Comparative Evaluation of Antimicrobial Efficacy of Commercially Available Herbal Dentifrices against Selected Putative Oral Microorganisms: An *In-vitro* Study

Dentistry Section

JAYESH PALEKAR¹, SAMEER ANIL ZOPE², GIRISH SURAGIMATH³, SIDDHARTHA A VARMA⁴, APURVA KALE⁵

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ABSTRACT

Introduction: Dental plaque accumulation has been identified as the primary cause of dental caries, periodontal diseases, and halitosis. Worldwide, a wide range of commercially available nonherbal and herbal dentifrices are used along with tooth brush as mechanical aids in plaque control. Owing to some undesirable side effects associated with non-herbal toothpastes, there has been an increased popularity of herbal toothpaste among the general population.

Aim: To assess and compare antimicrobial efficacy of three commercially available herbal toothpastes against selected putative cariogenic, periodontal and fungal microorganisms.

Materials and Methods: An in vitro assessment of antimicrobial efficacy of Sudanta, Dant Kanti, Colgate Cibaca Vedshakti toothpastes was done during the months of February to March 2017 against pure culture standard strains of *S. mutans, C albicans, P. gingivalis, A. actinomycetemcomitans* and *T.forsythia*. All the selected microorganisms were sub-cultured on specific culture media. Antimicrobial efficacy of the toothpastes was then tested in triplicate at full strength using standard disc-diffusion method. The antimicrobial efficacy was evaluated by measuring the zones of inhibition in millimetres surrounding disc containing the toothpastes. Means and standard deviations of the inhibitory zones were calculated for all herbal toothpastes and analysed using one-way ANOVA followed by Tukey's post-

hoc tests for differences amongst the different groups. The p-value \leq 0.05 was considered statistically significant.

Results: Sudanta and Dant Kanti toothpastes demonstrated higher antimicrobial activity against majority of the test microorganisms as compared to Colgate Cibaca Vedshakti toothpaste. Inter-group comparisons among the three toothpastes demonstrated that Dant Kanti toothpaste was significantly better in inhibiting *S. mutans and C. albicans* than Sudanta and Colgate Cibaca Vedshakti ($p \le 0.05$) while Sudanta toothpaste was significantly more effective against *A. actinomycetemcomitans* and *T. forsythia* than Colgate Cibaca Vedshakti toothpaste and Dant kanti ($p \le 0.05$). *A. actinomycetemcomitans* and *T. forsythia* were resistant to Colgate Cibaca Vedshakti toothpaste. However, Colgate Cibaca Vedshakti exhibited substantial antimicrobial activity against *P. gingivalis* when compared against Sudanta ($p \le 0.05$) and Dant kanti (p > 0.05).

Conclusion: Results of the present study revealed that Sudanta demonstrated better antimicrobial activity against selected putative periodontal microorganisms: *A. actinomycetemcomitans* and *T. forsythia*. Dant kanti toothpaste demonstrated better antimicrobial activity against selected putative cariogenic and fungal microorganisms: *S. mutans* and *C.albicans*. Whereas Colgate Cibaca Vedshakti toothpaste exhibited highest antimicrobial activity against P.gingivalis.

Keywords: Dental plaque, Oral disease, Oral health, Oral hygiene, Toothpaste

INTRODUCTION

Diseases such as dental caries and periodontal diseases affect the oral health of people across the globe. The reported prevalence of dental caries in India has been found to be 50-60% whereas periodontal diseases continue to affect 50% of Indians [1,2]. The quality of life and overall well being of individuals are greatly influenced by their oral health. The influence of oral microbiota on oral health conditions has been well established in the past [3].

