# Evaluation of Anti-Microbial Property of Toothpaste Containing Calcium, Fluoride, Xylitol and Herbs against Streptococcus Mutans

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**Abstract**
**Aim**
The aim of this study is to assess the antimicrobial activity of toothpaste containing calcium, fluoride, xylitol and herbs against streptococcus mutans.
**Objective**
Whether toothpaste helps us only to physically remove microbes from the oral cavity or do they really have Antimicrobial activity.
**Background**
Toothpaste gives a cleansing and refreshing effect in the mouth. It is made up of various chemical agents, some acts as detergents, some refreshes the mouth and some stimulates salivation. All these effects will enhance the cleansing effect in the mouth. The study is to find out the antimicrobial activity of toothpastes, besides other benefits.
**Reason**
Among the steps to maintain the oral hygiene, brushing the teeth is most important. In certain cases even with regular brushing, the oral hygiene is not properly maintained. Apart from the cleansing and refreshing effect, do the tooth pastes have antimicrobial property in it? So this study is conducted to know the antimicrobial activity if the tooth pastes made with different formulations.

**Keywords: Anti-microbial activity, toothpaste, dental caries and periodontal diseases.**

## Introduction

Dental caries is an aggressive microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavity formation. 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. 60-65% of the Indian population is affected with dental caries 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. 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, spirochetes and bacterioides. 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. Recently a number of plant and herbal extracts have been incorporated into oral hygiene agents because of their reportedly good antimicrobial activity.

But only very limited published data supports this idea and people tend to rely more on natural products these days, owing to their lack of side effects and high efficiency [1].
Periodontal disease and dental caries are essentially caused by the microorganism present in the dental plaque. The 'pioneer species' of dental plaque are streptococcus oralis, streptococcus sanguis and streptococcus mitis. Specific bacteria are closely related to specific dental diseases such as streptococcus mutans and porphyromonas gingivalis are associated with dental caries and periodontal diseases respectively.
Dental plaque is a complex biofilm that accumulates on the tooth surface in the oral cavity. Although over 500 bacterial species comprise plaque, colonization follows a regimented pattern with adhesion of initial colonizers to the enamel salivary pellicle followed by secondary colonization through interbacterial adhesion. A variety of adhesins and molecular interactions underlie these adhesive interactions and contribute to plaque accumulation and ultimately to pathological conditions such as caries and periodontal disease. Biofilm formation is a natural process in the oral cavity but still it needs to be controlled through regular brushing in order to prevent the development of dental caries and periodontal diseases [2].
Both mechanical and chemical oral hygiene aids are used for prevention and removal of plaque. Mechanical plaque control aids such as toothbrush, dental floss, interdental brushes and toothpicks are very popular and widely used in conjugation with chemical plaque control aids, e.g. Medicated toothpastes and mouth rinses. But in many people, brushing alone is adequate to remove the biofilm to an extent that the development of caries and periodontal diseases is prevented. Thus the toothpaste contains Antimicrobial activity augment mechanical plaque removal claims to maintain good oral hygiene [3].
The effectiveness of fluoride containing dentifrices in preventing dental caries is well documented. At fluoride levels of 1000 ppm, effects on the balance of demineralization and remineralisation are more important than any influence on bacterial metabolism. Past studies have found that different fluoride containing dentifrices have widely differing antimicrobial effects, which may explain why some confer greater protection than others. The antimicrobial actions of different forms of fluoride also vary considerably.
The tendency among world consumers, to seek natural products for a healthier lifestyle has increased the use these compounds in food, cosmetic, and pharmaceutical products. The marketing of these products contributes to an increasing in consumption, and natural dental products are also targets of such marketing with a wide variety of products available in all world. The consumer is often induced to buy these products without being aware of their efficacy with the presence of natural compounds by itself does not guarantee their antibacterial activity in the formulation, for example [4].
This is particularly important characteristic for toothpastes, which are expected to help in the control of the biofilm, including cariogenic and opportunistic bacteria, such as Streptococcus mutans, Enterococcus faecalis, and Pseudomonas aeruginosa.

Toothpastes have a wide range of pharmaceutical compositions and consistencies, including gel form that often incorporates natural compounds. Among the most common natural compounds in toothpastes is mint, which is used mainly for flavour, species of the genus. Menthes are also used for different medicinal purposes as an antiseptic, anti-inflammatory, and antimicrobial agent [5].

