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Comparative Evaluation of Antimicrobial Efficacy of Herbal Dentifrices *Vs* Chemical Dentifrices on *Streptococcus Mutans*: An *In-Vitro* Study

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Abstract

Context: Chemical dentifrices are known to have certain side effects. So, Herbal dentifrices are evaluated and compared for their anti-microbial efficacy against *Streptococcus mutans*.

Aim: To compare anti-microbial efficacy of dentifrices containing herbal combination versus dentifrices containing chemical combinations on caries initiating micro-organism *Streptococcus mutans* by agar well diffusion method.

Settings and design: Pure cultures of *Streptococcus mutans* (ATCC 25175) were sub-cultured on Mueller Hinton broth at 37^{0}C for 24hrs. The Blood agar plates were inoculated with 0.5 ml of 24 hour broth culture. Dilutions of the selected dentifrices containing herbal combinations and dentifrices containing chemical combinations were evaluated for their antimicrobial activity by agar well diffusion method. The agar plates were incubated at 37^{0}C for 24 hours. The diameter of obtained zones of inhibition were measured (in mm) using a graduated scale. The size of obtained zone of inhibition was considered to be directly related with the antimicrobial efficacy of the sample dentifrice.

Statistical analysis used: The results obtained were subjected to statistical analysis using One way ANOVA and Independent t-test.

Results: Amongst the herbal combinations, maximum zone of inhibition was observed with the dentifrice containing Clove, Pudina, Tomar, Ginger. Amongst chemical based dentifrices, Triclosan and Sodium fluoride containing dentifrice depicted maximum zone of inhibition against *S. mutans*. On comparing antimicrobial efficacy of Herbal *Vs* Chemical dentifrices, it was observed that there was no significant difference between them (P>0.001).

Conclusions: Herbal dentifrices are equally effective as Chemical dentifrices and unlike the latter they do not possess any side-effects.

Keywords: Agar well diffusion method; Anti-microbial efficacy; Dentifrice containing herbs; Dentifrices containing chemicals; *S. mutans*

Introduction

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by the demineralization of the inorganic portion and destruction of the organic substance of the tooth, leading to cavitation [1]. In India, the prevalence of dental caries is reported to be 50-60% [2].

For dental caries to occur, three components *viz*., tooth surface, fermentable carbohydrates and cariogenic micro-organisms have to be present. Hence, to control caries, removal of fermentable carbohydrates and control of cariogenic micro-organisms is required. This can be achieved by various mechanical and chemical measures [1].

For this, tooth brushing with dentifrices containing various chemicals are advocated. Commonly used chemicals in dentifrices are Triclosan, Sodium fluoride (Colgate Strong Teeth, Colgate-Palmolive India Ltd., Powai, India); Sodium monofluorophosphate (Kidodent, Indoco remedies Ltd., Solan, Himachal Pradesh, India); Chlorhexidine gluconate (Elgydium, Pierre Fabre Medicament, France); Metronidazole (Metrogyl DG gel, Lekar Pharma, Panoli, Gujrat, India); Calcium sodium phosphosilicate (Vantej, Dr. Reddy's Lab., Hyderabad, Telangana, India); Amine

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fluoride (Amflor, Group Pharma. Ltd., Malur, Karnataka, India) etc.

These dentifrices containing chemical agents are effective antimicrobial agents, but these chemicals are reported to have certain side effects like development of dental fluorosis etc [3]. Some chemicals like Triclosan are even reported to be carcinogenic [4].

Global thinking has a growing tendency to 'Go Natural'. It is believed that the natural phytochemicals present in plants could offer an effective alternative to synthetic preparations. Hence, despite the efficacy of dentifrices containing chemical formulations, there is an increased societal desire to rely on naturally occurring herbal formulations.

These herbal formulations have become further more appealing as they do not require alcohol, artificial preservatives, flavors or colors for their activity. Some of the active ingredients in the herbal dentifrices are Pomengranate, Triphala, Neem, Babool, Pippali, Pudina Satva, Clove oil, Ginger, Meswak, Akarkara, Tomar, Amla, Nimbu etc.

Various micro-organisms like *Streptococcus mutans, Escherichia coli and Candida albicans* play major role in dental caries. Out of these, *Streptococcus mutans* has the central role in caries initiation and it even plays role in progression of caries. Hence, control of *Streptococcus mutans* can play an important role in prevention of caries.

