

Antimicrobial Efficacy of Ten Commercially Available Herbal Dentifrices against Specific Oral Microflora – In Vitro Study

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ABSTRACT

Aim: To determine and compare the antimicrobial efficacy of ten commercially available *herbal dentifrices* against specific strains of oral microflora using a standard diffusion method at full strength and 1:1 dilution at 24 h.

Materials and Methods: The standard strains of *Streptococcus mutans* (ATCC 21293), *Streptococcus sanguis* (MTCC 442), *Actinomyces viscosus* (ATCC 3268), *Staphylococcus aureus* (ATCC 2592), *Streptococcus pyogenes* (MTCC 442) and *Candida albicans* (ATCC 183) were obtained. Antimicrobial efficacy of the dentifrices was tested in triplicate, at full strength and 1:1 dilution with the sterile water using a standard diffusion method for 24 h at 37°C. The antimicrobial efficacy was tested by observing the zones of inhibition in millimeters surrounding disk containing the dentifrice. Mean standard deviation and standard error of mean

of the inhibitory zones was calculated for each herbal dentifrice. $p < 0.05$ was considered statistically significant.

Results: Danth Kanthi (DK) was the most effective against all the microorganisms producing larger zones of inhibition at 24 h (F.S – 40 ± 1.5 ; 1:1 dilution – 40 ± 2.71). Amar Premium (AP) also produced larger zones of inhibition against all microorganisms except *S. aureus*. Of all the dentifrices, least zones of inhibitions i.e., around 5 mm was observed against *S. aureus* by Amar Premium (AP) and Dabur Babool (DB) at 24 h.

Conclusion: Based on the results of the present study, it can be concluded that all *herbal dentifrices* exhibited antimicrobial activity against the selected oral microorganisms, with DK being the most effective. Hence, it can be inferred that *herbal dentifrices* can also be recommended like the conventional formulations.

Keywords: Dental caries, Microorganisms, Oral hygiene, Oral diseases, Periodontal diseases

INTRODUCTION

Oral diseases like dental caries and periodontal diseases continue to be a major health problem globally. In India, the prevalence of dental caries was reported to be 50-60% [1] and periodontal diseases affecting more than 50% of the community [2]. Oral health significantly affects the quality of life and general well-being. The link between oral diseases and the activities of oral microbial species that form part of the microbiota of the oral cavity is well established [3].

All oral hygiene methods are aimed at reducing the pathogenic oral microflora. The common method for maintaining good oral hygiene is brushing the teeth with dentifrices that have antimicrobial properties and can prevent degradation of tooth enamel. Synthetic dentifrices commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use [4]. Consequently, oral hygiene could include any or all of the procedures that contribute to a state of good oral health [5]. Many techniques and products are designed to achieve improved oral health i.e., toothbrushes, rinses, floss and dentifrices [6].

Despite the efficacy of many toothpaste formulations with antibacterial properties, there is an increasing societal desire to rely on naturally occurring compounds for health, which has also found its way into dentistry [7]. Hence, dentifrices that contain extracts of medicinal plants and herbs are becoming popular [4].

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world [3]. According to World Health Organization Report, 70-95% of the population depends on traditional medicine for primary health care [8]. Ancient people chewed twigs from plants with high aromatic properties [9]. A number of studies have investigated the activity of plant extracts and products against specific oral

pathogens, while others have focused on their ability to inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the tooth surface [3].

Herbal medicines are plant-derived materials or products with therapeutic properties used since ancient times in folk medicine, involving both eastern and western medical traditions [10,11]. The use of these products in the prevention and treatment of oral conditions has increased recently and could benefit the low socio-economic urban and rural communities [12]. In an effort to improve the efficacy of the mechanical tooth-cleaning procedures, herbal extracts have been incorporated into the dentifrices.

Some of the proposed active ingredients in the herbal dentifrices include Azadirachta indica, Myrrh, Sage, Eucalyptus, Tea-tree oil, Miswak, Marica and Lavang, Akkal kadha, Chamomile, Cloves, Babul extract, Dalchini.

