



Evaluating the Antimicrobial Activity of Commercially Available Herbal Toothpastes on Microorganisms Associated with Diabetes Mellitus

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ABSTRACT

Aim: The present study was conducted to evaluate the efficacy of commercially available herbal toothpastes against the different periodontopathogens.

Materials and methods: Six herbal toothpastes that were commonly commercially available were included in the study. Colgate herbal, Babool, Meswak, Neem active, Dabur red toothpastes were tested for the study whereas sterile normal saline was used as control. Antimicrobial efficacies of dentifrices were evaluated against *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans*. The antimicrobial properties of dentifrices were tested by measuring the maximum zone of inhibition at 24 hours on the Mueller Hinton Agar media inoculated with microbial strain using disk diffusion method. Each dentifrice was tested at 100% concentration (full strength).

Results: The study showed that all dentifrices selected for the study were effective against the entire test organism but to varying degree. Neem active tooth paste gave a reading of 25.4 mm as the zone of inhibition which was highest amongst all of the test dentifrices. Colgate Herbal and Meswak dentifrices recorded a larger maximum zone of inhibition, measuring 23 and 22.6 mm respectively, compared to other toothpastes. All other dentifrices showed the zone of inhibition to be between 17 and 19 mm respectively.

Conclusion: The antibacterial properties of six dentifrices were studied *in vitro* and concluded that almost all of the dentifrices available commercially had antibacterial properties to some extent to benefit dental health or antiplaque action.

Keywords: Herbal dentifrices, Periodontopathogens, Antimicrobial susceptibility, Chronic periodontitis.

How to cite this article: Jenner F, Jaleel VA, Kulshrestha R, Maheswar G, Rao PK, Kranthi J. Evaluating the Antimicrobial Activity of Commercially Available Herbal Toothpastes on Microorganisms Associated with Diabetes Mellitus. J Contemp Dent Pract 2013;14(5):924-929.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to man for his life. The three important necessities of life food, clothing and shelter and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring healthcare.¹

Various medicinally important herbs have been used for centuries in the traditional systems of medicine. All most every part of plants is used in indigenous system of medicine as a health promoter and restorers. Herbal drugs were used in various aboriginal or traditional systems of medicine since the beginning of human civilization. In India along with Ayurveda and Yoga; Unani, Siddha and Homoeopathy, are practiced under the system of Ayush as a well established branch of medicine. With increasing incidence of drug resistance in prevalent pathogens and associated risk with chemotherapeutic agents makes it essential to find an alternative to existing drugs. If the dose response relations, interactions and the risk associated with the herbal medicines were fully evaluated, the herbs having known pharmaceuticals properties could be the best source of these alternative drugs. More emphasis was given to the antimicrobial properties of these herbs either in the form of active components derived from plants or in the form of crude extracts. In most of the indigenous system of medicines herbs were used in its crude form; it has also been reported

that the active substances of herbals are unstable in nature when fractionated. It has been reported in a number of *in vitro* studies that the herbs that were used to cure infectious diseases have wide antimicrobial spectrum.²

Periodontal diseases and dental caries are the two most prevalent oral infections affecting mankind worldwide. Endogenous oral bacterial species such as *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Streptococcus sorbius*, *Bacteroides* sp., *Prevotella* sp., *Fusobacterium* sp. and their metabolites play major roles in the initiation and progression of these infections.^{3,4} Effective prevention of these infections can be achieved by mechanical removal of dental plaque by proper tooth brushing and flossing. However, the majority of the population, particularly aged individuals, may not perform mechanical plaque removal sufficiently, and thus antimicrobial mouth rinses such as triclosan and chlorhexidine may be used to limit these two plaque-related oral infections.⁵ These chemical agents used in the form of either dentifrices or mouth rinses may have undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions.^{6,7} Although antibiotics are used routinely to prevent systemic infections originating from the oral cavity, they are not recommended for regular prevention of dental plaque formation because of the risk.

