EVALUATING THE ANTI- PLAQUE EFFICACY OF HERBAL DENTIFRICES- AN IN- VITRO STUDY

DR. PUNEET GUPTA[1], DR. NUPUR AGARWAL[2], DR. RAJNI SHARMA[3]

1.Sr. Lecturer, Department of Public Health Dentistry, Teerthankar Mahaveer Dental College & Research Centre, Moradabad

Email: puneet0408@yahoo.com

2.Sr. Lecturer, Department of Oral Medicine & Radiology, Teerthankar Mahaveer Dental College & Research Centre, Moradabad

3.Professor, Department of Microbiology, SMS Medical College, Jaipur

Email: sharmam@gmail.com

ABSTRACT

The arrays of oral health products available are on the increase and so is the strength of chemicals in them. The only alternate is to switch to herbal products. The Indian consumer today stands highly confused with the commercially available products for oral cleanliness. The present in vitro study was conducted to evaluate the efficacy of commercially available over the counter herbal dentifrices in comparison with conventional dentifrice on *Streptococcus sanguis.* Nine herbal dentifrices were included in the study. The herbal dentifrices were commonly commercially available over the counter products. Colgate Total toothpaste was used as the positive control for the study whereas sterile normal saline was used as negative control.

Antimicrobial efficacies of dentifrices were evaluated against *Streptococcus sanguis* (ATCC- 10556). The antimicrobial properties of dentifrices were tested by measuring the Maximum Zone of Inhibition at 24 hours on the blood agar plates inoculated with microbial strain using disc diffusion method. Each dentifrice was tested at 100% concentration (full strength). The results of the study showed that all dentifrices selected for the study were effective against the test organism but to varying degree. Colgate Total tooth paste gave a reading of 9.1 mm for the zone of inhibition which was higher than most of the test dentifrices. Only Meswak and Promise dentifrices recorded a larger Maximum Zone of inhibition, measuring 9.2 mm each, compared to Colgate Total toothpaste. All other dentifrices showed the Zone of Inhibition to be between 7 and 9 mm respectively.

KEY WORDS: Herbal Dentifrices, In- Vitro Study, Streptococcus sanguis

INTRODUCTION:

Complementary Alternative Medicine (CAM), natural, unconventional, integrative, and holistic are some of the terms used to describe medical and dental treatments that don't conform to mainstream/western/conventional health approaches.[1-3] With the growing field of alternative medicine, dentifrices based on plant extracts are available in the market.[2,4] Consumers who gravitate towards using herbal products often view these products as being safer than products that contain chemicals.

Dentifrices labeled as "natural" typically don't include ingredients such as synthetic sweeteners, artificial colors, preservatives, additives, synthetic flavors, and fragrances.[2,5] They are formulated from "naturally derived" components. For example, in "natural" tooth paste, 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.[1-3]

There is little or no research to prove or refute the efficacy of dentifrices containing combination of herbal components, in contrast with a plethora of such research for conventional ones. Hence, with the increased popularity of herbal dentifrices and severe paucity of data, dental professionals are not in a position to provide information to their patients and others about the efficacy of these products.[2, 5]

The purpose of this study was to evaluate the anti- plaque efficacy of commercially available herbal dentifrices compared to the conventional dentifrice, in-vitro. Streptococcus sanguis (ATCC- 10556) was the selected organisms as it is amongst the primary colonizers in plaque formation.

MATERIALS & METHOD

The ATCC strain of *Streptococcus sanguis* was obtained from Hi Media Laboratories. in a lyophilized form. To achieve the active stage, the organism was extracted from the vial and cultured on Mueller- Hinton + 5 % sheep blood agar (MHB), as per manufacturer's instructions.

Various general merchant shops and supermarkets in the city of Jaipur were visited and commonly available over the counter herbal dentifrices were selected. Nine herbal dentifrices were purchased from various shops in the city as they were commonly available.. Colgate Total was taken as the positive control and normal saline as the negative control for the study. The in-vitro study was carried out in the Department of Microbiology, SMS Medical College, Jaipur.

