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Laboratorial evaluation of antimicrobial efficacy of herbal dentifrices commercialized in India

Avaliação laboratorial da eficácia antimicrobiana de dentifrícios herbais comercializados na Índia

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Abstract

Objective: The purpose of this in vitro study was to evaluate antimicrobial efficacy of commercially available herbal dentifrices in comparison with conventional dentifrices against seven oral microbes, namely, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Actinomyces viscosus*, *Candida albicans*, and *Lactobacillus acidophilus*. **Materials and methods**: Antimicrobial properties of dentifrices were evaluated by measuring zone of inhibition at 48h on blood agar plates inoculated with microbial strains, using disc diffusion method. Each dentifrice was tested in 100% and 50% concentration. Sterile distilled water was used as control. One way Analysis of Variance followed by Tukey post hoc was used for multiple group comparisons. P value < 0.05 was considered statistically significant. **Results**: In both, 100% and 50% concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. **Conclusion**: Antimicrobial activity of Smyle™ herbal toothpaste is the best against microorganisms tested in present study.

Keywords: Herbal. Dentifrice. Oral microbes.

Resumo

***Objetivo**: O propósito deste estudo foi avaliar e comparar a eficácia antimicrobiana de dentifrícios herbais comerciais e convencionais contra sete micro-organismos:* Streptococcus mutans, Streptococcus mitis,

Streptococcus sanguinis, Streptococcus salivarius, Actinomyces viscosus, Candida albicans, *and* Lactobacillus acidophilus. ***Materiais e métodos****: Propriedades antimicrobianas dos dentifrícios foram avaliadas a partir da medição da zona de inibição utilizando o método de difusão em disco após 48h em placas de ágar sangue inoculadas com as cepas microbianas. Cada dentifrício foi testado nas concentrações de 100% e 50%. O controle consistiu em água destilada estéril. As comparações entre grupos foram realizadas por ANOVA a um critério e teste de Tukey post hoc, ambos com nível de significância de 5%.* ***Resultados****: Em ambas as concentrações de 100% e 50%, a zona de inibição máxima foi observada para o dentifrício herbal Smyle® seguido do dentifrício herbal Himalaya herbals®.* ***Conclusões****: O dentifrício herbal Smyle® apresentou o melhor resultado contra os micro-organismos avaliados no presente estudo.*

Palavras-chave*: Herbal. Dentifrício. Micro-organismos orais.*

Introduction

Self performed mechanical plaque removal is a well established method of controlling plaque and gingivitis. However, tooth brushing and flossing are difficult tasks, and most of the patients are not able to completely remove plaque from all tooth surfaces. Along with this, mechanical plaque control is time-consuming, and some individuals may lack motivation for these procedures. In an effort to improve the efficacy of mechanical tooth cleaning procedures, antimicrobial agents have been added to dentifrices like chlorhexidine, triclosan, essential oils, and fluorides. Safety and efficacy of these antimicrobial agents are well supported by researches (1, 2).

The public's interest in alternative health care, including use of herbal health care products has grown dramatically in the past few years (2-4).

With the growing field of alternative medicine, dentifrices based on plant extracts are available in the market. Many herbal dentifrices claim to have antimicrobial properties. This claim is almost always is based on *in vitro* studies of single ingredient(s), which shows that the active ingredient, when placed in a Petri dish with the target organism, can kill that organism. The company promoting the product urges the dental practitioner and consumer to extrapolate this "evidence" to the prevention or treatment of oral diseases like dental caries and gingivitis. However, the reliability of such documentation is disreputably poor (5, 6). Many things can occur during manufacturing of dentifrices like degradation of the active ingredient(s). One ingredient may affect the properties of another (6), for example detergents and abrasives may alter the antimicrobial potential of active ingredient(s) (2). Also, it is generally agreed that with the exclusion of fluoride from most herbal dentifrices, these dentifrices usually give up caries preventive benefits (7). But there is little or no research to prove or refute the efficacy of dentifrices containing combination of herbal components, in contrast with an overabundance of such research for conventional ones.