However, relatively few published articles in the literature have compared the effect of herbal based dentifrices on plaque associated bacteria [13-17]. Hence, the present study was carried out to determine and compare the antimicrobial efficacy of ten commercially available herbal dentifrices against specific strains of oral microflora using a standard diffusion method at full strength and 1:1 dilution at 24 h.

MATERIALS AND METHODS

The study was carried out from the month of August 2012 to September 2012 in Hyderabad City, India.

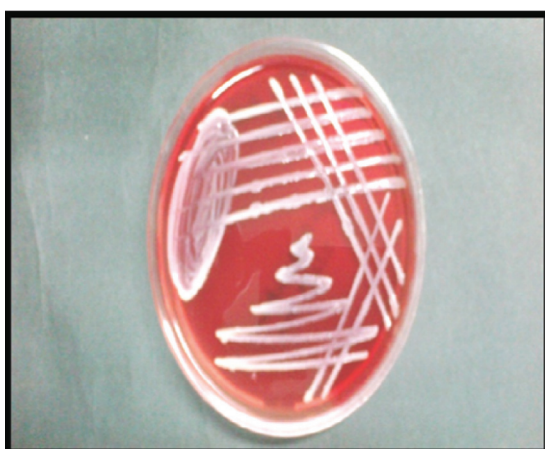
Herbal Dentifrices Used

Ten commercially available herbal dentifrices: Danth Kanthi (DK), Dabur Red (DR), Dabur Meswak (DM), Vicco Sugar Free (VSF), Vicco (V), Himalaya Herbals (HH), Colgate Herb (CH), Neem (N),

Amar Premium (AP) and Dabur Babool (DB) were employed in this study [Table/Fig-1].



[Table/Fig-1]: Herbal Dentifrices



[Table/Fig-2]: Growth of microorganisms on agar plate

Test Microorganisms

Strains of specific oral microflora of *Streptococcus mutans* (*S. mutans* – ATCC 21293), *Streptococcus sanguis* (*S. sanguis* – MTCC 442), *Actinomyces viscosus* (*A. viscosus* – ATCC 3268), *Staphylococcus aureus* (*S. aureus* – ATCC 2592), *Streptococcus pyogenes* (*S. pyogenes* – MTCC 442) and *Candida albicans* (*C. albicans* – ATCC 183) were obtained and utilized in the study.

Antimicrobial efficacy of the herbal dentifrices was tested in triplicate, at full strength and 1:1 dilution with the sterile water using standard diffusion method for 24 h at 37°C. Disc preparation was done using Whatman® filter paper 1 measuring 3mm diameter. Autoclaved filter disks were inoculated on to the Brain Heart Infusion (BHI) agar. At full strength (Fs), it involved applying a thin paper disk containing the *herbal dentifrice* (1 gm) on a culture of test microorganisms grown on the agar media. Dilution of 1:1 was prepared by mixing 1 gm of herbal dentifrice with 1 ml of sterile water. The disks were then incorporated into the diluted samples and subsequently were dried in hot air oven. Dried discs were then inoculated on to the Brain Heart Infusion (BHI) agar media for overnight incubation. Degree of sensitivity was measured by zones of inhibition (in millimeters) formed around the disc for susceptible microorganisms, representing the area where the microbial growth has been inhibited [Table/Fig-2].

STATISTICAL ANALYSIS

Mean, standard deviation and standard error of mean of the inhibitory zones was calculated for each *herbal dentifrice* at full strength and 1:1 dilution at 24 h. Statistical analysis was done using Statistical Package for Social Sciences software (SPSS version 19.0). Multi-group analysis was done using Analysis of Variance (ANOVA). T-test

was used for comparison between the groups at full strength and 1:1 dilution at 24 h. $p < 0.05$ was considered statistically significant.