It is well established that most infectious oral diseases such as dental caries and periodontal disease are linked to the microbial flora of the oral cavity. Dental caries is caused by acidogenic species of bacteria, mainly *S. mutans*, *Lactobacillus* and *Actinomyces*. These oral bacterial species metabolize sucrose to lactic and other organic acids in dental plaque produced on the surface of the tooth and dissolve calcium phosphate in the enamel, consequently giving rise to dental caries. Periodontal disease is mostly associated with anaerobic Gram-negative rods such as *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythus*, *Bacteroides*, *Prevotella* and *Fusobacterium* species. These periodontopathogens are frequently isolated from periodontal pockets of patients with periodontitis.

Dentifrices labeled as 'natural' typically do not include ingredients such as synthetic sweeteners, artificial colors, preservatives, additives, synthetic flavors and fragrances.⁸ They are formulated from 'naturally derived' components. For example, in 'natural' toothpaste, the fluoride comes from fluorspar, the abrasive system is ground calcium carbonate (chalk) instead of synthesized abrasive, the thickener is Carageenan (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.^{9,10}

MATERIALS AND METHODS (FIGS 1 TO 6)

Collection of Samples

The samples were taken by swabbing the oral cavity by rotating the sterile swab and where it had limitations, dental probes, endodontic paper points and scalers were used.

Cultivation

The samples that were collected were incubated at 37°C for 48 hours. Once dispersed samples were taken and Gram-staining was done, also they were spread on to a number of freshly prepared agar plates and incubated to allow cells to form microbial colony. The media used were nutrient agar, blood agar, trypticase soy agar and thioglycollate broth.

The above agar plates were inoculated by streak method and the anaerobes were kept in the McIntosh Jar and incubated at 37°C for 48 hours. After incubation period of 24 to 48 hours the colonies were identified by colony morphology, Gram-staining and biochemical reactions.^{11,12} The various microflora were identified by the hemolytic zones and pigmentation on blood agar, pigment production on nutrient agar and biochemical reactions such as IMViC test, fermentation test and other specific test (oxidase test, gelatine liquefaction, catalase test) were performed. For the organisms where fermentation and IMViC test was limitations, other specific tests such as bile test, esculin test was performed.^{15,16}

Various general merchant shops and supermarkets in the city of Bhilai were visited and commonly available herbal dentifrices were selected. Six herbal toothpastes were purchased from various shops in the city as they were commonly available. Normal saline was taken as the control for the study.

Antibiotic Sensitivity Testing

The breakpoints for susceptibility of anaerobes to the antibiotics were applied as recommended by the Clinical and Laboratory Standards Institute.¹⁹ The inoculums of test strains was adjusted to 1.5×10^8 CFU/ml equal to that of the 0.5 McFarland standard by adding sterile distilled water. The antimicrobial sensitivity of the test strains to five Herbal toothpastes were determined by Kirby-Bauer disc diffusion method.^{15,16} A 20 ml Muller Hinton agar autoclaved and cooled at 45°C was poured into sterile petriplates and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100 μ l inoculums (1.5×10^8 CFU/ml) with the help of a sterilized spreader onto the entire surface of agar plate. The plates were allowed to dry before applying the disk. A disk of 6 mm diameter (plain, sterile filter disk, obtained from Hi-Media Laboratories)



Fig. 1: Colony of *S. mutans* on mitis agar media



Fig. 4: Inoculating test dentifrices on the lawn culture of periodontopathogens



Fig. 2: Colony of *A. actinomycetemcomitans* on blood agar

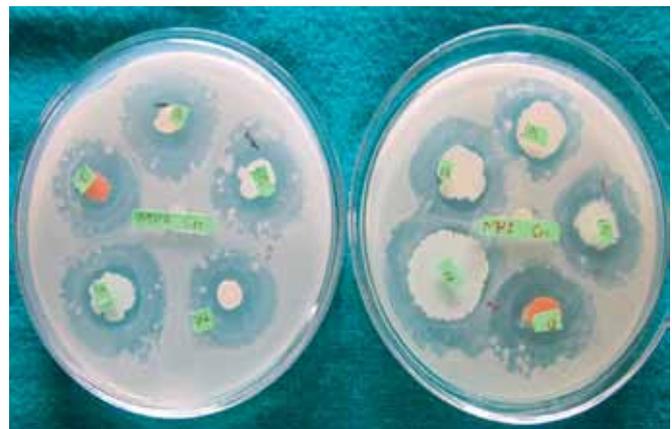


Fig. 5: Inhibition zone as shown by test dentifrices on *S. mutans*



Fig. 3: Dentifrices used for the study



Fig. 6: Inhibition zone as shown by test dentifrices on *A. actinomycetemcomitans*