Hence, with the increase popularity of herbal dentifrices, dental professionals are not in a position to provide information to their patients and others about the efficacy of these products. Thus this *in vitro* study was undertaken with an aim to evaluate the antimicrobial efficacy of commercially available herbal dentifrices in comparison with conventional dentifrices against seven oral microbes, namely, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Actinomyces viscosus*, *Candida albicans*, and *Lactobacillus acidophilus.*

Materials and methods

In present study antimicrobial efficacy of eight herbal dentifrices, namely Himalaya herbals™ dental cream, Smyle™ herbal toothpaste, Dabur™ red toothpaste, Vicco™ vajaradanti paste, Dabur™ Meswak toothpaste, Colgate™ herbal white™ toothpaste, Dabur™ Babool toothpaste, Neem™ active toothpaste; three conventional dentifrices namely Colgate™ super shakti dental cream, Pepsodent™ complete germi check™ toothpaste, New active gel Close up™; and control (sterile distilled water) was evaluated against *Streptococcus mutans* MTCC™ 890, *Streptococcus mitis* MTCC™ 2696, *Streptococcus sanguinis* ATCC™ 10556™,

Streptococcus salivarius ATCC™ 13419™, *Actinomyces viscosus* MTCC™ 7345, *Candida albicans* MTCC™ 183 and *Lactobacillus acidophilus* MTCC™ 447.

For the study first a market survey was conducted for a period of three months in twenty retail shops of Davangere city, Karnataka, India. On the basis of the results, eight most commonly used herbal dentifrices and three most commonly used conventional dentifrices were included in the study. Table 1 shows herbal ingredients of dentifrices as listed on packages.

Retrieving viable growth from freeze dried form of microbes

Brain Heart Infusion (BHI) broth was used to get the viable growth of microbes from freeze dried form. Turbidity in test tube containing BHI broth inoculated with freeze dried microbe confirmed the growth of microbe. Comparison of this turbidity was made with McFarland 0.5 turbidity standard (8).

Disc diffusion method for testing the antibacterial properties

Blood agar plates prepared with 5% defibrinated sheep blood were used in the study as a final growth medium for microorganisms.

With the help of sterile cotton swabs, individual microorganism grown in BHI broth was swabbed over the surface of blood agar plates (9). Filter discs uniformly loaded with 50 mg of dentifrice were placed with sterile tweezers onto the prepared plates (7). Four filter discs were placed in each blood agar plate. The blood agar plates were then incubated at 37 °C for 48 h. Incubation conditions were aerobic, as the literature received from MTCC™ and ATCC™ stated that all the microbes can be grown aerobically very well.

Each dentifrice was tested in 100% concentration and 50% concentration (50% w/w dilution using sterile distilled water).

Based on expected difference from pilot study, 5% level of significance (α), and 80% power of study