With regard to cariogenic bacteria, the extent of the antimicrobial activity of dentifrices is unclear. Accordingly, the aims of the present study were to measure the anti-S. Mutans activity of 10 over-the-counter fluoridated dentifrices and 6 herbal dentifrices, and compare this to the antimicrobial activity of key ingredients. These toothpastes were selected because they are popular among consumers and contain different ingredients [6].
Antimicrobials are added to mouth rinses and toothpastes in order to augment in achieving oral health through the killing of oral biofilm organisms. A wide range of Antimicrobials including triclosan, chlorohexidine, stannous fluoride, metal ions and enzymes can be added to mouth rinses. Toothpastes contains a variety of components, they are abrasives such as calcium hydroxide, calcium carbonate etc. Surfactants such as Sodium lauryl sulphate, antibacterial agents such as triclosan, flavorant, remineralisers etc. Chlorohexidine is considered to be the most effective oral antimicrobial. The aim of the study is to evaluate the antimicrobial efficacies of four toothpastes containing fluoride, xylitol, herbs and calcium against streptococcus mutans [7].
Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar 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 bacterioides are found to be the possible pathogens responsible for the disease. Toothpaste is a gel used along with the toothbrush as an accessory aid to clean and to properly maintain the aesthetics and periodontal health of teeth. Toothpaste is used to promote oral hygiene. Triclosan, an antibacterial agent, is a common toothpaste ingredient in the United Kingdom. Triclosan or zinc chloride prevents gingivitis and, according to the American Dental Association, helps reduce tartar and bad breath. Herbal toothpastes contain baking soda, aloe, eucalyptus oil, myrrh, plant extract, and essential oils. Various Oral micro floras include most commonly Escherichia coli and Candida albicans [8].

**Materials**

1. Bacterial suspension of streptococcus mutans with 0.5 mc farland std
2. Paste suspension
   A. 30ml of saline + calcium toothpaste →conical flask
   B. 30ml of saline +fluoride toothpaste→conical flask
   C. 30ml of saline + herbal toothpaste →conical flask
   D. 30ml of saline + xylitol toothpaste → conical flask
   All the flasks were covered and labelled properly and subjected to sterilisation.
3. Culture media – BHI

**Methods**

For each paste solution seven cuvettes were taken. Among seven, six cuvettes were filled with 1ml of the paste solution and one with 1ml of saline is used as a positive control. The six cuvettes containing paste suspension were taken, and in that six five cuvettes were used for test and one is used as negative control. To the five test cuvettes having the paste solution 10µl of the bacterial suspension made with 0.5 mc farland standard is added. The same methodology is followed for all the four paste categories.

After adding the bacterial suspension to the paste solution the cuvettes were incubated at 37°c for a period of 24hrs. After the incubation subculture is done from all the cuvettes to check for the viability of cells. 10µl of the suspension is transferred to the BHI agar and incubated overnight at 37°c to check the viability of the cells.

**Result**

After the incubation the CFU were counted from each plate of the subculture and the number of CFU were tabulated below. The tables are categorised as positive control (Table: 1), negative control (Table: 2), paste containing calcium (Table: 3), paste containing fluoride (Table: 4), paste containing herbs (Table: 5) and paste containing xylitol (Table: 6).

**Control**

**Table: 1 shows the bacterial indicator of the positive control**

| POSITIVE CONTROL | BACTERIAL INDICATOR |
|---|---|
| **Saline + bacteria** | 416 |

**Table: 2 show the bacterial indicator of the negative control**

| NEGATIVE CONTROL | BACTERIAL INDICATOR |
|---|---|
| **Saline + calcium toothpaste** | Negative |
| **Saline+ fluoride toothpaste** | Negative |
| **Saline+ herbal toothpaste** | Negative |
| **Saline+ xylitol toothpaste** | Negative |

**Test**

**Table: 3 show the bacterial indicator of the paste with calcium**

| PASTE WITH CALCIUM: | BACTERIAL INDICATOR |
|---|---|
| **Calcium 1** | Negative |
| **Calcium 2** | Negative |
| **Calcium3** | Negative |
| **Calcium 4** | Negative |
| **Calcium 5** | Negative |

**Table: 4 show the bacterial indicator of the paste with fluoride**

| PASTE WITH FLUORIDE: | BACTERIAL INDICATOR |
|---|---|
| **fluoride 1** | Negative |
| **fluoride2** | Negative |
| **fluoride3** | Negative |
| **fluoride4** | Negative |
| **fluoride5** | Negative |

**Table: 5 show the bacterial indicator of the paste with herbs**

| PASTE WITH HERBS: | BACTERIAL INDICATOR |
|---|---|
| **Herbal 1** | Negative |
| **Herbal 2** | Negative |
| **Herbal 3** | Negative |
| **Herbal 4** | Negative |
| **Herbal 5** | Negative |

**Table: 6 show the bacterial indicator of the paste with xylitol**

| PASTE WITH XYLITOL: | BACTERIAL INDICATOR |
|---|---|
| **Xylitol 1** | 45 |
| **Xylitol 2** | 50 |
| **Xylitol 3** | 27 |
| **Xylitol 4** | 38 |
| **Xylitol 5** | 59 |

**Discussion**

In this study, four toothpastes were evaluated for their antimicrobial activity against streptococcus mutants which is important in biofilm formation as a cause of dental caries or periodontal diseases.

Fluoride containing toothpastes due to triclosan / copolymer along with 1000ppm fluoride provides an effective antimicrobial activity.

Using herbal medicines has become an increasing trend which has made significant contributions to the medical practice. The antimicrobial activity of herbal toothpastes is due to its secondary metabolites such as lectins, flavonoids, tannins etc. These secondary metabolites help to strengthen the gingival tissue and periodontium [9].

