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*Original Article*

# Efficacy of Toothpastes on Bacteria Isolated from Oral Cavity

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## ABSTRACT

**Introduction**: Oral cavity comprises a diverse group of bacterial flora, some of these bacteria are known to cause oral diseases in humans. Toothpaste removes stain and drives away bad breath. Their formulations and active ingredients play an important role in preventing periodontal diseases and other oral infections. **Methods**: Present study aims to determine total bacterial count before and after brushing from saliva samples of 72 individuals of age group 20-22 years. Instructions were given to all participants regarding brushing techniques, brushing time, amount of toothpaste, sampling procedure and sampling time. They were advised to brush with the supplied toothpaste for 2 mins, twice daily, in the morning and in the evening during the test period. Evaluation of antimicrobial efficacy of six different kinds of toothpaste against the bacterial isolates was performed by agar well diffusion method. **Results**: The total microbial reduction ranges from 42.83 - 57.40 %, where Dabur Red being the highest. Predominant isolates were identified as *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Corynebacterium* sp., *Micrococcus* sp. and *Klebsiella* sp. Results of well diffusion test showed the anti-microbial efficacy of pepsodent over other brand of toothpastes. Herbal toothpaste like Dabur red, Babool and Himalaya are however effective, demonstrating significant zone of inhibition against *Streptococcus* sp., *Staphylococcus* sp. and *Micrococcus* sp. **Conclusion**: The active antimicrobial ingredients and formulations of synthetic toothpaste demonstrate their effectiveness in dental care, probably due to the presence of triclosan, fluoride, and other ingredients. However, the herbal toothpastes significantly reduce oral microbial load after brushing. Their natural origin as plant-derived antimicrobials provides opportunities in dental research and maintaining good oral health.
**Key words**: Bacteria, Saliva, Well diffusion test, Triclosan, Herbal toothpaste.

## INTRODUCTION

The oral cavity acts as a primary route of entry to the human digestive system and the oral biofilm harbours a diverse group of microbial community. The diversity of microorganisms in the oral cavity is greater than any other location in the human body.[1] Over 750 species of bacteria inhibits the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases.[2,3] Rozkiewicz, *et al*.[4] reported that the most common bacteria found in oral cavity are *Streptococcus mitis*, *Streptococcus oral*, *Streptococcus sanguis*, *Streptococcus mutans*, *Streptococcus gordonii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Veillonella* sp., *Neisseria sicca*, *Fusobacteria sp.*, *Actinomyces* sp., *Corynebacterium* sp., *Lactobacilli* sp. and *Prevotella* sp. Microorganisms from the oral cavity may cause dental problems which includes dental caries (tooth decay),[5] periodontitis (gum disease),[6] endodontic (root canal) infections[7] and formation of dental plagues.[8] In India, dental caries affects 60-65% of the general population[9] while the periodontal disease is estimated to occur in 50-90% of the population depending on age.[10]

Toothpaste removes stain caused by pigments contained in food and drinks[11] and prevents bad breath.[12] Triclosan is an antibacterial constituent in toothpaste used in the prevention of gum infections. Its antimicrobial and anti-inflammatory properties offer advantages in the management of periodontal diseases. Panagakos, *et al*.[13] demonstrated the effect of triclosan/copolymer/fluoride dentifrice on periodontitis, calculus, caries, whitening and stain removal, oral malodor, and on the oral microflora.

In recent times, it has been observed that many oral product manufacturers are formulating herbal or plant derived toothpastes. Studies showed that the leaf extracts of *Adhatoda vasica* Nees. improves gingival inflammation and reduces gum bleeding.[14] *Mimusops elengi* L. has long been used in indigenous systems of medicine as a specific cure for diseases of the gums and teeth.[15] Studies also demonstrated the anti-cancerous activity and suppression of oral squamous cell carcinoma by *Curcuma longa*[16] *Mentha spicata*,[17] *Withania somnifera*,[18] *Nigella sativa*[19] and *Azadirachta indica*.[20] Feng, *et al*.[21] reported that *Camellia sinensis* protects buccal cells from DNA damage caused by reactive oxygen species. Flower sap of *Echinacea* sp. and *Aloe Vera* stimulates oral immunity and treats gum wounds.[22,23] *Elettaria cardamomum* disinfects the oral cavity and drives away bad breath.[24] Antibacterial, antiviral, anti-inflammatory activity of *Centella asiatica* has been

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reported by Winston and Maimes [25]. The natural origin of herbal products together with antibacterial, antiviral, anti-inflammatory activity found its way into dentistry.[26,27] Thus, the present study aims to determine total bacterial count before and after brushing and evaluation of the antimicrobial efficacy of six different kinds of toothpaste against the isolated bacteria.