Table 1 - Herbal ingredients of dentifrices as listed on packages

S. No.	Herbal dentifrices	Herbal ingredients
1	Himalaya herbals™ dental cream	Extracts of Pomegranate (*Punica granatum*), Suterberry (*Zanthoxylum armatum*), Gum Arabic (*Acacia arabica*), Indian Gooseberry (*Emblica officinalis*), Chebulic Myrobalan (*Terminalia chebula*), Beleric Myrobalans (*Terminalia belerica*), False Black Pepper (*Embelia ribes*), Chaste tree (*Vitex negundo*), Indian Lilac (*Azadirachta indica*), Carom seeds (*Trachyspermum copticum*), Toothbrush tree (*Salvadora persica*), Sweet acacia (*Acacia farnesiana*), Catechu (*Acacia catechu*), Indian Medlar (*Mimusops elengi*).
2	Smyle™ herbal toothpaste	Clove (*Syzygium aromaticum*) oil, Indian Lilac (*Azadirachta indica*) extract
3	Dabur™ red toothpaste	Black pepper (*Piper nigrum*), Long pepper (*Piper longum*), Ginger (*Zingiber officinale*), Suterberry (*Zanthoxylum armatum*), Clove (*Syzygium aromaticum*) oil, Camphor (*Cinnamomum camphora*), Spearmint (*Mentha spicata*).
4	Vicco™ vajaradanti paste	Gum Arabic (*Acacia arabica*), Blackberry (*Syzigium Jambolanum*), Clove (*Syzygium aromaticum*) oil, Bengal Madder (*Rubia cordifolia*), Cinnamon (*Cinnamomum cassia*), Jujube Fruit (*Zizyphus jujuba*), Porcupine (*Barleria prionitis*), Walnut (*Juglans regia*), Catechu (*Acacia catechu*), Sappan Wood (*Caesalpinia sappan*), Mayweed plant (*Anacylus pyrethrum*), Indian Medlar (*Mimusops elengi*), Sweetwood (*Glycyrrhiza glabra*), Cubeb (*Zanthoxylum rhetsa*), Indian Sarsaparila (*Hemidesmus indicus*), Oak (*Quercus infectoria*), Indian Gooseberry (*Emblica officinalis*), Chebulic Myrobalan (*Terminalia chebula*), Beleric Myrobalans (*Terminalia belerica*), Carom seeds (*Trachyspermum copticum*).
5	Dabur™ Meswak toothpaste	Toothbrush tree (*Salvadora persica*) extract
6	Colgate™ herbal white™ toothpaste	Myrrh (*Commiphora myrrha*) tincture, Chamomile (*Matricaria recutita*) tincture, Sage (*Salvia officinalis*) oil, Eucalyptus (*Eucalyptus globulus*) oil, Tea tree (*Melaleuca alternifolia*) oil.
7	Dabur™ Babool toothpaste	Gum Arabic (*Acacia arabica*) extract
8	Neem™ active toothpaste	Indian Lilac (*Azadirachta indica*) extract, Tea tree (*Melaleuca alternifolia*) oil.

Freeze dried form of microbes were purchased from Microbial Type Culture Collection (MTCC™) and Gene Bank, Chandigarh, India and American Type Culture Collection (ATCC™) Virginia, United States of America (USA).

(1−β), for each dentifrice (100% concentration and 50% concentration) and control experiment was conducted for twelve times.

Measurement of zone of inhibition

The zone of microbial inhibition was measured at 48 h underside of the Petri dishes using vernier calipers. The zone of inhibition on the growth of test strains were defined by the area where visible growth had been inhibited if that is obvious; if it was not then measurement was made to the point of 80% inhibition of growth (8, 10). First, the whole of the diameter of the zone of inhibition was measured and later the diameter of filter disc was deducted from it. This gave the actual zone of inhibition around the filter disc (8).

All the measurements of zone of inhibition were carried out by a single examiner. Intra examiner agreement was determined using kappa statistics (k). Intra examiner agreement score (k) was 0.94.

Statistical analysis

Mean and Standard Deviation (SD) of zone of inhibition were calculated. One way Analysis of Variance (ANOVA) test was used for multiple group comparisons followed by Tukey post hoc for group wise comparisons. P-value < 0.05 was considered statistically significant.

Results

Table 2 shows effect of dentifrices (100% and 50% concentrations) and control against *Streptococcus mutans* MTCC™ 890 at 48 h. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effect of Dabur™ Babool toothpaste was significantly lower than any other dentifrice tested in the present study. There was no significant difference between conventional dentifrices in both the concentrations. In 100%

Table 2 - Effect of dentifrices and control against *Streptococcus mutans* MTCC™ 890