RESULTS

[Table/Fig-3] represents the details of the *herbal dentifrices* used in the study. [Table/Fig-4] illustrates the mean diameter of the zones of inhibition formed by *herbal dentifrices* against the microorganisms at 24 h at full strength and 1:1 dilution. Among the ten *herbal dentifrices* tested, Danth kanthi was the most effective against most of the microorganisms both at Fs and 1:1 dilution producing larger zones of inhibition (mean 40 mm) except for *A. viscosus* (25 ± 12.6). Concurrently, Amar premium also produced greater zones of inhibition against all microorganisms except *S. aureus*. Of all the *herbal dentifrices*, least zones of inhibitions i.e., around 5 mm were produced against *S. aureus* by AP and DB. The most susceptible microorganism for all the tested herbal dentifrices was *S. pyogenes* in both concentrations (Fs and 1:1 dilution) at 24 h.

Within group comparison at Fs and 1:1 dilution demonstrated significant difference against *C. albicans* by AP only ($p=0.009$). No other significant difference was observed for the test dentifrices against any of the microorganisms at both concentrations.

Between group comparison in Fs revealed significant difference between the dentifrices for all microorganisms. Likewise, at 1:1 dilution except for *S. pyogenes*, significant difference was noted for all.

Results of Post-hoc Analysis at Fs and 1:1 Dilution Based on Microorganisms

S. mutans

At both Fs and 1:1 dilution, significant difference was noticed only for DK and AP with all other dentifrices except each other.

S. sanguis

At Fs, DK and AP produced significantly larger zones of inhibition as compared to other dentifrices; DB produced significantly smaller zones of inhibition than DR, VSF, CH, N, AP whereas DR showed significant difference only with DM, V and HH. CH showed larger zones of inhibition compared to V ($p=0.0001$).

At 1:1 dilution, DK and AP showed significant difference with all except each other. DR produced significant difference with DM, VSF, V, N, and DB at 24 h. CH and HH produced greater zones of inhibition than DM and DB. VSF, V and N revealed lesser zones of inhibition as compared to CH ($p=0.0001$).

A. viscosus

DK revealed larger inhibitory zones against all except CH, AP and N whereas AP produced greater inhibitory zones against DB only at Fs ($p=0.02$). At 1:1 dilution, significant difference was noted only for DK with all dentifrices.

S. aureus

Comparison at Fs, DK, DR, AP and DB produced significantly greater zones of inhibition. However, AP and DB were equally effective as no significant difference was noted between each other. Apart from this, DM, VSF and HH produced significant difference with N. At 1:1 dilution, only DK and DR produced significant difference with all.

S. pyogenes

At Fs, DK showed significant difference with DR ($p=0.03$), HH ($p=0.01$) and CH ($p=0.01$). DM, N, AP and DB produced greater inhibitory zones than DR. DM produced significant difference with HH and CH. N, AP, DB and V revealed significantly larger zones of inhibition as compared to HH and CH. At 1:1 dilution, no significant difference was observed amongst any of the dentifrices.