Kirby- Bauer Disk- diffusion method[6,7] was followed. Three to five colonies were suspended in 4-5 ml of tryptic soy broth and incubated at 35 degrees Celsius to achieve 0.5 McFarland standards.[5,6,7] The turbidity of actively growing cells when adjusted to 0.5 McFarland standards using sterile saline provided an

inoculums of approximately 12 x 10^8 CFU/ml. (CLSI procedure). A lawn culture of the organism was obtained by streaking the agar plate with cotton swab dipped in normal saline solution, while maintaining sterile conditions.

In the pilot run a disc of 6 mm diameter (plain, sterile filter disc, obtained from Hi-Media Laboratories)[8] was loaded with approximately 50 mg of the test dentifrice[5]. The loaded disc was placed manually on the culture plate immediately after the streaking and pressed to obtain proper contact with the media. Each blood agar plate was loaded with 5 filter discs. 3 discs were loaded with the test dentifrice, one disc with positive control and one disc with negative control (sterile normal saline). (FIGURE- 1)

The blood agar plate was placed in the candle jar at 37 degree Celsius which was placed in the incubator for 24 hrs. After 24 hrs, the maximum zone of inhibition (FIGURE-2) was measured using a zone measuring scale. On the instances that contamination was found on the plates, the tests were repeated. The contaminated plates were discarded immediately.

The test dentifrices were blinded for the study. The blinding procedure was carried out by the moderator of the study. Coding was done using alphabetical order for the test dentifrices. De-coding for the dentifrices was done after the results were obtained.

RESULTS

A uniform lawn of *Streptococcus sanguis* was established in an even layer, obtained through the streaking method, and a well defined zone of inhibition, which could be measured accurately, was observed. The development of a clear zone around the disk after 24hrs of incubation indicated antibacterial activity against *Streptococcus sanguis*.

In the test plates, on de-coding, Promise and Meswak were found to be more effective than the positive control. All the other test dentifrices showed inhibitory effect against the growth of *Streptococcus sanguis* but were not as effective as the positive control dentifrice. The test results showed wide variations based on the in-vitro study carried out. (TABLE- 1 and GRAPH- 1)

DISCUSSION

The main aim of the study was to evaluate the anti- plaque efficacy of commercially available herbal dentifrices. *Streptococcus sanguis* (ATCC 10556) was chosen as the test organism on the basis that in the oral cavity, it is amongst the first colonizers in the formation of dental plaque in the oral cavity. The specific adhesion mechanisms operating *in vivo* are the 'corncob' structures seen in plaque, and possibly the selective, initial colonization of teeth by *Streptococcus sanguis*. The corncob mechanism appears to involve *Streptococcus sanguis* or *Streptococcus mitis* and *Actinomyces naeslundi*.[9]

The early colonizers of teeth, mainly *Streptococcus sanguis* and *Actinomyces naeslundi*, adhere to the salivary pellicle on the tooth surface and are localized at sites on the pellicle which are thermodynamically most favourable for their growth. This depends on interactions that include Vander Waal's forces, electrostatic interactions and hydrogen bonds. The higher affinity interactions often impart specificity of colonization; for e.g. *Streptococcus sanguis* adheres strongly to tooth pellicle but poorly to cells of the dorsum of the tongue as a result of which it is able to colonize the teeth but not the tongue.[9]

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 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 micro-organisms that are responsible for plaque formation. They do not stop the biofilm from forming but they help slow down the process.[9]

It is a well known fact that plaque is a multi-factorial entity and in vivo conditions vary from the in- vitro conditions. *S. sanguis* being the primary colonizer of the plaque film in the oral cavity, is placed in the deeper layers of plaque and therefore the dentifrices may not be able to stop its colonization (because by the time the dentifrice is applied, plaque biofilm has already formed), 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 micro-organisms.

Lee et al[5] 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 percent of cases of endocarditis for patients with damaged heart valves or other cardiac abnormalities.

Moran et al[10] 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 anti-plaque 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[11] and the addition of newer substances in the dentifrices with better anti- bacterial and anti- plaque properties.

In the current study the measurement of the zone of inhibition was found to be less than that reported by Lee et al[5] for Colgate Total. The inhibition zone reported was nearly 2.5- 3 times that of the current study. The difference in the values of the result may be due to the variations in the manufacturing guidelines of the product, locally and internationally.