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	5.10 ± 0.01	3.31 ± 0.04
Smyle™ herbal toothpaste (2)	6.81 ± 0.03	4.17 ± 0.01
Dabur™ red toothpaste (3)	3.44 ± 0.01	2.23 ± 0.01
Vicco™ vajaradanti paste (4)	3.48 ± 0.01	2.27 ± 0.01
Dabur™ Meswak toothpaste (5)	4.95 ± 0.01	3.08 ± 0.01
Colgate™ herbal white™ toothpaste (6)	3.46 ± 0.02	2.25 ± 0.02
Dabur™ Babool toothpaste (7)	2.54 ± 0.01	1.64 ± 0.01
Neem™ active toothpaste (8)	2.59 ± 0.01	1.69 ± 0.01
Colgate™ super shakti dental cream (9)	4.98 ± 0.01	3.19 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	5.00 ± 0.05	3.18 ± 0.01
New active gel Close up™ (11)	4.99 ± 0.01	3.20 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	37418.59 ($p < 0.001$)	21107.61 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 > 1, 3-12 3, 4, 6 > 7,8,12 5, 9, 11 > 3, 4, 6-8, 12 7 > 12 8 > 7, 12 10 > 3-8, 12	1 > 3-12 2 > 1, 3-12 3,6 > 7, 8,1 2 4 > 3, 7, 8, 12 5 > 3, 4, 6-8, 12 7 > 12 8 > 7, 12 9, 10, 11 > 3-8, 12

concentration, zones of inhibition of Colgate™ super shakti dental cream and New active gel Close up™ were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste. For the same concentration zone of inhibition of Pepsodent™ complete germi check™ toothpaste was significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste and Himalaya herbals™ dental cream. In 50% concentration, zones of inhibition of three conventional dentifrices were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste and Himalaya herbals™ dental cream.

Table 3 shows effect of dentifrices (100% and 50% concentrations) and control against *Streptococcus mitis* MTCC™ 2696. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effect of Dabur™ Babool toothpaste was significantly lower than any other dentifrice tested in the present study. There was no significant difference between conventional dentifrices. In both the concentrations, zones of inhibition of conventional dentifrices were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste.

Table 4 shows effect of dentifrices (100% and 50% concentrations) and control against *Streptococcus sanguinis* ATCC™ 10556™. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. In 100% concentration effects of Dabur™ Babool toothpaste and Neem™ active toothpaste were significantly lower than any other dentifrice tested in the present study. While in 50% concentration smallest zone of inhibition was observed with Dabur™ Babool toothpaste. In both the concentrations, effect of Colgate™ super shakti dental cream was significantly greater than other two conventional dentifrices. In 100% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly greater than all the dentifrices (herbal

Table 3- Effect of dentifrices and control against *Streptococcus mitis* MTCC™ 2696

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	7.18 ± 0.01	4.33 ± 0.01
Smyle™ herbal toothpaste (2)	7.87 ± 0.01	4.65 ± 0.01
Dabur™ red toothpaste (3)	4.71 ± 0.01	2.98 ± 0.01
Vicco™ vajaradanti paste (4)	4.88 ± 0.01	3.04 ± 0.03
Dabur™ Meswak toothpaste (5)	6.33 ± 0.01	3.84 ± 0.01
Colgate™ herbal white™ toothpaste (6)	4.78 ± 0.02	3.02 ± 0.05
Dabur™ Babool toothpaste (7)	2.67 ± 0.01	1.46 ± 0.02
Neem™ active toothpaste (8)	3.05 ± 0.03	1.69 ± 0.01
Colgate™ super shakti dental cream (9)	6.31 ± 0.02	3.82 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	6.29 ± 0.01	3.84 ± 0.01
New active gel Close up™ (11)	6.30 ± 0.01	3.82 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	125126.92 ($p < 0.001$)	26753.83 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 > 1, 3-12 3 > 7, 8, 12 4 > 3, 6-8, 12 5 > 3, 4, 6-8, 10, 12 6 > 3, 7, 8, 12 7 > 12 8 > 7, 12 9, 10, 11 > 3, 4, 6-8, 12	1 > 3-12 2 > 1, 3-12 3,6 > 7, 8, 12 4 > 3, 7, 8, 12 5 > 3, 4, 6-8, 12 7 > 12 8 > 7, 12 9, 10, 11 > 3, 4, 6-8, 12