Brand Name	Batch No	Composition	Manufacturing Date	Manufactured by
Dant Kanthi (DK)	P 0030	Anacyclus pyrethrum - 20 mg, Azadiracta indica - 20 mg, Acacia Arabica - 20 mg, Xanthoxylum alatum - 20mg, Menthe spicata - 10mg, Syzygium aromaticum - 10mg, Piper longum - 10mg, Barleria prionitis - 10mg, Mimiosops elengi - 10 mg, Embelia ribes - 10 mg, Curcuma longa - 10mg, Salvadoria persica - 10 mg, Quercus infectoria - 5 mg	04/12	Patanjali Ayurved Ltd
Dabur Red (DR)	RU0641	Herbal extract – 2.5% w/ - Maricha/ marica (piper nigrum) - 31.04g, - Pippalai (piper longum) - 31.04 g - Shunthi (zingiber officinale) - 31.04 g - Tejovati/tomar (zanthoxylum armatum) - 31.04 g Lavang / laung oil (Syzygium aromaticum) - 0.50g, Karpoo (Cinnamomm camphora) - 0.50g, Pudina sathwa (mentha spicata) - 0.50 g, Gairic powder - 1.80 g , Sweetner (sodium saccharin), Preservatives (methyl parraben, propyl paraben, sodium benzoate), Excipient q.s	05/12	Dabur India Ltd
Dabur Meswak (DM)	BD0397	Calcium Carbonate, Sorbitol, water, silica, sodium laurel sulphate, flavor, Meswak Extract, cellulose gum, carrageenan, sodium silicate, sodium saccharin, formaldehyde, foaming, non-flouridated tooth paste.	1/12	Dabur India, Ltd
Vicco Sugar Free (VSF)	002	Extracts of : Babhul - 1.8g, Bor - 1g, Akkal Kadha - 0.26g, Jambhul - 1g, Vajradanti - 0.5g, Bakul - 3.2g, Lavang - 0.06g, Acrod - 0.06g, Jeshthamadh - 1.3g, Manjishtha - 2.6g, Khair - 1.64g, Kavab-Chini(Chirfal) - 1.94g, Dalchini - 0.16g, Patang - 2.4g, Anant Mul - 0.4g, Ajwan - 0.02g , Maifal - 0.06g, Trifala (amala, harda, behada) - 1.6g , Excipients: Calcium carbonate - 46.4 g, Tragacanth gum - 1.56 g, Sorbitol - 26 g, Methyl paraben sodium - 0.12 g, Propyl paraben sodium - 0.12 g, Sodium lauryl sulphate - 2.36 g, Sodium hydroxide - 0.02 g, Flavor q.s., water q.s.	1/12	Vicco Laboratories, Goa, India
Vicco (V)	118	Babhul - 1.8g, Jambhul - 1g, Lavang - 0.06g, Manjishtha - 2.6g , Dalchini - 0.16g, Bor - 1g, Vajradanti - 0.5g, Acrod - 0.06g, Khair - 1.64g, Patang - 2.4g, Akkal Kadha - 0.26g, Bakul - 3.2g, Jeshthamadh - 1.3g, Kavab-Chini(Chirfal) - 1.94g, Anant Mul - 0.4g, Maifal - 0.06g, Trifala (amala, harda, behada) - 1.6g, Excipients q.s.	10/11	Vicco Laboratories, Goa, India
Himalaya Herbals (HH)	L – 108	Dadima (Punica granatum) - 2.57mg, Tumburu (Zanthoxylum alatum) - 1.80mg, Babbula (Acacia arabica) - 1.71mg, Triphala - 1.71mg,Vidanga (Embelia ribes) - 1.71mg, Nirgundi (Vitex negundo) - 1.14mg,Vaikranta bhasma - 2mg, Nimba (Azadirachta indica) - 1.44mg, Ajamoda satva - 1mg, Processed in Pilu (Salvadora Persica), Irameda (Acacia farnesiana), Khadira (Acacia catechu) , Bakula (Mimosops elengi). Inactives: Sodium Benzoate IP, Bronopol IP. Saccharine Sodium IP 0.5%.	05/12	The Himalaya Drug Company
Colgate Herb (CH)	B28 CP	Calcium Carbonate, Sorbitol, Silica, Sodium Lauryl Sulphate, Polyethylene Glycol, Flavor, Sodium Carboxy Methyl cellulose, Sodium Silicate, Sodium Monofluorophosphate, Sodium Saccharin, Xanthan Gum, Pigment Green No.7 (Cl74260), Myrrh Tincture, Chamolmile Tincture, Tea Tree Oil, Sage Oil, Eucalyptus , in aqueous base	03/12	Colgate-Palmolive India
Neem (N)	285	Precipitated Calcium Carbonate, Sorbitol, Aqua, Glycerine, Hydrated Silica, Sodium Lauryl Sulphate, Flavor, Sodium Silicate, Sodium Carboxymethyl cellulose, Carrageenan, Sodium Saccharin, Methylparaben, Propylparaben, Neem Extract, Sodium Monofluoro Phosphate, Sodium Dihydrogen Phosphate, Tea Tree Oil, Cl 19140, Cl 42090. Natural & Nature derived ingredients: Contains 1000 ppm max. of available fluoride packed Foaming fluoridated toothpaste	12/11	Henkel Marketing India
Amar Premium (AP)	035	Jethimadh - 7mg, Godavaj - 19mg, Mayuphal - 19mg, Harde - 22mg, Aritha - 25mg, Piper - 12mg, Babul - 25mg, Amar Danti - 22mg,Thomer - 6mg, Kalamari - 19mg, Bheda - 21mg, Samudraphal -16mg, Phatakadi - 11mg, Sindhav - 7mg, Plus Flavour, colour and cream base q.s.	01/11	Amar Remedies LTD
Dabur Babool (DB)	N1818	Calcium Carbonate, Sorbitol, Water, Silica, Sodium Lauryl Sulphate, Flavor, Babul Extract, Cellulose Gum, Carrageenan, Sodium Silicate, Sodium Saccharin, Formaldehyde, Foaming, Non-Flouridated tooth paste.	06/11	Dabur India Limited