## MATERIALS AND METHODS

### *Collection of saliva samples*

Saliva samples were collected from 72 individuals of age group 20-22 years, who were not on medication or any antibiotic dosage for the past six months. Participants were randomly allocated into 6 groups, consisting of 12 individuals in each group and were given one of the following types of toothpastes: Dabur red, Babool, Himalaya, Close-up, Colgate and Pepsodent (Manufacture information are given in Table 1). All participants were advised to brush with the supplied toothpaste for 2 mins, twice daily, in the morning and in the evening during the test period of 10 days. They were instructed to use the same amount of toothpaste as they did usually. Pre-bushing and post-brushing saliva samples were collected from all participants on day 11 at approximately 9.00 a.m in sterile container, and immediately brought to the laboratory for isolation, enumeration and identification of oral bacteria. Similar process was repeated for all other brands of toothpastes in each group with a test period of 10 days, so as to minimize the error between groups and to reduce the likelihood of any carry over effects.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee. Instructions were given to all participants regarding brushing techniques, brushing time, amount of toothpaste, sampling procedure, sampling time etc. Formal consent was obtained from all individual participants included in the study.

### *Determination of total microbial count and identification of bacteria*

Serial dilution of saliva samples was made up to $10^{-3}$ using sterile distilled water. 50 µl of the sample was inoculated on nutrient agar plates and incubated at 37°C for 24 h. The bacterial colonies were counted using a colony counter and were further sub-cultured to obtain a pure culture. The bacterial isolates were identified by colony morphology (shape, structure, colour, pattern, size) gram staining and biochemical tests (indole production, MR, VP, citrate utilization, starch hydrolysis and Triple Sugar Iron) following the standard procedure.[28,29]

### *Antibacterial efficacy of toothpaste*

The effectiveness of different toothpaste against the isolated bacteria was studied by well diffusion method. Mueller-Hinton Agar (MHA) plates were prepared, and wells were made using a sterile syringe puncture. The cultured bacterial samples were spread onto the plates using a sterile spreader, and the wells were filled with toothpaste using micropipettes. The antibacterial activity was evaluated by measuring the zone of inhibition in millimetre.

**Table 1: Toothpaste brands and their active ingredients.**

| Brand Name | Manufacturer | Ingredients |
|---|---|---|
| Colgate | Colgate-Palmolive India Limited | Calcium carbonate, sorbitol, sodium lauryl sulphate, silica, titanium dioxide, sodium silicate, flavor, carrageenan, sodium monofluorophosphate, sodium bicarbonate, benzyl alcohol, sodium saccharin, triclosan, in aqueous base. |
| Himalaya | The Himalaya Drug Company | Sorbitol, aqua, hydrated silica, glycerin, silica, sodium lauryl sulphate, bromelain, xanthan gum, titanium dioxide, flavor, sodium saccharin, sodium benzoate, potassium sorbate, papain, menthol, *Salvadora persica* stem extract, sodium citrate, *Prunus amygdalus dulcis* shell extract, *Cinnamomum zeylanicum* bark oil, *Eugenia caryophyllus* bud oil. |
| Pepsodent | Hindustan Unilever Limited | Calcium carbonate, water, sorbitol, sodium lauryl sulphate, hydrated silica, flavor, magnesium aluminium silicate, sodium monofluorophosphate, potassium nitrate, benzyl alcohol, sodium silicate, cellulose gum, triclosan, sodium saccharin |
| Close up | Hindustan Unilever Limited | Sorbitol, water, hydrated silica, sodium lauryl sulphate, cocamidopropylbetain, flavor, cellulose gum, sodium saccharin, sodium fluoride, zinc sulphate, sodium hydroxide |
| Babool | Dabur India limited | Calcium carbonate, sorbitol, water, silica, sodium lauryl sulphate, flavor containing clove oil, babul (*Acacia arabica*) extract, cellulose gum, xanthan gum, sodium silicate, sodium saccharin. |
| Dabur Red | Dabur India Limited | Clove oil, *Pudina sativa*, Tomar beej (*Zanthoxylum alatum*), Sunthi (Ginger) |

**Table 2: Percentage reduction of oral bacteria by different kinds of toothpaste.**

| Toothpaste Brand | Reduction of Bacteria (in %) |
|---|---|
| Dabur Red | 57.40 |
| Close-up | 56.42 |
| Babool | 53.44 |
| Himalaya | 48.82 |
| Colgate | 45.46 |
| Pepsodent | 42.83 |

## RESULTS AND DISCUSSION

### *Reduction in total microbial count by toothpastes*

The present study evaluates the effectiveness of different toothpaste by percentage microbial reduction. The total microbial reduction ranges from 42.83 to 57.40% (Table 2); where Dabur Red being the highest, followed by Close-up (56.42 %), Babool (53.44 %) and Himalaya (48.82 %). Toothpaste containing triclosan as active ingredients (i.e., Colgate and Pepsodent) were found to reduce 45.46 % and 42.83% of oral bacteria after brushing. Several researchers also reported that triclosan is retained in the oral cavity 12 h after brushing, thereby allowing prolonged control of oral bacteria that forms plaque, gingivitis and oral malodor.[13,30] Oral rinses and toothpaste manufacturers have incorporated plant-derived antimicrobials into their formulations, thereby demonstrating efficacy in reducing oral bacteria similar to that of non-herbal toothpastes containing triclosan, sodium fluoride, sodium saccharin or other ingredients.