Table 4 - Effect of dentifrices and control against *Streptococcus sanguinis* ATCC™ 10556™

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	4.98 ± 0.01	3.26 ± 0.01
Smyle™ herbal toothpaste (2)	6.20 ± 0.01	3.81 ± 0.01
Dabur™ red toothpaste (3)	2.75 ± 0.01	1.13 ± 0.01
Vicco™ vajaradanti paste (4)	2.78 ± 0.01	1.17 ± 0.01
Dabur™ Meswak toothpaste (5)	4.10 ± 0.01	2.70 ± 0.01
Colgate™ herbal white™ toothpaste (6)	2.76 ± 0.02	1.14 ± 0.02
Dabur™ Babool toothpaste (7)	1.57 ± 0.01	0.89 ± 0.01
Neem™ active toothpaste (8)	1.59 ± 0.01	0.91 ± 0.01
Colgate™ super shakti dental cream (9)	4.13 ± 0.01	2.72 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	3.98 ± 0.01	2.42 ± 0.01
New active gel Close up™ (11)	4.03 ± 0.01	2.64 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	131985.74 ($p < 0.001$)	64671.36 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 > 1, 3-12 3, 6 > 7, 8, 12 4 > 3, 7, 8, 12 5 > 3, 4, 6-8, 10-12 7, 8 > 12 9 > 3-8, 10-12 10 > 3, 4, 6-8, 12 11 > 3, 4, 6-8, 10, 12	1 > 3-12 2 > 1, 3-12 3, 6 > 7, 8, 12 4 > 3, 6-8,12 5, 9 > 3, 4, 6-8, 10-12 7 > 12 8 > 7, 12 10 > 3, 4, 6-8, 12 11 > 3, 4, 6-8, 10, 12

and conventional) except Smyle™ herbal toothpaste and Himalaya herbals™ dental cream. In 50% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly larger than all the dentifrices (herbal and conventional) except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste.

Table 5 shows effect of dentifrices (100% and 50% concentrations) and control against *Streptococcus salivarius* ATCC™ 13419™. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effects of Dabur™ Babool toothpaste and Neem™ active toothpaste were significantly lower than any other dentifrice tested in the present study. There was no significant difference between conventional dentifrices. In both the concentrations, zones of inhibition of conventional dentifrices were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste.

Table 6 shows effect of dentifrices (100% and 50% concentrations) and control against *Actinomyces viscosus* MTCC™ 7345. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effects of Dabur™ Babool toothpaste and Neem™ active toothpaste were significantly lower than any other dentifrice tested in the present study. In 100% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly greater than all the dentifrices (herbal and conventional) except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream, Dabur™ Meswak toothpaste and New active gel Close up™. In 50% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly greater than all the dentifrices (herbal and conventional) except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste.

Table 7 shows effect of dentifrices (100% and 50% concentrations) and control against *Candida albicans* MTCC™ 183. In both the concentrations

Table 5 - Effect of dentifrices and control against *Streptococcus salivarius* ATCC™ 13419™