Assays to test antimicrobial activity are generally based on Agar Well Diffusion method [5], Disk Diffusion method [2], Linear Regression method [6], measuring Salivary Streptococcus Count [7] or Minimum Inhibitory Concentration [8], *via in-vivo* [7] and *in-vitro* studies [9]. Out of all these methods, Agar Well Diffusion method proves to be the most readily available, easy to accomplish as it allows for easy manipulation and provides quick results.

Sparse knowledge of various ingredients used in herbal dentifrices and their antimicrobial efficacy compared to chemical dentifrices is present. Amongst the commonly used herbal dentifrices in today's era, few have been evaluated earlier for their anti-microbial potential [2]. Some herbs are not yet assessed. These include Pomengranate, Bishop's weed, False black pepper, Five leaved chaste (Himalaya Complete Care) and Amra, Nimbu (Himalaya HiOra).

Hence, in this *in-vitro* study, anti-microbial efficacy of various combination of herbs available as dentifrices were compared with various combinations of chemical agents available in dentifrices on caries initiating micro-organism *Streptococcus mutans* by agar well diffusion method.

Materials and Methods

Herbs and chemicals

Different dentifrices containing combinations of various herbs (Group I)/ chemicals (Group II) were selected for the study. Herbal dentifrices were grouped as IA: Pomengranate, Bishop's weed, False black pepper, Neem, Triphala, Five leaved chaste (Himalaya Complete Care), IB: Babool (Dabur Babool), IC: Clove, Pudina, Tomar, Ginger (Dabur Red), ID: Meswak (Dabur Meswak), IE: Akarkara, Pippali, Babool,Tomar, Pudina, Clove, Neem, Haldi, Vajradanti, Vidang (Patanjali Dantkanti) and IF: Triphala, Amra, Babool, Nimbu, Clove (Himalaya HiOra). The chemical dentifrices were grouped as IIA: Triclosan, Sodium fluoride (Colgate Strong Teeth), IIB: Metronidazole (Metrogyl DG gel), IIC: Sodium monofluorophosphate (Kidodent), IID: Chlorhexidine gluconate (Elgydium), IIE: Calcium sodium phosphosilicate (Vantej) and IIF: Amine fluoride (Amflor) (Figure 1).

Herbal formulations

IA Anar, Babool, Neem, Triphala, Ajwain, Kababchini (Himalaya Complete Care)

IB Babool (Dabur Babool)

IC Lavang, Pudina, Tomar, Adrak (Dabur Red)

ID Meswak (Dabur Meswak)

IE Akarkara, Neem, Babool, Tomar, Pudina, Lavang, Pippli, Vajradanti, Bakul, Vidang, Haldi, Pilu, Majuphal (Patanjali Dantkanti)

IF Pilu, Triphala, Babool, Amra, Lavang, Nimba (Himalaya HiOra)

Chemical formulations

IIA Triclosan, Sodium fluoride (Colgate Strong Teeth)

IIB Metronidazole, Chlorhexidine gluconate (Metrogyl DG gel)

IIC Sodium monofluorophosphate (Kidodent)

IID Chlorhexidine gluconate, Calcium carbonate (Elgydium)

IIE Calcium sodiumphosphosilicate (Vantej)

IIF Amine fluoride (Amflor)

Figure 1: Composition of dentifrices evaluated in the study.

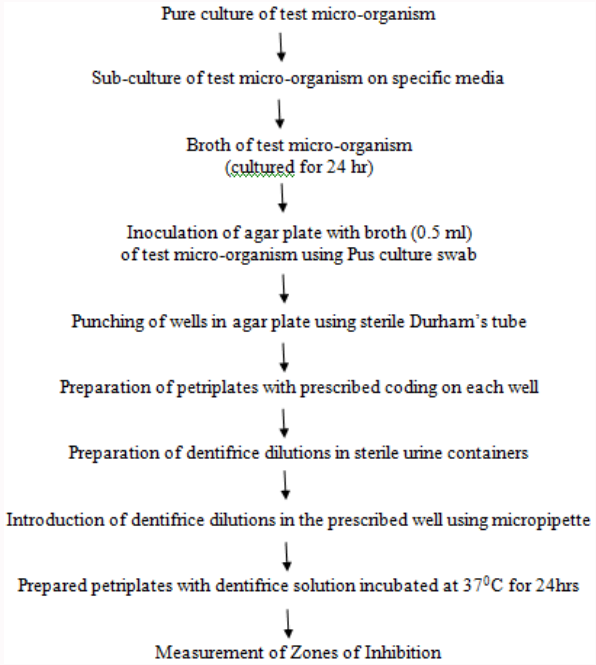

Figure 2: Methodology of study.