Williams et al[12] have reported Colgate Total to be a better toothpaste with prolonged retention and efficacy against oral micro-organisms. The study under discussion has also found Colgate Total to be an effective anti- plaque agent, but the maximum zone of inhibition for two herbal dentifrices was greater compared to Colgate Total.

Dumas et al[13] 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[14] as two herbal dentifrices were found to have a larger maximum zone of inhibition. This may be due to the variation in the method that was adopted for conducting the study.

Poureslami et al[14] 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 *S. sanguis*.

Acharaya et al[15] 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 Dant Manjan showed good results and no contamination or extra growth of organisms was observed. Also, in the current study, the herbal dentifrices showed an overall better result compared to the study by Acharaya et al.[15] The reason for extra growth in the study by Acharaya et al[15] may be microbial contamination.

Okpalugo et al[16] reported that the toothpaste brands considered for their study were not effective in reducing the oral micro-organisms whereas the results of our study showed inhibition of *S. sanguis* by all brands, and are in accordance with George et al.[17]

Limitations of the Study:

There are limitations associated with the current study. Bacterial and fungal pathogenicity is a multi-factorial process, involving microbial virulence and host response, along with genetic and environmental factors such as saliva buffering and diet. Some researchers have looked at whether detrimental shifts in periodontopathic cariogenic or opportunistic flora and increases in resistant strains have resulted from antibacterial ingredients in regular dentifrices.[5]

It is known that a balance exists in each person's oral microbial population. If that balance is lost, opportunistic microorganisms proliferate, enabling the initiation of disease process. Therefore, the dentifrice that was identified as having the largest microbial inhibition zone—and thus probably the strongest antibacterial and antifungal properties—may not be necessarily superior to those found to have smaller diameter inhibition zones. Because a dentifrice used in vivo likely is diluted by saliva, the level to which

antimicrobial properties are buffered or lost in dilution in vitro may vary, and so will the effect of the dentifrice.[5]

CONCLUSION

No earlier study has compared the efficacy of Meswak and Promise against Colgate Total toothpaste for anti- plaque and anti- gingivitis efficacy, and 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.

TABLE 1 MAXIMUM ZONE OF INHIBITION FOR DENTIFRICES

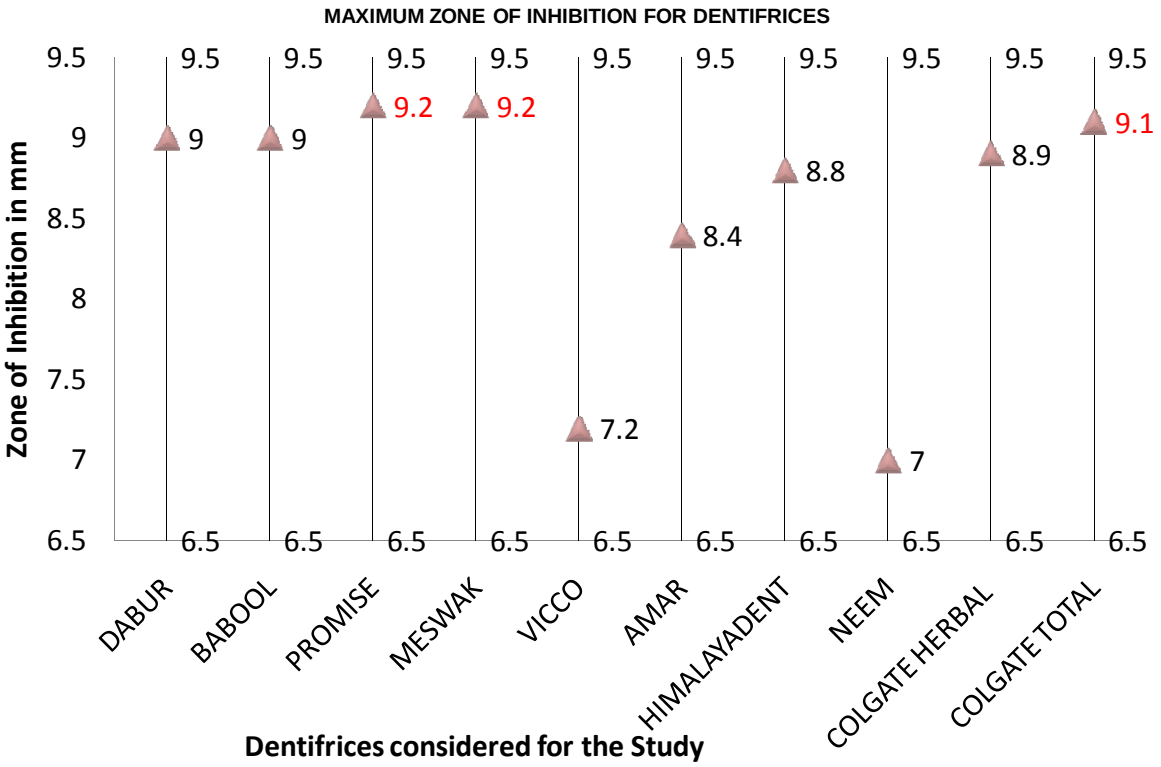
S. No.	TEST DENTIFRICE	MAIN ACTIVE INGREDIENTS	INHIBITION ZONE (maximum diameter)
1	Dabur Red Dant Manjan	Clove Oil	9 mm
2	Babool	Babul extract	9 mm
3	Promise	Clove Oil	9.2 mm
4	Meswak	Meswak extract	9.2 mm
5	Vicco Vajradanti	Babul	7.2 mm
6	Amar	blend of Karanj , Neem and Babool	8.4 mm
7	Himalayadent	Bishop's Weed	8.8 mm
8	Neem active	Neem	7 mm
9	Colgate Herbal	Chamomile & Sage	8.9 mm
10	Colgate Total (positive control)	Triclosan	9.1 mm
11	Normal Saline (negative control)	---	0 mm