[Table/Fig-3]: Details of the Herbal Dentifrices used in the study

C. albicans

For *C. albicans* at Fs, DK produced significant difference with all dentifrices. Likewise, AP produced significant difference with all except CH. DB on other hand, revealed significantly lesser inhibitory zones with majority of the dentifrices (DR, HH, CH, N, AP). DM and VSF showed lesser inhibitory zones compared to CH, HH and N. V showed significant difference with CH.

At 1:1 dilution, DK produced significant differences with all except for DR, CH, AP. Comparing with other dentifrices, DR exhibited greater inhibitory zones than DM, VSF and V. DM, VSF and HH showed significant difference against CH and AP for *C. albicans*. In addition, V and AP showed significant difference as compared to N.

DISCUSSION

Herbal extracts have received special attention because of being non-chemical and non-synthetic in nature, and have been used in traditional medicine [18-20]. Interest in natural-based toothpastes has been increased recently [21]. Dental plaque is a significant

risk factor for the development of dental and periodontal disease [22]. The use of herbal dentifrices led to a considerable reduction in dental plaque accumulation both on smooth and approximal tooth surfaces [21]. Probably, the active ingredients of the *herbal dentifrices* penetrate the biofilm and prevent plaque accumulation, thereby potentially preventing the colonization of the oral bacteria on the tooth surfaces [23]. However, very few studies [4, 13, 15] have evaluated the microbial efficacy of commercially available *herbal dentifrices* against oral microflora. Therefore, this study was aimed to determine the zones of inhibition formed by ten *herbal dentifrices* against specific oral microflora using the standard diffusion method at Fs and 1:1 dilution at 24 h.

In the oral cavity, most commonly encountered pathogens are *S. mutans*, *S. sanguis*, *A. viscosus*, *S. aureus*, *S. pyogenes* and *C. albicans*. *S. mutans* has been implicated as the most important bacteria to initiate dental caries. During the first year of life other species of streptococci, staphylococci and actinomyces also colonize. *S. mutans* and *S. sanguis* initially colonize on the dental