The major goal of oral hygiene methods is to reduce the pathogenic microbial load in the oral cavity. The most common method to do so is by brushing the teeth regularly using dentifrices. Majority of the dentifrices have ingredients with antimicrobial properties and can prevent degradation of tooth enamel by dental caries. Commonly used toothpastes contain chemical agents like triclosan and sodium lauryl sulphate, which are known to exert harmful side effects like altered taste sensation, abrasion, cancer on repeated use [4]. Oral hygiene methods include those procedures which are aimed to achieve a state of good oral and dental health [5]. Various techniques and products such as toothbrushes, dentifrices, mouthrinses and dental floss have been designed to improve oral health [6].

Around the world, herbal or plant derived medicines have been used traditionally for the treatment of various human diseases since time immemorial [7]. According to the report of World Health Organissation, 70-95% of the global population utilises holistic medicines for primary health care [8]. There has been a long history of the use of aromatic plant twigs or chewing sticks with anti-bacterial properties for oral hygiene maintenance [9]. In an effort to improve the efficacy of the conventional mechanical tooth-cleaning procedures, dentifrices incorporated with herbal extracts were introduced which became very popular due to minimal toxicity and proven effectiveness [3,4]. There has been a growth in popularity of these products recently for the prevention and treatment of oral health conditions especially among the low socioeconomic urban populations and people residing in rural areas [10]. In recent years, several authors have investigated the activity of herbal extracts and their products against specific oral pathogens. The main focus of the research was the ability of herbal extracts to inhibit the dental biofilms formation by exerting antimicrobial action and by reducing the adhesion of oral pathogens to the tooth surface [3,7,11,12].

However, there are no studies in existing literature that have compared the antimicrobial efficacy of herbal based dentifrices used in current study to the best of our knowledge. Hence, this study was carried out to assess and compare the antimicrobial efficacy of three commercially available herbal toothpastes against selected putative cariogenic, periodontal and fungal microorganisms.

MATERIALS AND METHODS

This in-vitro study was carried out during the months of February to March 2017 after due approval from the Ethics Committee of Krishna Institute of Medical Sciences Deemed University (vide letter no: KIMSDU/IEC/04/2016 Dated. 28/12/2016). Three toothpastes (labeled A, B, and C) for the purpose of blinding of the microbiologist obtained from local ayurvedic pharmacies in Karad, Maharashtra, India were selected for assessment of their in-vitro antimicrobial activities. Toothpaste A was non-fluoridated while toothpaste B and C were fluoridated herbal preparations. Compositions of all the herbal toothpastes used in the study are mentioned in [Table/ Fig-1].

Toothpaste	Commercial name	Ingredients			
А	Sudanta	Mayaphal, Lavanga, Maricha, Bakul, Dalchini.			
В	Dant Kanti	Vajradanti, Pilu, Bakul, Babool, Akarkara Extract, Majuphal, Karpur, Vidang Extract, Nimb or Neem Extract, Lavang Extract, Fluoride from plant origin			
С	Colgate cibaca vedshakti	Lemon, Clove, Cinnamon leaf oil, Basil, Camphor, Thymol, Aloe Vera extract, Honey, Sodium Monofluorophosphate, Zinc oxide, Sodium lauryl sulphate			
[Table/Fig-1]: Detailed Composition of herbal toothpastes used in study.					

Preparation of Subcultures of Microorganisms

Pure laboratory cultures of S. mutans (SM) (ATCC 25175), C albicans (CA) 9 (ATCC. 2091), P. gingivalis (PG) (ATCC 33277), A. actinomycetemcomitans (AA) (NCTC 9710), and T.forsythia (TF) (ATCC 43037) were obtained from the Department of Microbiology, Maratha Mandal's Dental College, Belgaum. These test microorganisms were then sub-cultured on specific culture media. In anti-fungal disc diffusion method, Sabouraud's agar medium was used while for S. mutans trypticase soy agar was used and for P. gingivalis, A. actinomycetemcomitans, and T.forsythia Brain heart infusion agar was used. Sabouraud agar has been found to be the most helpful for selective cultivation of C. albicans when the specimen contains mixed population of aerobic, anaerobic, gramnegative and gram positive bacteria in addition to yeast. When pure cultures of S. mutans are used with traditional culture-based media, trypticase soy agar has been found to support superior bacterial recovery than other media like Mitis-Salivarius Bacitracin (MSB). The Brain heart infusion agar medium has been found to favour the growth of gram negative anaerobic periodontal pathogens [13-15].