Preparation of broth

Pure cultures of *Streptococcus mutans* (ATCC 25175) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India and were sub-cultured in Mueller Hinton broth (HiMedia Laboratories Pvt. Ltd.) at 37°C for 24 hours. The identification of the strain was confirmed by standard biochemical and staining methods. All the

Figure 3: Observed Zones of Inhibition.

procedures were done on laminar air flow to maintain asepsis. To standardize the inoculum density for a susceptibility test, a barium sulphate turbidity standard, equivalent to a 0.5 McFarland standard was used. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.

Preparation of dilution

The dilution of selected specimens was made by mixing the calculated amount of dentifrice (2.0 g) *via* use of weighing machine in measured volume (2 ml) of pyrogen free distilled water via disposable syringe to obtain 1:1 dilution in sterile urine containers (Figure 2).

Agar well diffusion assay

The antimicrobial activity of different dentifrices was determined by agar well diffusion method. Culture plates were poured with Blood agar within 15 minutes (optimally) after adjusting the turbidity of the inoculum. They were allowed to solidify to make a base layer. A sterile pus culture swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This procedure removed the excess inoculum from the swab. The dried surface of agar plate was inoculated with 0.5 ml of 24 hr broth culture by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The plates were then allowed to dry for about one hour.

After confirming complete drying of the agar plates, four wells at equidistance were punched in each agar plate using sterile Durham's tube (inner diameter of 7 mm). Dentifrice dilution (0.1 ml) was introduced in respective wells. The fourth well of each petriplate was filled with same volume (0.1 ml) of pyrogen free distilled water (Control). The petriplates were then incubated at 37^0C for 24 hours. Zone of inhibition were observed around the wells (Figure 3) and the diameter of these zones were measured (in mm) using graduated scale (Kirby-Bauer method, National Committee for Clinical Laboratory Standards approved) [10].

Only those petriplates were selected in which uniform circular zone of inhibition was observed with a confluent lawn of growth. Petriplates with tear in agar during punching of holes or those with individual colonies of micro-organisms were discarded. The size of obtained zone of inhibition was considered to be directly related with the antimicrobial efficacy of the sample dentifrice. After one day, the intra-observer readings were verified and inter-observer verification was done by a microbiologist. Kappa statistics was applied on the collected data and the coefficient of co-relation was found to be in the range of 0.9 and 0.8 for intra and inter observer. All the co-relation coefficient values were found to be high reflecting high degree of concordance.

After the evaluation of zones of inhibition, the culture plates were collected in Yellow color coded bags and sent to the Bio-medical waste management company for further disposal. The results obtained were tabulated and subjected to statistical analysis.**3.5. Statistical method**

Statistical analysis was performed by SPSS version 22.0. Statistical comparison was done using One way Analysis of Variance (ANOVA). Post-Hoc Tukey's test and Independent t-test were done for intra and inter group comparisons. Significance level was kept at 95% confidence interval ($p<0.05$).

Results

IC group dentifrice containing herb combination: Clove, Pudina, Tomar, Ginger depicted maximum mean zone of inhibition (11.25 mm) against *Streptococcus mutans*. IA group dentifrice with combination of Pomengranate, Bishop's weed, False black pepper, Neem, Triphala, Five leaved chaste depicted 10.65 mm mean zone of inhibition; IF group dentifrice with combination of Triphala, Amra, Babool, Nimbu, Clove depicted 10.20mm mean inhibition zone; IE group dentifrice with combination of Akarkara, Pippali, Babool,Tomar, Pudina, Clove, Neem, Haldi, Vajradanti, Vidang depicted 10.15 mm mean zone of inhibition; IB group dentifrice containing Babool depicted 9.95 mm mean zone of inhibition and ID groupdentifrice containing Meswak depicted 9.65 mm mean zone of inhibition.

