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Antimicrobial activity of commercially available five ayurvedic dentifrices

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Abstract

Background: Dental caries is one of the most leading cause of oral diseases among the globe, numerous dentifrices and mouthwashes come to the market for the remedy. Thus, more research are focussed on natural system of medicine like Ayurveda and its use for curbing oral health problems.

Aim and Objectives: To assess the antimicrobial activity of commercially available five dentifrices against streptococcus mutans.

Materials and Method: For the present study we used strains of micro-organisms- Streptococcus mutants Strain- 890 from MTCC. To revive the strains Brain Heart Infusion Agar (BHI agar media) was used. Double blinding was done and samples were coded with alphabets to avoid examiner bias. The plates were kept in incubation chamber for 24 hours. After 24 hours, 48 hours, 72 hours the zone of inhibition was measured with the help of Hi Antibiotic Zone scale from HiMedia Laboratories Limited, Mumbai.

Observations and Results: After assessing the antimicrobial activity for 24 H, 48 H and 72 H, DantKanti was found to be most effective with the highest zone of inhibition 28.16 ± 0.75 , followed by Meswak. Whereas, Dabur lal and Himalaya had the least effect when compared to their counterparts.

Conclusion: The result of the current study demonstrated that Dantikanti has the maximum antimicrobial effect against *S.mutans* followed by Meswak and Babol, remaining dentifrices like Himalaya and Dabur lal had the least effect against the microbes. Thus, when compared with all other commercially available ayurvedic dentifrices Dantikanti proved to be more effective at 24 hours, 48 hours and 72 hours among all others.

Keywords: Ayurvedic dentifrices, *S.mutans*, MTC-890

Introduction

Oral cavity of humans consists of large number of Gram positive and gram negative microorganisms. Several studies have shown that oral cavity act as a paradise with its warm and moist environment, growth, proliferation and multiplication. A biofilm inside the oral cavity is responsible for caries activity, gingivitis and periodontitis. The epidemiological studies evidently reflect a noticeable increase in the prevalence of dental caries in many developed and developing countries. Streptococcus mutans is one of the main opportunistic pathogens of dental caries. The pathogens of dental caries, play a central role in fermenting carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel (Ajithkrishnan *et al.*, 2014; Chhina, 2016) ^[1, 4].

The problem of dental caries can be prevented by various documented methods of plaque control which may be chemical, mechanical or a combination of both. Most commonly used method is use of dentifrices along with proper brushing techniques. Nowadays a wide range of dentifrices are available in the market for maintaining good oral hygiene using various mechanisms. Similarly various toothbrushes are available to synergise the effect of these dentifrices for efficient removal of plaque and debris. Also there is a paradigm shift in the use of herbal and ayurvedic products recently, and the oral hygiene products are not an exception to it. The market is flooded with herbal dentifrices even from big brands. Many studies have justified the use of antimicrobial chemotherapeutic agents as a means of reducing the levels of oral bacteria, specifically Streptococcus mutans. Though many herbal dentifrices claim to have antimicrobial properties similar to that of other chemotherapeutic agents but if we search

the literature very little research has been conducted to prove the same. Hence, the present study was undertaken to investigate antimicrobial efficacy of different herbal toothpastes against *S.mutans* by using standard agar well diffusion method (Bafna, 2015; Latiyan *et al.*, 2017; Bafna *et al.*, 2015)^[2, 3].

Aim and Objectives: To assess the antimicrobial activity of commercially available ayurvedic dentifrices against the streptococcus mutans.

Materials and Methods

This *in-vitro* study was conducted to evaluate and compare the efficacy of herbal toothpastes in reducing the colonization of streptococcus mutans commercially available herbal toothpastes were taken and to blind fold the study, they were labelled as A, B, C, D, E to minimize the observers bias and increase the internal validity of the study. Samples were collected from them and their efficacy against *S.mutans* was evaluated using modified agar well disk diffusion method. Standard protocol of dilution was used for each of them, each dilution was made by adding 3ml of distilled water in 3gm of toothpaste.

Bacteria strains: For the present study we used strains of micro-organisms- Streptococcus mutans Strain- 890 from MTCC. To revive the strains Brain Heart Infusion Agar (BHI agar media) was used. The inoculation of *S.mutans* in liquid medium (BHI media) from its single colony was done to ensure its best growth at 37 °C in peptide rich conditions. All these steps were performed using sterile technique to avoid contamination. Using a sterile, disposable inoculating loop or wooden stick, one colony was picked from tube containing viable *S.mutans* colonies. The colony was suspended in liquid BHI medium in a sterile 15 ml screw cap conical tube. Cap on tube was tightened to prevent release of CO₂ during growth. Volume was close to maximum of tube to reduce the available air space. Since the tube was sealed, culture was placed in non- CO₂ (ambient air) incubator. Incubation was done statically overnight at 37 °C. Culture was checked under a light microscope at 40× magnification for presence of *S.mutans*. The method used was by modified agar well disk diffusion method. This method was used because it is gold standard for in vitro study of anti-microbial properties of specific culture. This method is widely used in other studies and research related for anti-microbial effects in specific culture (Chhina, 2016; Bafna, 2015; Bafna *et al.*, 2015; Singh *et al.*, 2014)^[4, 2, 3, 9, 10].