Determination of Antimicrobial Assay

The antimicrobial activity of different dentifrices (A, B, C) against putative oral bacteria was determined by using the disc diffusion method. The microbiologist was blinded regarding the identity of toothpastes as the toothpaste samples were provided in a sterile container with nomenclature as A, B and C, respectively.

In this method, nutrient agar plates were seeded with 0.5 mL of 24 hour broth cultures of *S. mutans (SM), C albicans (CA), P. gingivalis (PG), A. actinomycetemcomitans (AA)*, and *T.forsythia (TF)*. Within 15 minutes of adjusting the inoculums to a McFarland 0.5 turbidity standard it was swabbed on the entire surface of agar plate three times, rotating the plates approximately 60° between streaking to ensure even distribution. Inoculated plate was allowed to stand for at least 3 minutes. A sterile 5 mm hollow tube was used to cut three wells at equidistance in each of the plates [16].

Discs impregnated with the toothpastes (5 mm diameter) were placed in prepared wells on Petridishes containing culture media which was inoculated with five indicative microorganisms. Plates were incubated immediately for period of 24 hours at 37°C in incubator. In order to compare the efficacy of the various toothpastes tested, the diameters of the halo formed by the growth inhibition were measured and compared at 24 hours following initial incubation. The mean diameters of inhibition zones were determined for all toothpastes. To measure the inhibition zone, plate was first placed on a non-reflective surface. A ruler that measures in millimetres was placed at the "0" in the centre of the impregnated disc. Radius of the zone of inhibition was measured from the centre of the disc to the edge of inhibition area with zero growth. This measure was multiplied by two in order to get the diameter. Antimicrobial efficacy of the dentifrices was tested in triplicate, at full strength.

STATISTICAL ANALYSIS

The collected data was analysed with Statistical Package for the Social Sciences (SPSS Software Version 19, Armonk, New York; IBM. Corporation USA). One-way ANOVA test was used for comparison of antimicrobial efficacy of three herbal toothpastes followed by Turkey's Post-hoc tests for differences among the various groups. The level of significance was set at p<0.05.

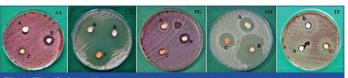
RESULTS

The present in-vitro study was conducted to evaluate the antimicrobial efficacy of three commercially available herbal toothpastes. [Table/ Fig-2,3] shows the mean zones of inhibition (in millimeters) obtained from toothpastes A, B and C respectively at 24 hours at full strength. There was a significant difference observed in the antimicrobial activity among the three toothpaste groups (p≤0.05). All the toothpastes demonstrated antimicrobial activity against all the test microorganisms except toothpaste C to which *A. actinomycetemcomitans* and *T. forsythia* were resistant.

Test organisms	Toothpaste A	Toothpaste B	Toothpaste C	F value	p- value
S. mutans	38±10	45±10	35±10	79.0	0.01*
C albicans	43±10	50±10	39±10	93.0	0.001*
P. gingivalis	25±10	28±10	30±10	19.0	0.002*
A. actinomycetemcomitans	23±10	20±10	0 (R)	703.5	0.001*
T. forsythia	30±10	28±10	0 (R)	1266	0.001*

[Table/Fig-2]: Comparison of antimicrobial efficacy (zone of inhibition in mm) of toothpastes.