Microorganisms	Concen. Tested	Herbal Dentifrices										p-value
		DK	DR	DM	VSF	V	HH	CH	N	AP	DB	
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
<i>Streptococcus mutans</i> (ATCC 21293)	Fs	40±1.5	18± 5.6	16 ±5.7	17±6.0	13±3.5	14±1.3	19±1.3	16±1.3	38± 1.3	15±1.3	0.0001*
	1:1	40±2.71	13± 5.6	13 ± 2.7	14± 3.9	12±3.9	17±1.8	15±1.3	13±1.3	40±1.3	15±1.3	0.0001*
	p-value	1.0	1.0	0.79	0.82	1.0	1.0	1.0	1.0	1.0	1.0	
<i>Streptococcus sanguis</i> (MTCC 442)	Fs	40±2.7	18±5.7	13±5.6	16±0.6	12±1.8	28±1.5	17±1.3	16±1.3	24±1.3	10±1.3	0.0001*
	1:1	40±1.5	18±3.1	12±0.5	16±0.3	12±2.2	13±0.6	17±1.3	16±1.3	24±1.3	9±1.3	0.0001*
	p-value	1.0	1.0	0.77	1.0	1.0	0.40	1.0	1.0	1.0	0.40	
<i>Actinomyces viscosus</i> (ATCC 3268)	Fs	25±12.6	15±1.3	16±0.8	17±3.2	15±0.1	15±1.3	20±1.3	18±1.5	22±1.3	13±1.3	0.0001*
	1:1	25±12.6	15±0.5	16±0.5	16±6.0	15±0.6	15±1.3	19±1.3	18±1.5	22±1.3	25±12.6	0.01*
	p-value	1.0	1.0	1.0	0.81	1.0	1.0	0.40	1.0	1.0	1.0	
<i>Staphylococcus aureus</i> (ATCC 25923)	Fs	40±1.5	30±0.8	10±2.5	10±1.3	11±2.5	10±1.3	12±1.5	10±1.3	5±1.3	5±1.3	0.0001*
	1:1	40±1.8	30±2.2	10±1.8	10±0.5	10±2.5	10±1.3	12±1.8	10±1.3	15±2.2	5±1.8	0.0001*
	p-value	1.0	1.0	1.0	1.0	0.63	1.0	1.0	1.0	1.0	1.0	
<i>Streptococcus pyogenes</i> (MTCC 442)	Fs	40±2.7	30±12.3	40±1.5	36±11.4	38±1.5	28±1.5	28±1.3	40±1.3	40±2.2	38±1.3	0.04*
	1:1	40±1.5	30±9.7	40±2.7	36±7.7	37±1.5	28±2.1	28±1.3	40±1.0	40±0.5	38±1.3	0.22
	p-value	1.0	1.0	1.0	1.0	0.46	1.0	1.0	1.0	1.0	1.0	
<i>Candida albicans</i> (MTCC 183)	Fs	40±1.8	20±5.5	12±2.5	10±0.8	15±8.7	20±1.3	23±1.3	19±1.3	26±2.5	11±1.3	0.0001*
	1:1	40±1.5	18±6.5	11±1.3	10±2.5	18±8.6	20±1.3	18±1.3	19±1.8	26±1.3	11±1.3	0.001*
	p-value	1.0	0.70	0.57	1.0	0.69	1.0	0.10	1.0	0.009*	1.0	

Table/Fig-4: Comparison of mean inhibitory zones at Full strength & 1:1 dilution at 24 hrs – Within group and Between groups

pellicle through adhesions. Later on, additional plaque bacteria such as actinomyces also bind. *C. albicans* is the most common fungus isolated from mouth [24]. Hence, the standard strains of this specific oral microflora (American Type Culture Collection - ATCC and Microbial Type Culture Collection - MTCC) were utilized in the study.

In the present study, standard diffusion (in vitro method) was employed as it is a well-established technique commonly used in screening the antimicrobial efficacy of chemicals before in vivo testing. In vivo use of the *herbal dentifrices* is likely to be diluted by saliva, thereby antimicrobial properties can be buffered or lost in dilution, hence testing in 1:1 dilution was also carried out. Since, the most common practice is to brush at least once a day; evaluation of the zones of inhibition was done at 24 h.

The results of this study revealed that among all the tested herbal dentifrices, Danth Kanthi (DK) possessed the greatest antimicrobial activity against all the microorganisms both at Fs and 1:1 dilution at 24 h except for *A. viscosus*. As well, Amar Premium (AP) also produced larger zones of inhibition at Fs and 1:1 dilution, whereas AP and DB demonstrated lesser zones of inhibition (5 mm) against *S. aureus*.