TABLE 2 Beneficial Properties claimed by Ingredients in Herbal Toothpastes

Natural or Herbal ingredient	Oral condition, disease or health property claimed to be benefitted by product
Aloe Vera	Anti-inflammatory, wound healing, stomatitis, analgesic, immunostimulant, gingival health, varicella-zoster virus, viracidal to herpes simplex types 1 and 2
Babul extract *(Acacia Arabia*)	Swelling and bleeding of gums**,** keeping your gums healthy **and** teeth strong.
Bakul (*Mimusops elengi*)	Astringent
Bee Propolis	Anti-inflammatory, wound healing, antiviral action on herpes simplex virus type 1, antibacterial against *Streptococcus mutans*
Bishop's Weed (*Carum copticum*)	Mouth-freshening, antiseptic, astringent and carminative properties.
Cinnamon	Antimicrobial, analgesic, antifungal, anesthetic, toothache, halitosis
Clove (Caryophyllus aromaticus)	Aromatic, Antiseptic, Local, Anaesthetic, internally increases circulation, stimulates the Skin, Stimulates Salivary Gland
Echinacea	Boosts immune system; in tincture form, may numb aches of herpes and other sores of the oral soft tissues; used also for periodontal disease and abscesses
Fluorspar (Vaikranta bhasma)	Natural source of fluorine, which prevents dental caries.
Green Tea	Antimicrobial and thus combats halitosis, tooth caries and periodontal disease; anti-inflammatory
Indian Gum-Arabic Tree (*Acacia arabica*)	External application for mouth ulcers.
Jambhul (Syzigium jambolanum)	Astringent, Anti Inflammatory, Haemostatic, Healing
Juniper	Anti-inflammatory, antimicrobial, antihypotensive, antiviral, antiexudative, halitosis

Karanj *(Milletia pinnata)*	biocidal activity as well an anti-inflammatory, antinociceptive (reduction in sensitivity to painful stimuli) and antipyretic (reduction in fever) properties.
Meswak *(Salvadore Persica)*	Reduce tarter & plaque, fights germs & bacteria to keep gum healthy, helps prevent tooth decay, eliminates bad breath and ensure strong teeth.
Myrrh	Gingivitis, periodontal disease, mouth ulcers
Neem or Nimba	antiseptic and antifungal activity
Nirgundi (*Vitex negundo*)	analgesic.
Pomegranate (*Punica granatum*)	astringent and antibacterial activity.
Rosemary	Analgesic for mouth injuries
Sage Oil	Periodontal disease, bactericidal against wide range of bacteria
Sanguinarine	Antimicrobial, antiplaque, gingivitis, anti-inflammatory
Seaweed	Antihypertensive
Teaberry	Anti-inflammatory
Toothache Tree or Tumburu (*Zanthoxylum alatum*)	Used for bleeding gums and sensitive teeth.
Triphala (*Emblica officinalis*)	Astringent, which arrests bleeding of gums and healing of ulcers.
Vidanga (*Embelia ribes*)	antibacterial activity against *E.coli* and possesses anti-inflammatory properties.