Amongst chemical combinations, IIA dentifrice containing combination of Triclosan, Sodium fluoride depicted maximum mean zone of inhibition (12.35 mm) against *Streptococcus mutans*. This was followed by IID dentifrice containing combination of Chlorhexidine and Calcium carbonate depicted mean zone of inhibition of 10.50 mm; IIE dentifrice containing Calcium sodium phosphosilicate depicted mean zone of inhibition of 9.90 mm; IIC dentifrice containing Sodium monofluorophosphosilicate depicted mean zone of inhibition of 8.55 mm; IIF dentifrice containing Amine fluoride depicted mean zone of inhibition of 8.05 mm and IIB dentifrice containing combination of Metronidazole and Chlorhexidine depicted mean zone of inhibition of 7.20 mm (Table 1).

Discussion

Streptococcus mutans plays a major role in the tooth decay by

Table 1: Mean zone of inhibition (mm) and standard deviation of Herbal and Chemical based dentifrices against *Streptococcus mutans*.

Dentifrice	N	Mean	S.D	Minimum	Maximum
IA	10	10.65	0.78	9.00	12.00
IB	10	9.95	0.98	8.00	11.50
IC	10	11.25	0.89	10.00	13.00
ID	10	9.65	0.97	7.50	11.00
IE	10	10.15	0.75	9.00	11.00
IF	10	10.20	0.54	9.50	11.00
IIA	10	12.35	1.36	11.00	15.00
IIB	10	7.20	0.48	7.00	8.50
IIC	10	8.55	0.55	8.00	9.50
IID	10	10.50	0.53	10.00	11.50
IIE	10	9.90	1.29	8.50	12.00
IIF	10	8.05	1.17	7.00	11.00

Table 2: Comparison of mean zone of inhibition (mm) against *Streptococcus mutans* with different herbal and chemical groups by One way ANOVA.

		Sum of Squares	Df	Mean Square	F	Sig.
Herbal group (Group I)	Between Groups	16.021	5	3.204	4.611	P<0.001**
	Within Groups	37.525	54	0.695	-	-
	Total	53.546	59	-	-	-
Chemical group (Group II)	Between Groups	175.438	5	35.088	37.170	P<0.001*
	Within Groups	50.975	54	0.944	-	-
	Total	226.413	59	-	-	-

* The mean difference is significant at the 0.05 level.

metabolizing sucrose to lactic acid. It has the ability to metabolize dietary sucrose and synthesize glucan by cell surface and extracellular glucosyl transferase which in turn helps in the establishment of *S. mutans* in the dental plaque [3].

To prevent plaque accumulation, many mechanical plaque control measures are available. Tooth brushing alone will remove only about 50% of dental plaque, so additional mechanical measures are required to further reduce the bacterial load. For this, incorporation of dentifrices containing chemical agents is advocated but as they have certain side effects and microbial resistance to most antibiotics is also reported, therefore, herbal based products are in more demand recently. A wide variety of medicinal plants used traditionally have not yet been systematically investigated against various microbial pathogens. The effect of plant extracts on bacteria has been studied by a large number of researchers in different parts of the world.

In the present study, the antimicrobial activity of plant extracts and phytochemicals was evaluated and compared with chemical based products (Table 2). All herbal dentifrices exhibited antimicrobial efficacy against *Streptococcus mutans*. As this micro-organism is responsible for caries initiation, therefore, controlling it may help in prevention of dental caries. Out of all the herbal dentifrices, IC group dentifrice containing Clove, Pudina, Tomar, Ginger was most effective followed by group IA and IE, but the difference in antimicrobial efficacy of these three dentifrices was not significant (p>0.05) (Table 3). Herbs like Clove, Pudina, Tomar and Ginger when added to oral products, kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. They prevent bacterial aggregation, slow multiplication and release endotoxins.

Clove (Syzygium aromaticum) is a median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. It represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material. Researchers have found that eugenol significantly inhibits acid production of *S. mutans*, reduces the synthesis of water insoluble glucans, markedly suppresses the adherence of the bacteria to the saliva-coated hydroxyapatite beads, and reduces severity and incidence of carious lesions [11].