Colgate herbal (CH) produced significant zones of inhibition against all the microorganisms except *S. aureus* at Fs and 1:1 dilution. This finding is supported by the study done by George et al., [25] wherein, Colgate herbal toothpaste was as effective as the conventionally formulated dentifrices in the control of plaque and gingivitis. On the contrary, a study by Peck MT et al., [14] stated that Colgate herbal produced inhibitory zones that were significantly smaller than other tested toothpastes (Aquafresh® Herbal and Dentazyme® Herbal) except for Nature fresh.

Poureslami HR et al., [26] demonstrated that Meswak extract, alone or in combination with toothpaste, can affect the growth of dental plaque bacteria. Likewise, study by Adwan G et al., [27] reported that Dabur Meswak herbal toothpaste exhibited antifungal activity against *C. albicans*. On the other hand, in this study, Dabur Meswak (DM) except for *S. pyogenes* (40 mm) formed significantly smaller zones of inhibition against the specific oral microflora as compared to other tested *herbal dentifrices*.

S. pyogenes was the most susceptible microorganism for all the tested *herbal dentifrices* at Fs and 1:1 dilution. On the other hand,

another Indian study reported that all *herbal dentifrices* showed greater zones of inhibition against *S. sanguis* [28].

This study revealed that herbal dentifrices possesses significant antimicrobial activity which may reduce the development of dental plaque, thereby influencing the adhesion of bacteria to the tooth surfaces. Even though, the *herbal dentifrices* demonstrated the antimicrobial activity, the study presents with certain limitations. Pathogenicity of bacteria and fungi is a multi factorial process based on microbial virulence and host response, wherein genetic and environmental factors such as saliva buffering, diet play an important role. Moreover, as in vitro test was performed, it cannot be assumed that the results could be proportional or transferrable to the oral cavity and translated into the clinical effectiveness.

CONCLUSION

Based on the results of the present study, it can be concluded that all herbal dentifrices exhibited antimicrobial activity against the selected oral microorganisms, with Danth Kanthi (DK) being the most effective. Hence, it can be inferred that herbal dentifrices can also be recommended like the conventional formulations. However, future research efforts are needed for the evaluation of quality and efficacy of the herbal dentifrices for its regular use in the oral hygiene products. Further studies on the safety and efficacy of such product need to be established.

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REFERENCES

- [1] Shah N. Oral and dental diseases: Causes, prevention and treatment strategies: Burden of disease, National Commission on Macroeconomics and Health. 2005;275-98.
- [2] Agarwal V, Khatri M, Singh G, Gupta G, Marya CM, Kumar V. Prevalence of periodontal diseases in India. *J Oral Health Comm Dent*. 2010;4:7-16.
- [3] Palombo EA. Traditional Medicinal plant extracts and natural products with activity against ora bacteria: potential application in the prevention and treatment of oral diseases. *Evid Based Complement Alternat Med*. 2011; 1-15.