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	5.11 ± 0.01	3.24 ± 0.01
Smyle™ herbal toothpaste (2)	5.24 ± 0.01	3.35 ± 0.01
Dabur™ red toothpaste (3)	2.33 ± 0.01	1.26 ± 0.01
Vicco™ vajaradanti paste (4)	2.34 ± 0.01	1.26 ± 0.01
Dabur™ Meswak toothpaste (5)	4.53 ± 0.01	2.94 ± 0.01
Colgate™ herbal white™ toothpaste (6)	2.34 ± 0.01	1.27 ± 0.01
Dabur™ Babool toothpaste (7)	0.52 ± 0.01	0.22 ± 0.01
Neem™ active toothpaste (8)	0.54 ± 0.01	0.23 ± 0.01
Colgate™ super shakti dental cream (9)	4.5 ± 0.01	2.94 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	4.5 ± 0.01	2.95 ± 0.01
New active gel Close up™ (11)	4.5 ± 0.01	2.94 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	246026.73 ($p < 0.001$)	95870.48 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 >1, 3-12 3, 4, 6 > 7, 8, 12 5 > 3, 4, 6-12 7, 8 > 12 9, 10, 11 > 3, 4, 6-8, 12	1 > 3-12 2 > 1, 3-12 3 > 7, 8, 9, 12 4, 6 > 7, 8, 12 5, 9, 10, 11 > 3, 4, 6-8, 12 7, 8 > 12

maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effects of Neem™ active toothpaste were significantly lower than any other dentifrice tested in the present study. In 100% concentration there was no significant difference between conventional dentifrices. Zones of inhibition of conventional dentifrices were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste. In 50% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly greater than all the dentifrices (herbal and conventional) except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream, Dabur™ Meswak toothpaste and New active gel Close up™.

Table 8 shows effect of dentifrices (100% and 50% concentrations) and control against *Lactobacillus acidophilus* MTCC™ 447. In both the concentrations maximum zone of inhibition was observed with Smyle™ herbal toothpaste followed by Himalaya herbals™ dental cream. Effects of Dabur™ Babool toothpaste and Neem™ active toothpaste were significantly lower than any other dentifrice tested in the present study. In 100% concentration there was no significant difference between conventional dentifrices. Zones of inhibition of conventional dentifrices were significantly greater than all the herbal dentifrices except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream and Dabur™ Meswak toothpaste. In 50% concentration, zones of inhibition of Colgate™ super shakti dental cream were significantly greater than all the dentifrices (herbal and conventional) except Smyle™ herbal toothpaste, Himalaya herbals™ dental cream, Dabur™ Meswak toothpaste and New active gel Close up™.

There was no effect of control (zone of inhibition = 0 mm) (Figure 1).

Figure 2 shows effect of Smyle™ herbal toothpaste against *Actinomyces viscosus* MTCC™ 7345.

Discussion

Oral cavity is continually attacked by the various rudiments of nature, and its eternal warm and moist climate serves as an ideal site for the residence and development of numerous microbial populations (10). To contest these invading and potentially harmful microbes, ingredients possessing antimicrobial activity, such as chlorhexidine, triclosan, essential oils,

Table 6 - Effect of dentifrices and control against *Actinomyces viscosus* MTCC™ 7345

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	17.53 ± 0.01	9.46 ± 0.01
Smyle™ herbal toothpaste (2)	23.51 ± 0.01	13.93 ± 0.01
Dabur™ red toothpaste (3)	14.12 ± 0.01	8.07 ± 0.01
Vicco™ vajaradanti paste (4)	14.11 ± 0.02	8.06 ± 0.01
Dabur™ Meswak toothpaste (5)	16.85 ± 0.01	9.10 ± 0.01
Colgate™ herbal white™ toothpaste (6)	14.09 ± 0.01	8.05 ± 0.01
Dabur™ Babool toothpaste (7)	11.93 ± 0.01	7.10 ± 0.01
Neem™ active toothpaste (8)	11.91 ± 0.01	7.12 ± 0.01
Colgate™ super shakti dental cream (9)	16.78 ± 0.01	8.72 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	16.74 ± 0.01	8.68 ± 0.01
New active gel Close up™ (11)	16.76 ± 0.02	8.69 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	1137868.35 ($p < 0.001$)	438238.07 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 > 1, 3-12 3 > 6-8, 12 4, 6 > 7, 8, 12 5 > 3, 4, 6-12 7, 8 > 12 9 > 3, 4, 6-8, 10, 12 10, 11 > 3, 4, 6-8, 12	1 > 3-12 2 > 1, 3-12 3 > 6-8, 12 4, 6 > 7, 8, 12 5 > 3, 4, 6-12 7, 8 > 12 9 > 3, 4, 6-8, 10-12 10, 11 > 3, 4, 6-8, 12