was loaded with approximately 50 mg of the test dentifrice. The loaded disk was placed manually on the culture plate immediately after the streaking and pressed to obtain proper contact with the media. The disks were firmly applied to

the surface of agar plates within 15 minutes of inoculation. All the agar plates were incubated at 37°C, anaerobically inside McIntosh chamber using Gas pack, for 24 hours. If antimicrobial activity was present on the plates, it was

indicated by an inhibition zone surrounding the dentifrices disk. The diameter of the inhibition zones in millimeter at 24 hours were measured using an antibiotic zone reader scale, Hi-Media. The experiments were conducted in triplicate for each test pathogen. An organism was interpreted as highly susceptible if the diameter of inhibition zones was more than 30 mm, intermediate if diameter was 25 to 30 mm and resistant if diameter was less than 25 mm. Each plate was loaded with 6 filter disks with the test dentifrice at the periphery of the petriplates and one disk with control (sterile normal saline) at the center.^{17,18}

RESULTS (TABLES 1 TO 3)

A uniform lawn of the four periodontopathogens was established in an even layer, obtained through the lawn culture method, and a well defined zone of inhibition, which could be measured accurately, was observed after 24 hours. The development of a clear zone around the disk after 24 hours of incubation indicated antibacterial activity against the test organisms. In the test plates Neem active toothpaste was found to be more effective. All the other test dentifrices showed inhibitory effect against the growth of test organisms but were not as effective as the Neem active dentifrice. Colgate Herbal and Meswak toothpaste also showed sensitive zone of inhibition.

DISCUSSION

The main aim of the study was to evaluate the anti-microbial efficacy of commercially available herbal dentifrices. *Streptococcus mutans* and *Actinobacillus*

actinomycetemcomitans, was chosen as the test organism on the basis that in the oral cavity, they are amongst the predominant colonizers of the oral cavity. Oral diseases seem to appear after an imbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria.^{19,20}

Periodontal disease is one of the most prevalent afflictions worldwide. The most serious consequence is the loss of the periodontal supporting structures which includes gingiva, cementum, periodontal ligament and alveolar bone.³ Periodontal disease is an all-encompassing term used to describe all disorders of the supporting structures of the teeth. These structures include the gingiva, periodontal ligament and the underlying alveolar bone. The level of infection can range from gingivitis, which is the inflammation of the gingiva all the way to full-blown periodontitis that can result in tooth loss. Unlike most illness, bacteria that are foreign to the body do not cause periodontal diseases. Today, periodontal disease is considered primarily a polybacterial manifestation connected with certain bacterial pathogens. Rather it's the microbes that inhabit the oral cavity which are responsible for periodontal disease. The attack is triggered by a shift in the balance of oral microflora toward more invasive microbes. These microorganisms can cause inflammation leading to periodontal disease, by either directly invading the surrounding tissues or indirectly by emitting a toxin.

The diversity of microorganisms that have been detected in the oral cavity is greater than at any other location in the human body.³⁻²¹ Research has demonstrated that bacteria in biofilm such as plaque have decreased sensitivity to antibacterial agents. It is due to the fact that plaque is a thick

Table 1: Maximum zone of inhibition of test dentifrices

S. no.	Test dentifrice	Main active ingredient	Inhibition zone (Max. diam.) Aa	<i>Streptococcus mutans</i>
1	Babool	Babool extract	17.2 mm	28.2 mm
2	Colgate herbal	Chamomile and sage	23 mm	28.6 mm
3	Meswak	Meswak extract	25.4 mm	28.4 mm
4	Neem	Neem	26.8 mm	34.4 mm
5	Dabur red	Clove oil	19.8 mm	27.4 mm
6	Control	Normal saline	Resistant	Resistant

Table 2: Zone of inhibition for *Actinobacillus actinomycetemcomitans* in mm of the dentifrices considered for the study