- [4] Vyas YK, Bhatnagar M, Sharma K. In vitro evaluation of antibacterial activity of an herbal dentifrice against *Streptococcus mutans* and *Lactobacillus acidophilus*. *Indian J Dent Res*. 2008;19(1):26-28.
- [5] Caldwell RC and Stallard RE. A Textbook of Preventive Dentistry. West Washington Square Philadelphia; W.B. Saunders Publishers 1977: 214.
- [6] Barnes VM, Richter R, De Vizio W. Comparison of the short-term antiplaque/antibacterial efficacy of two commercially available dentifrices. *J Clin Dent*. 2010;21:101-04.
- [7] Deshpande RR, Kachare P, Sharangpani G, Varghese VK, Bahulkar SS. Comparative evaluation of antimicrobial efficacy of two commercially available dentifrices (fluoridated and herbal) against salivary microflora. *Int J Pharm Pharm Sci*. 2014;6:72-74.
- [8] Verkaik MJ, Busscher HJ, Jager D, Slopm AM, Abbas F, Van der Mei HC. Efficacy of natural antimicrobials in toothpaste formulations against oral biofilms in vitro. *J Dent*. 2011;39:218-24.
- [9] The world medicine situation 2011. Traditional Medicines: Global situations, issues and challenges. Traditional Medicines WHO, Geneva 2011.
- [10] Walter C, Shinwari ZK, Afzal I, Malik RN. Antibacterial activity in herbal products used in Pakistan. *Pak J Bot*. 2011;43:155-62.
- [11] Vahabi S, Najafi E, Alizadeh S. In vitro antimicrobial effects of some herbal essences against oral pathogens. *Journal of Medicinal Plants Res*. 2011;5(19):4870-78.
- [12] Botelho MA, Nougéria NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, et al. Antimicrobial activity of the essential oil from *Lippia Sidiodes*, carvacrol and thymol against oral pathogens. *Braz J Med Biol Res*. 2007;40(3):349-56.
- [13] 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;135:1133-41.
- [14] Peck MT, Africa CWJ, Stephen LXG, Marnewick JM, Abdul M. An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers. *Int Journal of Clinical Dental Science*. 2011;2(3):28-32.
- [15] Wu-Yuan CD, Green L, Birch WX. In vitro screening of Chinese medicinal toothpastes: their effects on growth and plaque formation of mutans streptococci. *Caries Res*. 1990;24:198-202.
- [16] Ozaki F, Pannuti CM, Imbrono AV, Pessotti W, Saraiva L, Maria de Freitas N, et al. Efficacy of a herbal toothpaste on patients with established gingivitis - a randomized controlled trial. *Braz Oral Res*. 2006;20:172-77.
- [17] Netuschil L, Brex M, Heumann C, Hoffman T. Clinically controlled 6- month study of the influence of toothpastes with anti-inflammatory ingredients on plaque and gingivitis. *Quintessenz*. 2005;56:1277-86.
- [18] Elvin-Lewis M. Plants used for teeth cleaning throughout the world. *J Prevent Dent*. 1980;6(1):61-70.
- [19] Willershausen B, Gruber I, Hamma G. The influence of herbal ingredients on the plaque index and bleeding tendency of the gingiva. *J Clin Dent*. 1991;2(3):75-8.
- [20] Marsh PD, Bradshaw DJ. Microbiological effects of new agents in dentifrices for plaque control. *Int Dent J*. 1993;43(4 Suppl 1):387-97.
- [21] Al-Kholani AI. Comparison between the efficacy of herbal and conventional dentifrices on established gingivitis. *Dent Res J*. 2011;8(2):57-63.
- [22] Beck JD, Arbes SJ. Epidemiology of Gingival and Periodontal diseases. In: Newman M.G. eds. Carranza's Clinical Periodontology 9th edition. Philadelphia: WB Saunders, 2002: 74-94.
- [23] Teles RP, Teles FR. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control. *Braz Oral Res*. 2009;23(Suppl 1):39-48.
- [24] Baveja CP. Textbook of Microbiology for Dental students. 2nd ed. India; Arya Publications 2006: 333-34.
- [25] George J, Hegde S, Rajesh KS, Kumar A. The efficacy of a herbal-based toothpaste in the control of plaque and gingivitis; A clinic-biochemical study. *Indian J Dent Res*. 2009;20(4):480-82.
- [26] Poureslami HR, Makarem A, Mojab F. Paraclinical effects of miswak extract on dental plaque. *Dent Res J*. 2007;4(2):106-10.
- [27] Adwan G, Salameh Y, Adwam K, Barakat A. Assessment of antifungal activity of herbal and conventional toothpastes against isolates of *Candida albicans*. *Asian Pacific Journal of Tropical Biomedicine*. 2012;375-79.
- [28] Gupta P, Agrawal N, Sharma R. Evaluate the anti-plaque efficacy of herbal dentifrices – An in-vitro study. *Int JAPS BMS*. 2012;1(2):150-59.

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