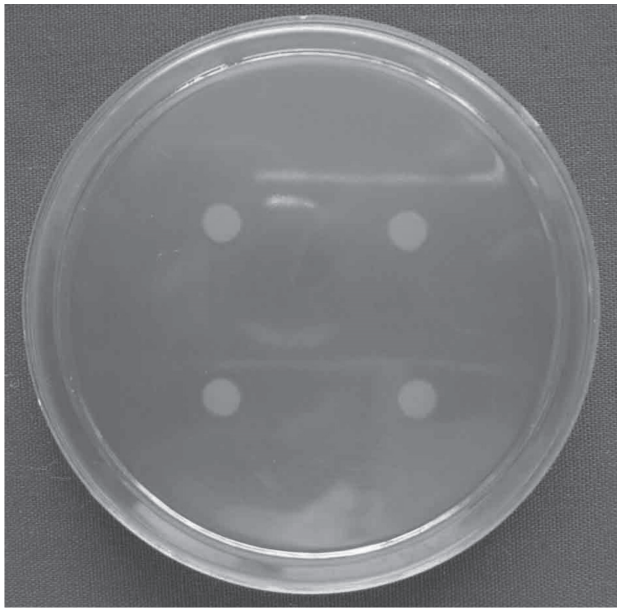

Figure 1 - Effect of control against *Streptococcus mutans* MTCC™ 890

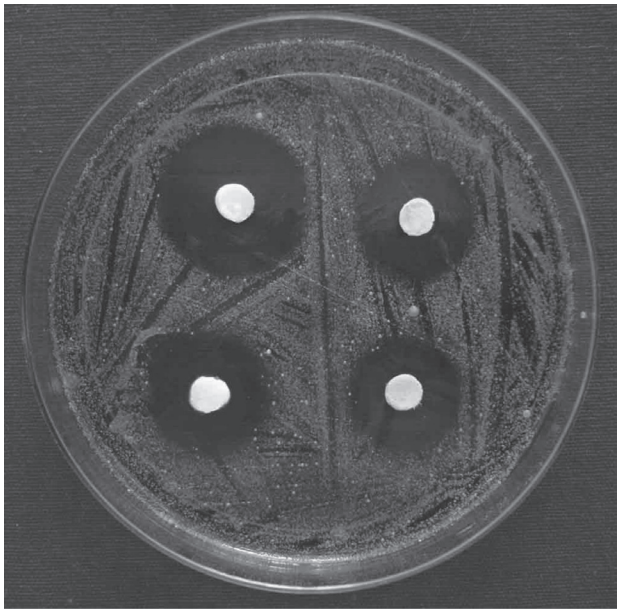

Figure 2 - Effect of Smyle™ herbal toothpaste against *Actinomyces viscosus* MTCC™ 7345