*Statistically significant; p≤0.05; A: Sudanta; B: Dant Kanti; C: Colgate CibacaVedshakti; R: Resistant; [#]One-Way ANOVA



[Table/Fig-3]: Disc diffusion method for determination of antimicrobial activity of different dentifrices (A, B, C) against *A. actinomycetemcomitans, C. albicans, P. gingivalis, S. mutans* and *T. forsythia.*

Intergroup comparisons using post-hoc test analysis revealed, toothpaste A and B demonstrated better antimicrobial activity than toothpaste C against *S. mutans, C albicans, A. actinomycetemcomitans,* and *T. forsythia* (p≤0.05) while toothpaste C was better in inhibiting *P. gingivalis* than toothpaste A (p≤0.05) and B (p>0.05) [Table/Fig-4]. Toothpaste B produced significantly larger zones of inhibition against *S. mutans* and *C albicans* than toothpaste A (p ≤0.05) and C (p ≤0.05) while Toothpaste A demonstrated greater zone of inhibition against *A. actinomycetemcomitans* and *T. forsythia* than toothpaste B (p≤0.05) and C (p≤0.05) [Table/Fig-4].

Organism	Toothpaste	Other toothpaste	p-value			
	А	В	0.001*			
S. mutans		С	0.024*			
	В	С	0.001*			
	А	В	0.001*			
C. albicans		С	0.006*			
	В	С	0.001*			
	А	В	0.020*			
P. gingivalis		С	0.002*			
	В	С	0.108†			
	А	В	0.009*			
A. actinomycetemcomitans		С	0.001*			
	В	С	0.001*			
	А	В	0.05*			
T. forsythia		С	0.001*			
	В	С	0.001*			
[Table/Fig-4]: Inter group comparisons between different toothpastes in relation to test microorganisms by Post-hoc test. *Statistically significant; p<0.05; *Non-significant; p>0.05; A: Sudanta; B: Dant Kanti; C: Colgate CibacaVedshaktir #Tikev's Post-hoc test						

DISCUSSION

Most common cause of dental caries and periodontal disease is the accumulation of dental plaque secondary to inadequate oral hygiene [17]. An unequivocal method of controlling dental plaque is self-performed mechanical plaque removal. Efficacy of brushing is usually dependent on patient's compliance hence efforts are made to improve its efficacy of plaque control by the adjunctive use of toothpastes [18].

In recent years, awareness about the harmful effects of various chemical constituents of conventional (non-herbal) toothpastes has increased interest in use of traditional herbal medicine in prevention and control of dental caries and periodontal diseases [19]. Minimal side effects and lack of synthetic active ingredients as well as flavouring agents make the herbal toothpaste preparations the preferred choice especially for people living in rural areas [12].

Majority of the studies available have evaluated the antimicrobial efficacy of commercially available herbal toothpastes against selected oral pathogens mainly *streptococcus* spp., *staphylococcus* spp., *candida* spp. etc., but there is no study in already existing literature that has evaluated the antimicrobial activity of herbal toothpastes against putative periodontal pathogens [20-22]. To the best of our knowledge, this is the first study to assess and compare the antimicrobial activity of Colgate Vedshakti, Sudanta and Dant Kanti toothpastes against putative cariogenic, periodontal and fungal pathogens.

The results of the present study revealed that all the herbal dentifrices showed substantial antibacterial activity against *S. mutans*. Patanjali Dant Kanti had the highest antibacterial activity, followed by Sudanta and Colgate Vedshakti out of these toothpastes. Among all the tested herbal toothpastes, Dant Kanti and Sudanta possessed better antimicrobial activity against all test pathogens other than *P. gingivalis* while *A. actinomycetemcomitans* and *T. forsythia* were resistant to Colgate Cibaca Vedshakti. However, Colgate Cibaca vedshakti had the highest antimicrobial activity against *P. gingivalis*.

Studies by Vyas S and Kulkarni S., Shaheen SS et al., have reported substantial antimicrobial activity of Dant Kanti toothpaste against *S. mutans* and *C. albicans* in comparison to various herbal toothpastes [23,24]. These results are in accordance with the current study. Toothpastes containing herbal components as well as fluoride were found to be more effective against cariogenic microorganisms than toothpastes containing herbal components only. It can be attributed to presence of fluoride and active herbal components like *Vajradanti, Bakul, Babool, Neem, Lavang* which act synergistically [23].