GRAPH 1



REFERENCES

1. Storehagen, Silje, Nanna Ose & Shilpi Midha. Dentifrices and Mouthwashes Ingredients and their use. Det odontologiske fakultet, Universitetet i Oslo, 2003. Available from URL: http://www.duo.uio.no/publ/odontologi/2003/17975/Storehagen_Ose_Midha.pdf Accessed on 19th November, 2011.
2. Goldstein BH, Epstein JB. Unconventional dentistry: Part IV. Unconventional dental practices and products. J Can Dent Assoc. 2000;66(10):564-8.
3. Jacobsen PL, Epstein JB, Cohan RP. Understanding "alternative" dental products. Gen Dent. 2001;49(6):616-20; quiz 621-2.
4. Ozaki F, Pannuti CM, Imbronito AV, Pessotti W, Saraiva L, de Freitas NM, Ferrari G, Cabral VN. Efficacy of a herbal toothpaste on patients with established gingivitis--a randomized controlled trial. Braz Oral Res. 2006;20(2):172-7.
5. Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: results of an in vitro diffusion method study. J Am Dent Assoc. 2004;135(8):1133-41.

6. McKee. Disk diffusion methods. In, McKee (ed). Diagnostic Microbiology and Infectious Diseases. 1st edition. New York, McGraw-Hill Book Co, 1987;166-177

7. Bailey & Scott. Diffusion methods in laboratory investigations. In, William Robert Bailey, Elvyn G. Scott (ed). Diagnostic Microbiology, 11th edition. London, 2002; 17-18

8. Joshi A, Iyer V, Balasubramanyam U, Kagal A, Bhradwaj R. Comparison of efficacy of three antibiotic discs. Indian Journal of Medical Microbiology, 2008; 26 (2), 160-2.

9. Busscher H.J. & Evans L.V. Oral Microflora. In Busscher (ed). Oral Biofilms and Plaque Control,1st edition, AITBS Publication, 1998; 222-231.

10. Moran J. and Addy M. The antibacterial properties of some commercially available toothpastes *in-vitro*. Journal of dental research, 1984; vol no.156, pg-175

11. Adjunctive Dental Therapies for the Reduction of Plaque and Gingivitis; ADA Council of Scientific Affairs, September 1997

12. Williams M & Ummins DC (Colgate-Palmolive Company, Piscataway, NJ, USA); The technology behind Colgate Total toothpaste; Oral Diseases 2005;11(Suppl. 1), 98–121

13. Dumas E.R., Engelman E.E., and Venell J.A. *In vitro* Antibacterial Comparison of Dentifrices Claiming Antigingivitis and Gum-Healing Properties. Abstract # 0389. Tom‘s of Maine, Kennebunk, Maine, USA. Available from URL: http://www.mattmetzgar.com/matt_metzgar/files/AntigingivitisAntibacterialComparison.pdf

14. Poureslami Hamid Reza, Abbas Makarem, Faraz Mojab. Paraclinical Effects of Miswak Extract on Dental Plaque. Dent Res J 2007; 4(2): 106-110

15. Acharya A, Subedi B, Paudyal B, Jnawali M, Shakya P, K.C.U, Shrestha UT, Tiwari KB Agrawal V. Assessment of Antimicrobial Activity of Different Toothpastes Against Oral Isolates.

16. Okpalugo J, Ibrahim K, Inyang US. Toothpaste formulation efficacy in reducing oral flora. Tropical Journal of Pharmaceutical Research, February 2009; 8 (1): 71-77.

17. George Dilip, Bhat Sham S., Antony Beena. Comparative evaluation of the antimicrobial efficacy of aloe vera tooth gel and two popular commercial toothpastes: An in vitro study. General Dentistry 2009; May/June, pg-238