### *Identification of bacteria*

The predominant isolates were identified as *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Corynebacterium* sp., *Micrococcus* sp. and *Klebsiella* sp. These microflora on the enamel constantly adapts to changing environment within protective biofilms that predominate mucosal surface of the oral cavity,[31] *Bacillus* and *Corynebacterium* sp. are

**Table 3: Antibacterial efficacy of toothpaste.**

| Isolated bacteria | Zone of inhibition (in mm) | | | | | |
|---|---|---|---|---|---|---|
| | Dabur Red | Babool | Close-up | Himalaya | Colgate | Pepsodent |
| *Klebsiella* sp. | 7±0.32 | 11±0.71 | 5±0.45 | 6±0.45 | 2±0.45 | 7±0.45 |
| *Staphylococcus* sp. | 12±0.86 | 12±0.63 | 12±1.0 | 13±0.32 | 9±0.45 | 17±0.63 |
| *Corynebacterium* sp. | 9±0.95 | 10±0.55 | 10±0.55 | 8±0.32 | 4±0.32 | 16±0.20 |
| *Bacillus* sp. | 13±1.0 | 7±0.32 | 8±0.32 | 10±0.45 | 5±0.71 | 16±0.84 |
| *Streptococcus* sp. | 17±0.55 | 8±1.0 | 13±0.32 | 10±0.45 | 14±0.63 | 15±0.45 |
| *Enterobacter* sp. | 4±0.32 | 6±0.45 | 2±0.63 | 3±0.32 | 5±0.20 | 12±0.32 |
| *Micrococcus* sp. | 13±0.71 | 10±0.45 | 12±0.77 | 12±0.71 | 7±0.32 | 2±0.55 |

*Values are the mean ± standard error of 5 replicates

frequently observed in the oral cavity,[32] *Staphylococcus aureus* in the oral cavity and perioral region[33] and *Streptococcus mutans* are reported to be one of the main opportunistic pathogens of dental caries.[34] *Klebsiella* and related Enterobacteriaceae degrade proteinaceous substance resulting in bad breath.[35] The oral cavity comprises of microflora that may exist as nonpathogens but may become opportunistic to cause various oral cavity associated infections.

### *Antibacterial efficacy of toothpaste*

In most cases, brushing alone is inadequate to remove oral biofilms which cause periodontal disease.[36,37] Therefore, the study attempts to find out the efficacy of different kinds of toothpaste on oral bacteria that harbours the oral cavity which is depicted in Table 3. Among all the tested toothpaste *in-vitro*, Pepsodent was more effective showing a significant zone of inhibition against most of the isolated bacteria. *Staphylococcus* sp. and *Streptococcus* sp. were found to be susceptible against all the tested brands of toothpaste. Prasanth[38] observed that Triclosan, sorbitol, and sodium monofluoro phosphate has an inhibitory effect on oral pathogens *Streptococcus mutans* and *Escherichia coli*. Toothpaste containing fluoride is effective in reducing the adherence capability of *Streptococcus mutans* strains.[39] Herbal toothpaste like Dabur red, Babool and Himalaya are however effective, and showed significant results against isolated bacteria. Chowdhury, *et al.*[40] formulated toothpaste with active natural ingredients of clove oil, lemon peel, orange peel, banana peel etc. Laboratory test results showed that the toothpaste can whitens the teeth, kills germs and imparts freshness feel inside mouth. Natural toothpastes and plant extracts also showed significant results to cure various diseases of teeth like gingivitis,[41,42] tooth decay,[43] cavity,[44] gum bleeding,[45,46] bad breath[47] and dental-caries[48] as well as anti-cancerous properties. The present study reported the efficacy of herbal toothpaste in reducing oral bacterial flora, which is also evident by several studies.[31-35] However, these findings may not correspond to actual *in vivo* potentialities and behaviour of the toothpaste.

## CONCLUSION

Present study showed that herbal toothpastes are capable of combating periodontal microorganisms, thereby preventing periodontal diseases. Several studies have proven the medicinal values of herbal products. However, the active antimicrobial ingredients and formulations of synthetic toothpaste demonstrate their effectiveness in dental care. This study suggests the use of herbal toothpaste over synthetic toothpaste as they are of natural origin and have significant antimicrobial properties. The study also suggests that efforts must be made to find plants and formulate herbal toothpaste capable of preventing or minimizing the effects of dental or oral diseases.

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## CONFLICT INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**MR:** Methyl Red; **VP:** Voges Proskauer; **MHA:** Mueller Hinton Agar

## SUMMARY

Oral bacteria cause infections leading to dental problems in humans. The aim of this study was to determine total bacterial count before and after brushing and to evaluate antimicrobial efficacy of six different kinds of toothpaste against the oral bacteria. The total microbial reduction after brushing ranged from 42.83 - 57.40 %, where Dabur Red being the highest. Predominant isolates were identified as Staphylococcus sp., Streptococcus sp., Bacillus sp., Enterobacter sp., Corynebacterium sp., Micrococcus sp. and Klebsiella sp. The active antimicrobial ingredients and formulations of synthetic toothpaste demonstrate their effectiveness in dental care and reduction in oral microbial load, probably due to the presence of triclosan, fluoride, and other ingredients.

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