Pudina (Mentha arvensis), a perennial aromatic herb belonging to the family Labiatae and genus Mentha is an important plant with immense medicinal use. Today, Pudina is cultivated in North America, Africa, Australia, and Asia mainly for its pharmaceutical and medicinal uses. The entire plant is antibacterial. It yields an essential oil and menthol. Its effect is lightly anesthetic and anodyne local effect, through the rapid evaporation [12]. It has menthol, menthone, methyl esters and terpenoids which are responsible for antibacterial

Table 3: Comparison of mean zone of inhibition (mm) against *Streptococcus mutans* within herbal dentifrices by Tukey Post Hoc test.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.
Herbal group	IA	IB	0.7000	0.3728	0.427
		IC	-0.6000	0.3728	0.596
		ID	1.0000	0.3728	0.096
		IE	0.5000	0.3728	0.761
		IF	0.4500	0.3728	0.832
	IB	IA	-0.7000	0.3728	0.427
		IC	-1.3000*	0.3728	0.012
		ID	0.3000	0.3728	0.965
		IE	-0.2000	0.3728	0.994
		IF	-0.2500	0.3728	0.984
	IC	IA	0.6000	0.3728	0.596
		IB	1.3000*	0.3728	0.012
		ID	1.6000*	0.3728	0.001
		IE	1.1000	0.3728	0.050
		IF	1.0500	0.3728	0.070
	ID	IA	-1.0000	0.3728	0.096
		IB	-0.3000	0.3728	0.965
		IC	-1.6000*	0.3728	0.001
		IE	-0.5000	0.3728	0.761
		IF	-0.5500	0.3728	0.681
	IE	IA	-0.5000	0.3728	0.761
		IB	0.2000	0.3728	0.994
		IC	-1.1000	0.3728	0.050
		ID	0.5000	0.3728	0.761
		IF	-0.0500	0.3728	1.000
	IF	IA	-0.4500	0.3728	0.832
		IB	0.2500	0.3728	0.984
		IC	-1.0500	0.3728	0.070
		ID	0.5500	0.3728	0.681
		IE	0.0500	0.3728	1.000

* The mean difference is significant at the 0.05 level.

effect. Menthol ($C_{10}H_{20}O$), the active constituent present in Pudina, perhaps is largely responsible for the therapeutic potentials of Pudina. The oil is antiseptic, carminative, refrigerant, stimulant and diuretic. The other important constituents include 4.5 to 10% esters-menthyl acetate and 15 to 20% of ketones. The antimicrobial activity of Pudina against *Streptococcus mutans* can be attributed to these constituents. Recent studies have shown that although Chlorhexidine was more effective when compared to Pudina extract, it has several well-

Table 4: Comparison of mean zone of inhibition (mm) against *Streptococcus mutans* within Chemical dentifrices by Tukey Post Hoc test.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.
Chemical group	IIA	IIB	5.1500*	0.4345	0.000
		IIC	3.8000*	0.4345	0.000
		IID	1.8500*	0.4345	0.001
		IIE	2.4500*	0.4345	0.000
		IIF	4.3000*	0.4345	0.000
	IIB	IIA	-5.1500*	0.4345	0.000
		IIC	-1.3500*	0.4345	0.034
		IID	-3.3000*	0.4345	0.000
		IIE	-2.7000*	0.4345	0.000
		IIF	-0.8500	0.4345	0.380
	IIC	IIA	-3.8000*	0.4345	0.000
		IIB	1.3500*	0.4345	0.034
		IID	-1.9500*	0.4345	0.001
		IIE	-1.3500*	0.4345	0.034
		IIF	0.5000	0.4345	0.858
	IID	IIA	-1.8500*	0.4345	0.001
		IIB	3.3000*	0.4345	0.000
		IIC	1.9500*	0.4345	0.001
		IIE	0.6000	0.4345	0.738
		IIF	2.4500*	0.4345	0.000
	IIE	IIA	-2.4500*	0.4345	0.000
		IIB	2.7000*	0.4345	0.000
		IIC	1.3500*	0.4345	0.034
		IID	-0.6000	0.4345	0.738
		IIF	1.8500*	0.4345	0.001
	IIF	IIA	-4.3000*	0.4345	0.000
		IIB	0.8500	0.4345	0.380
		IIC	-0.5000	0.4345	0.858
		IID	-2.4500*	0.4345	0.000
		IIE	-1.8500*	0.4345	0.001

* The mean difference is significant at the 0.05 level.