Calcium is added to the toothpastes for its abrasive quality, these abrasives assist in removing residual surface stains and debris. The antimicrobial activity of calcium containing toothpastes is due to the presence of triclosan which is an active ingredient and it is most effective [10].

Xylitol is a natural sweetener derived from the fibrous part of plants. It can help keep a neutral pH level in the mouth and does not break down like sugar (sucrose). It prevents bacteria from sticking to the tooth surface. The bacteria in the mouth which causes caries and periodontal disease are unable to digest xylitol, their growth is greatly reduced [11].

In vitro studies have demonstrated that S. mutans produces less acid when a low concentration of fluoride is constantly present. Fluoride concentrates in dental plaque and inhibits metabolizes of carbohydrate (by cariogenic bacteria), leading to lesser production of lactic acid. It also affects the bacterial production of adhesive polysaccharides [12].

Colgate Total, which contains both agents as well as fluoride, gave the largest zone of growth inhibition. This dentifrice contains more antimicrobial agents than the other toothpastes tested. The result for Colgate Total is in agreement with previous assessments of triclosan/copolymer dentifrices which showed significantly stronger antimicrobial effects compared to the dentifrices that did not contain triclosan. Likewise, researchers found that this particular triclosan/copolymer/fluoride dentifrice provided more effective plaque control when compared with non-triclosan containing dentifrices. Dentifrices labelled as "natural" typically don't include ingredients such as synthetic sweeteners, artificial colours, preservatives, additives, synthetic flavours, and fragrances. They are formulated from "naturally derived" components. For example, in "natural" tooth paste, the fluoride comes from fluorspar, the abrasive system is ground calcium carbonate (chalk) instead of synthesized abrasive, the thickener is Carrageenan (derived from seaweed) instead of a product such as methyl cellulose, and the sweetener is Xylitol (a product extracted from birch tree) instead of saccharin. There is little or no research to prove or refute the efficacy of dentifrices containing combination of herbal components, in contrast with a plethora of such research for conventional ones. Hence, with the increased popularity of herbal dentifrices and severe inadequacy of data, clinicians are not in a position to provide proper information to their patients and others about the efficacy of these oral hygiene aids [13].

Another natural compound that deserves attention is propolis. A study demonstrated that the chemical composition of propolis, especially total flavonoids, is dependent on a variety of factors. A characteristic of propolis is that its concentration of the natural compounds varies depending on the vegetation throughout the region where it originates. A study observed that the samples of propolis extract from three different states in Brazil showed different antibacterial activities against S. mutans. The sample from the state of Parana did not show any antibacterial activity; however, those from the states of São Paulo and Minas Gerais had antimicrobial activities though with different minimum inhibitory concentrations (MIC). Researchers evaluated different concentrations of propolis from the Northeast Brazil and reported that propolis showed important antimicrobial activity on E. faecalis with an inhibition halo of 16 mm by a 7.5% solution of red propolis (150 mg). The origin red propolis can be from Cuba, Venezuela, and Brazil. Furthermore, a research found a decrease in the plaque index and gingival inflammation in patients who used oral products with propolis. We observed in our data that the dentifrice VIII (propolis) exhibited antimicrobial activity against S. mutans and E. faecalis similar the positive controls [14, 15].

The performances of products VIII (propolis) and V (propolis/melaleuca) were not similar, even though both gels contain propolis. E. faecalis exhibited resistance to product V (propolis/melaleuca) and sensitivity to product VIII (propolis). A conflicting result can be justified that the propolis in these two products did not have the same origin and concentration [16].

It was estimated that there would be sensibility to E. faecalis to both toothpastes regardless of the presence of M. alternifolia. Another research found that the E. faecalis is sensitive to essential oil the M. alternifolia in contrast to the present study. S. mutans, although sensitive, also showed significantly different responses toward products V and VIII. S. mutans was highly sensitive to product V (propolis/melaleuca = 13.3 mm), and the less responsive to the product VIII (propolis = 8.0 mm) compared to the other gels. These results demonstrated the proven antimicrobic activity of M. alternifolia, corroborated by comparing antiseptic solutions and reported that M. alternifolia product had the strongest antimicrobial activity against S. mutans and other microorganisms. These researchers, however, observed that the residual effect was inferior to those of other solutions tested [17, 18, 19].

The substances added to the dental products kill the micro-organisms by disrupting their cell wall and inhibiting their enzymatic activity. They prevent the accumulation 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. The antimicrobials also keep the growth of micro flora 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 Clinical investigations suggest that the use of a dentifrice with triclosan (0.3%) may reduce halitosis, biofilm formation supragingival calculus formation, reduced clinical signs of inflammation and gingivitis [20, 21].

**Conclusion**

The level of pathogenic organisms in the oral cavity is one of the etiological factors for dental caries and other periodontal diseases. There are number of toothpastes available in the market that claim to have antimicrobial potential. This study shows that xylitol in the toothpaste has less potency as an antimicrobial agent but it can be used in children to avoid fluoride toxicity. Other benefits of xylitol have their own credit, but their antimicrobial property is very much limited when incorporated in the toothpaste.

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