Antimicrobial Assay: Brain heart infusion agar media was fixed in the lab and then poured into the sterile streak plates and was incubated for 1 hour at 37°C until it gets solidifies. Test tube containing Streptococcus mutans was opened and diluted in 1:1 ratio with distilled water. For the present study we used strains of micro-organisms- Streptococcus mutans [MTCC-890]. The organisms were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics. Whole procedure was done under horizontal laminar flow hood for making the condition sterile and prevent contamination. Using a sterile cotton swab, Streptococcus mutans was spread over the media and was cultured in brain heart infusion agar and incubated at 37°C for 48 hours. The toothpaste was diluted with pathogenic free distilled water by mixing 3gm of toothpaste with 3 ml of distilled water. Totally

15 petridishes (3 each for 5 dentifrices) were prepared and Streak plates were divided into 3 equal parts (equal in surface area) and their centres were marked. By ditch method using sterile gel puncher was used to cut 3 wells equidistant from each other and was filled with different toothpaste samples (2 ml) with micropipette. The plates were kept in incubation chamber for 24 hours. After 24 hours, 48 hours, 72 hours The zone of inhibition was measured with the help of Hi Antibiotic Zone scale from Hi Media Laboratories Limited, Mumbai, which is certified to International Standards Organization and World Health Organization (WHO) Good Manufacturing Practice^[7-10].

Statistical analysis: The results were entered into the Microsoft excel 2010 for data analysis and interpretation. Descriptive statistics (Mean and S.D) were calculated and one way ANNOVA analysis with Bonferroni correction was done to analyse mean difference among the five dentifrices using IBM SPSS Version 23.

Results

Table 2 represents the zones of microbial inhibition demonstrated by the five commercially available dentifrices at full strength against the *S. mutans* with mean zone of inhibition. DantKanti and Meswak represents the highest mean diameter against *S. mutans* of 27.83±1.16 and 23.58±1.96 respectively and Himalaya had the lowest zone of inhibition of 7.83±0.75 at 24 hours.

Table 1: Sample with active ingredients

Label	Sample	Active Ingredient
A	Himalaya (Total Sensitive)	Triphala
B	DantKanti (Patanjali)	Neem, Clove, Pudina & Babool
C	Meswak	Calcium carbonate & Sorbitol
D	Babool	Lavage oil, Pudina, Camphor
E	Dabar Lal	Clove oil, Pudina, Ginger

Table 2: Descriptive statistics for mean zone of inhibition

		N	Mean± Std. Deviation
24 Hours	Himalaya	6	7.83±0.75
	DantKanti	6	27.83±1.16
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabaralal	6	8.00±0.63
48 Hours	Himalaya	6	8.00±0.63
	DantKanti	6	28.16±0.75
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabaralal	6	8.00±0.63
72 Hours	Himalaya	6	8.00±0.63
	DantKanti	6	28.16±0.75
	Meswak	6	23.58±1.96
	Babool	6	13.83±0.75
	Dabaralal	6	8.00±0.63

Similarly, at 48 hours and 72 hours, Dant Kanti and meswak had the highest zone of inhibition compared to other dentifrices. The maximum zone of inhibition was demonstrated at 48 hours by Dantikanti (28.16±0.75) which was unchanged till 72 hours. Comparison of different dentifrices using one- way analysis of variance (ANOVA) showed that the difference in microbial inhibition was significant for all the five dentifrices against the *S. mutans*

which can be inferred from Table 3. Tables 4 indicates the Multiple comparisons using Bonferroni correction at 24, 48 and 72 hours represents statistically significant difference

in mean zone of inhibition compared to others, except for the Dabur Lal – Himalaya group (p=0.999, p=1.000).

Table 3: One – way annova analysis for mean comparison

		F	p-value	Sig.
24 Hours	Between Groups	370.111	.000	S
	Within Groups Total			
48 Hours	Between Groups	438.608	.000	S
	Within Groups Total			
72 Hours	Between Groups	438.608	.000	S
	Within Groups Total			

Bonferroni correction used for mean comparison between the different dentifrices. The mean difference is significant at ≤ 0.05 . S: Significant; NS: Non significant