Table 7 - Effect of dentifrices and control against *Candida albicans* MTCC™ 183

Dentifrice	Zone of inhibition (mm, mean ± SD)	
	100% concentration	50% concentration
Himalaya herbals™ dental cream (1)	12.81 ± 0.01	7.07 ± 0.01
Smyle™ herbal toothpaste (2)	13.03 ± 0.01	7.27 ± 0.01
Dabur™ red toothpaste (3)	8.07 ± 0.01	4.32 ± 0.01
Vicco™ vajaradanti paste (4)	8.08 ± 0.01	4.34 ± 0.01
Dabur™ Meswak toothpaste (5)	10.46 ± 0.01	5.89 ± 0.01
Colgate™ herbal white™ toothpaste (6)	8.07 ± 0.01	4.33 ± 0.01
Dabur™ Babool toothpaste (7)	3.21 ± 0.01	1.98 ± 0.01
Neem™ active toothpaste (8)	3.18 ± 0.01	1.95 ± 0.01
Colgate™ super shakti dental cream (9)	9.11 ± 0.01	5.14 ± 0.01
Pepsodent™ complete germi check™ toothpaste (10)	9.12 ± 0.01	5.11 ± 0.01
New active gel Close up™ (11)	9.12 ± 0.01	5.13 ± 0.01
Control (12)	0.00 ± 0.00	0.00 ± 0.00
ANOVA (p value)	833576.23 ($p < 0.001$)	280682.78 ($p < 0.001$)
Tukey's *post hoc*	1 > 3-12 2 > 1, 3-12 3, 4, 6 > 7, 8, 12 5 > 3, 4, 6-12 7 > 8, 12 8 > 12 9, 10, 11 > 3, 4, 6-8, 12	1 > 3-12 2 > 1, 3-12 3, 4, 6 > 7, 8, 12 5 > 3, 4, 6-12 7 > 8, 12 8 > 12 9 > 3, 4, 6-8, 10, 12 10, 11 > 3, 4, 6-8, 12

fluorides, and herbal extracts are added in several oral hygiene products including dentifrices (1, 2, 10).

Streptococcus mutans, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Actinomyces viscosus*, *Candida albicans*, and *Lactobacillus acidophilus* microorganisms were selected for present study because they have been implicated in oral diseases (11-15).

In regards to the testing of zones of inhibition, many methods have been devised and employed with concerns for cost and time. One of the most popular methods used today, as explained by Cormican et al., is to measure zones of inhibition by a disc diffusion method. This method is quick, easy, and inexpensive, making it ideal for research done on low budget with time constraints (10).

Among the dentifrices tested in present study, Himalaya herbals™ dental cream, Colgate™ herbal white™ toothpaste, Neem™ active toothpaste, New active gel Close up™, Colgate™ super shakti dental cream, and Pepsodent™ complete germi check™ toothpaste were fluoridated; and Smyle™ herbal toothpaste, Dabur™ red toothpaste, Vicco™ vajaradanti paste, Dabur™ Meswak toothpaste, Dabur™ Babool toothpaste were non fluoridated dentifrices. Fluoride is the most commonly used and widely accepted therapeutic agent added to dentifrices, which aids in prevention of dental caries (16). Sheen et al. stated that with the exclusion of fluoride from most natural herbal dentifrices, these dentifrices usually forfeit caries preventive benefits (7). Results of our study show best overall antimicrobial activity of a non fluoridated dentifrice, Smyle™ herbal toothpaste against oral microbes. Results similar to present study were reported by Lee et al. (7), Leyster (10), and Wu-Yuan et al. (17). In their studies, effects of some non fluoridated herbal dentifrices against oral microbes were significantly greater than fluoridated dentifrices. Herbal ingredients may be an alternative for fluoride in dentifrices.

Herbal dentifrices tested in present study vary in number and type of herbal ingredients. For example, total number of active herbal ingredients in Vicco™ vajaradanti paste, Himalaya herbals™ dental cream, Smyle™ herbal toothpaste and Dabur™ Meswak toothpaste were twenty, fourteen, two and one respectively.

Results of present study indicate that numbers of active herbal ingredients do not correspond to the antimicrobial activity. Antimicrobial activity of a dentifrice may be the outcome of interaction between different herbal ingredients. Further research efforts are needed to rule out the best possible combination of herbal ingredients in a dentifrice.

Conclusion

The authors conclude that antimicrobial activity of Smyle™ herbal toothpaste is the best against microorganisms tested in present study, but *in vivo* studies are needed to prove or refute its efficacy and safety in real environmental circumstances. Future research efforts are also needed to scrutinize the effectiveness of these dentifrices against other oral microbes.

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