Sudanta toothpaste, which is non-fluoridated herbal toothpaste has shown promising results in terms of antimicrobial activity against selected oral microorganisms. Superior antimicrobial activity of Sudanta toothpaste against periodontal pathogens can be attributed to the presence of Mayaphal. Mayaphal is rich in gallotannic acid, gallic acid and ellagic acid which have shown strong inhibitory effects against microorganisms like *S. mutans*, *S. salivarius*, *P. gingivalis*, and *F. nucleatum* [25].

Inhibition of *P. gingivalis* by Colgate Cibaca Vedshakti was found to be greater than Sudanta and Dant Kanti toothpaste. This can be attributed to the presence of Zinc Oxide which has shown strong suppression of volatile sulphur compound producing pathogens like *P. gingivalis, F. nucleatum, P. intermedia* thus also reducing oral malodour [26].

The antimicrobial activity of the herbal pastes is due to the presence of Plant Secondary Metabolites (PSMs) such as alkaloids, flavonoids, polyphenols, and lectins. Antimicrobial activity of PSMs includes inhibition of protein or cell wall synthesis, induction of cell wall leakage and interference with DNA/RNA synthesis/function [27]. Herbal toothpastes potentially prevent the colonisation of the oral bacteria on the tooth surfaces most likely by penetration of the active herbal ingredients into the plaque biofilm and attenuating further plaque maturation [28].

Several studies have shown similar antimicrobial efficacy of herbal tooth pastes to that of conventional fluoridated chemical tooth pastes [22,29,30] while few authors reported that herbal toothpastes are less effective than conventional toothpastes [17,31,32].

As seen from the composition of toothpastes used in present study, variation in the herbal contents of the toothpastes and addition of compounds like fluoride, zinc oxide, Karpur etc., might have influenced the study results [23,26]. Antimicrobial efficacy of toothpastes with numerous herbal ingredients is found to be more than the toothpastes with less number of herbal ingredients [33]. Infrequently, owing to the presence of multiple herbal components mucosal irritation can be encountered in some users [34].

Limitation(s)

The antimicrobial properties of only herbal toothpastes were compared in the present in-vitro study. Further studies can be carried out comparing various herbal toothpastes with conventional non-herbal toothpastes. As this study was carried out against isolated strains of oral microorganisms, it would be interesting to test its efficacy in oral cavity against microorganisms present in the organised plaque biofilm.

CONCLUSION(S)

Based on the findings of present in-vitro study, the study hereby conclude that all the three herbal toothpastes demonstrated antimicrobial activity against the selected oral pathogens, with Dant Kanti being the most efficacious as a herbal fluoridated toothpastes and Sudanta as a herbal non-fluoridated toothpaste. Owing to the benefits of herbal toothpastes like minimal toxicity, and proven antibacterial activity as also demonstrated by current study, they can be considered as an alternative to conventional toothpastes. However, future research is required for the clinical evaluation of efficacy of the herbal dentifrices for its regular use in the oral hygiene maintenance.

Declaration: The study does not promote any company. It is completely meant to promote research and scientific evidence.

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PARTICULARS OF CONTRIBUTORS:

- 1. Under Graduate Student, Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Karad, Maharashtra, India.
- 2. Reader, Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Karad, Maharashtra, India.
- 3. Professor and Head, Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Karad, Maharashtra, India.
- 4. Reader, Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Karad, Maharashtra, India.
- 5. Senior Lecturer, Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Karad, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR: Sameer Anil Zope,

Department of Periodontology, School of Dental Sciences, KIMS Deemed to be University, Malkapur, Karad, Maharashtra, India. E-mail: aoldentist@gmail.com

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