S. no.	Tooth paste	<i>Actinobacillus actinomycetemcomitans</i>					Average (mm)
		1 (mm)	2 (mm)	3 (mm)	4 (mm)	5 (mm)	
1	Babool	16	19	17	17	17	17.2
2	Colgate herbal	21	24	24	22	24	23
3	Meswak	13	17	13	17	17	25.4
4	Neem	27	27	27	26	27	26.8
5	Dabur red	17	18	18	18	18	19.8
6	Control	Res	Res	Res	Res	Res	Res

Res: Resistant/No zone of inhibition

Table 3: Zone of inhibition for *Streptococcus mutans* in mm of the dentifrices considered for the study

S. no.	Tooth paste	<i>Streptococcus mutans</i>					Average (mm)
		1 (mm)	2 (mm)	3 (mm)	4 (mm)	5 (mm)	
1	Babool	16	19	17	17	17	17.2
2	Colgate herbal	21	24	24	22	24	23
3	Meswak	13	17	13	17	17	25.4
4	Neem	27	27	27	26	27	26.8
5	Dabur red	17	18	18	18	18	19.8
6	Control	Res	Res	Res	Res	Res	Res

Res- Resistant/No zone of inhibition

film and has to be removed manually and the chemical agents play a minor role in the removal of the plaque film. The role of the dentifrices is limited to the reduction in number of microorganisms that are responsible for plaque formation. They do not stop the biofilm from forming but they help slow down the process.²²

It is a well known fact that plaque is a multifactorial entity and *in vivo* conditions vary from the *in vitro* conditions, but still the effective dentifrices will be able to lower the number of *S. sanguis* in the oral cavity, thereby leading to slower formation of biofilm with lower number of microorganisms. Lee et al²³ have reported that *S. sanguis* is considered to be an opportunistic bacterium in the oral cavity. It may induce significant health risks when it enters sites in which abscesses develop, such as the brain and the heart. The authors reported that the viridians streptococci such as *S. sanguis*, which entered the bloodstream through an oral infection wound or an extraction site, caused 40 to 50% of cases of endocarditis for patients with damaged heart valves or other cardiac abnormalities.¹⁸

Moran et al²⁴ evaluated the antibacterial properties of many dentifrices *in vitro* and concluded that not even 50% of the dentifrices available commercially had antibacterial properties to benefit dental health or antiplaque action whereas our results showed otherwise. This may be due to the changes in the manufacturing guidelines that were set after the study was conducted and the addition of newer substances in the dentifrices with better antibacterial and antiplaque properties.²⁵

Williams et al²⁶ have reported Colgate Total to be better toothpaste with prolonged retention and efficacy against oral microorganisms. The study under discussion has found Neem active toothpaste better than Colgate Total to be an effective antimicrobial agent having the maximum zone of inhibition as compared to control. Dumas et al²⁷ have reported Colgate Total to be a better dentifrice as the MIC observed against *S. sanguis*, for Colgate Total, was less compared to the MIC reported for the Herbal dentifrices. The results of the current study are not in accordance with the

study by Dumas et al as two herbal dentifrices were found to have a larger and maximum zone of inhibition. This may be due to the variation in the method that was adopted for conducting the study. Poureslami et al²⁸ have reported that the minimum concentration of Meswak that is required for effectively killing *S. sanguis* was 7.4 mg/ml and that Meswak was effective against *S. sanguis*. The current study also has found the toothpaste Meswak (with Meswak as the main component of the dentifrice) to be highly effective against test pathogens but not as effective as Neem active and Colgate herbal but better than other dentifrices. Acharaya et al²⁹ have reported that the Dabur red powder showed growth of unidentified microorganisms on and around the samples in all test plates. In the current study Dabur red toothpaste showed good results and no contamination or extragrowth of organisms was observed. Also, in the current study, the herbal dentifrices showed an overall better result. Okpalugo et al³⁰ reported that the toothpaste brands considered for their study were not effective in reducing the oral microorganisms whereas the results of our study showed inhibition of periodontopathogens by all brands, and are in accordance with George et al.^{18,31,32}

CONCLUSION

The antibacterial properties of five dentifrices were studied *in vitro* and concluded that almost all of the dentifrices available commercially had antibacterial properties to some extent to benefit dental health or antiplaque action. From the above study it is concluded that further *in vitro* and *in vivo* research is advised. Further research efforts are also needed to establish manufacturing guidelines to ensure the efficacy and safety of herbal dentifrices available freely over the counter.

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