Table 4: Multiple comparison using bonferroni correction for mean comparison

Dependent Variable	Dentifrice	Dentifrice	P-value	Sig.
24 Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.999	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Meswak	Himalaya	.000	S
		DantKanti	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Babool	Himalaya	.000	S
		DantKanti	.000	S
		Meswak	.000	S
		Dabarlal	.000	S
Dabarlal	Himalaya	.999	NS	
	DantKanti	.000	S	
	Meswak	.000	S	
	Babool	.000	S	
48 Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	1.000	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Meswak	Himalaya	.000	S
		DantKanti	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Babool	Himalaya	.000	S
		DantKanti	.000	S
		Meswak	.000	S
		Dabarlal	.000	S
Dabarlal	Himalaya	1.000	NS	
	DantKanti	.000	S	
	Meswak	.000	S	
	Babool	.000	S	
72 Hours	Himalaya	DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	1.000	NS
	DantKanti	Himalaya	.000	S
		Meswak	.000	S
		Babool	.000	S
		Dabarlal	.000	S
	Meswak	Himalaya	.000	S
		DantKanti	.000	S
		Babool	.000	S
		Dabarlal	.000	S

		Dabarlal	.000	S
	Babool	Himalaya	.000	S
		DantKanti	.000	S
		Meswak	.000	S
		Dabarlal	.000	S
	Dabarlal	Himalaya	1.000	NS
		DantKanti	.000	S
		Meswak	.000	S
		Babool	.000	S

Bonferroni correction used for mean comparison between the different dentifrices.

The mean difference is significant at ≤ 0.05 . S: Significant; NS: Non significant

Thus there was significant difference among the mean of all the groups and highest was reported for Dantkanti.

Discussion

The current research was carried out to assess the antimicrobial activity of commercially available five ayurvedic dentifrices to assess the effectiveness against the *S. mutans*. According to the results, DantKanti has showed the maximum zone of inhibition with the mean diameter of 28.16 ± 0.75 at 72 hours, whereas babool, daburlal and Himalaya showed minimal changes in the zone of inhibition. the active component of DantKanti (neem), have demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties in the literature. Menthol ($C_{10}H_{20}O$), the active constituent present in Pudina, perhaps is largely responsible for the therapeutic potentials of Pudina. It is used to treat liver and spleen diseases, asthma and jaundice.

The oil is antiseptic, carminative, refrigerant, stimulant and diuretic. Clove oil has Longley been used directly to the gums to ease toothache. There is evidence that the eugenol in clove oil is effective at fighting several known oral bacteria. Medicines containing eugenol are widely used in dentistry and some research suggests that clove gel may reduce the pain of needle insertion in dentistry. Thus, there can be a synergistic effect of the combination of clove, neem, babool and pudina which is present in the DantKanti. Research carried out by various investigators have also demonstrated that they have significant antimicrobial activity and also reported reduction in plaque and improved periodontal health. In modern India, with the advent of research activities into the naturopathy system of medicine has elaborated the beneficial effect of these herbs in oral health. These components are also used in the mouthwashes for the analysis of the antimicrobial property and it has proved to be effective against *S. mutans* and *C. albicans* (Singh, 2018; Tomar, 2018; Jyoti, 2018; Singh, 2014; Shah, 2018) [8, 11, 5, 9, 10, 7].

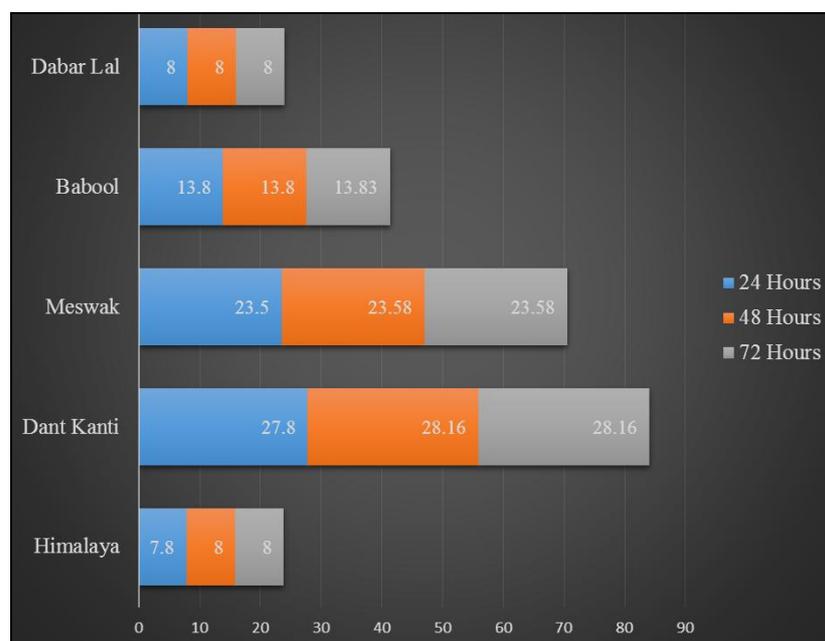


Fig 1: Showed the maximum zone of inhibition with the mean diameter of 28.16 ± 0.75 at 72 hours

Conclusion

The result of the current study demonstrated that Dantikanti has the maximum antimicrobial effect against *S. mutans* followed by Meswak and Babool, remaining dentifrices like Himalaya and Dabar lal had the least effect against the microbes. Thus, when compared with all other commercially available ayurvedic dentifrices Dantikanti proved to be more effective at 24 hours, 48 hours and 72 hours among all others.

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