Conclusion

Toothpastes containing fluoride and triclosan are most effective but herbal tooth pastes have the prospect of becoming safer and effective alternative and may provide an ideal home care anti-cariogenic regime.

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