known side effects like staining of teeth and restoration, alteration of taste sensation, development of resistant microorganisms, which may limit the long term use of Chlorhexidine. In comparison to it, herbal medicine Pudina is abundantly available, easily accessible, economically feasible, and culturally acceptable and may possess minimal side effects and hence can be recommended for long term use. In a study, it was found that the anti-microbial effects of Pudina extract are better as compared to other herbal products like Neem, Mango and Tulsi. A recent research evaluated the alcoholic extract of pudina leaves at three different concentrations, 5%, 10%, and 50% and depicted zone of inhibition at 10% and 50% concentrations only. The alcoholic extract of pudina leaves at 10 mg/ml was more active against *S. mutans*. In contrast, another study evaluating alcoholic extracts of garlic and pudina leaves at 1 mg/ml and 2 mg/ml concentrations, did not show any antibacterial effect against *Streptococcus mutans*. The reason for this could be the low concentration of test extracts that were used (1to 2 mg/ml). The antimicrobial activity of clove and ginger when evaluated against *Streptococcus mutans* using agar well method, was found to be very high in a study.

Table 5: Comparison of mean zone of inhibition (mm) of herbal dentifrice with different chemical dentifrices against *Streptococcus mutans* by Independent t-test.

Streptococcus mutans	Mean	S.D	Mean	S.D	P-value
I-A to II A	10.65	0.78	12.35	1.36	0.000 *
I-A to IIB	10.65	0.78	7.20	0.48	0.000*
I-A to IIC	10.65	0.78	8.55	0.55	0.000*
I-A to IID	10.65	0.78	10.50	0.53	0.620
I-A to IIE	10.65	0.78	9.90	1.29	0.130
I-A to IIF	10.65	0.78	8.05	1.17	0.000*
I-B to II A	9.95	0.98	12.35	1.36	0. 000*
I-B to IIB	9.95	0.98	7.20	0.48	0.000*
I-B to IIC	9.95	0.98	8.55	0.55	0.000*
I-B to IID	9.95	0.98	10.50	0.53	0.14
I-B to IIE	9.95	0.98	9.90	1.29	0.92
I-B to IIF	9.95	0.98	8.05	1.17	0.000*
I-C to II A	11.25	0.89	12.35	1.36	0.05
I-C to IIB	11.25	0.89	7.20	0.48	0.00*
I-C to IIC	11.25	0.89	8.55	0.55	0.00*
I-C to IID	11.25	0.89	10.50	0.53	0.03*
I-C to IIE	11.25	0.89	9.90	1.29	0.01*
I-C to IIF	11.25	0.89	8.05	1.17	0.00*
I-D to II A	9.65	0.97	12.35	1.36	0.000*
I-D to IIB	9.65	0.97	7.20	0.48	0.000*
I-D to IIC	9.65	0.97	8.55	0.55	0.01*
I-D to IID	9.65	0.97	10.50	0.53	0.03*
I-D to IIE	9.65	0.97	9.90	1.29	0.63
I-D to IIF	9.65	0.97	8.05	1.17	0.00*
I-E to II A	10.15	0.75	12.35	1.36	0.00*
I-E to IIB	10.15	0.75	7.20	0.48	0.00*
I-E to IIC	10.15	0.75	8.55	0.55	0.00*
I-E to IID	10.15	0.75	10.50	0.53	0.24
I-E to IIE	10.15	0.75	9.90	1.29	0.60
I-E to IIF	10.15	0.75	8.05	1.17	0.00*
I-F to II A	10.20	0.54	12.35	1.36	0.00*
I-F to IIB	10.20	0.54	7.20	0.48	0.00*
I-F to IIC	10.20	0.54	8.55	0.55	0.00*
I-F to IID	10.20	0.54	10.50	0.53	0.22
I-F to IIE	10.20	0.54	9.90	1.29	0.50
I-F to IIF	10.20	0.54	8.05	1.17	0.00*

*The mean difference is significant at the 0.05 level.

Methanolic extracts of Zanthoxylum armatum (Tomar) were reported to be effective against the strains of *Streptococcus mutans* and incorporation of the plant extracts having potent antimicrobial (anti- *S. mutans*) activity with dentifrices was recommended.

All chemical dentifrices exhibited antimicrobial efficacy against *Streptococcus mutans*. Out of these, IIA group was most effective (Table 4). The difference in its antimicrobial efficacy with the other five dentifrices of this group was statistically significant ($p<0.05$).

The active ingredient in IIA is Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol]. It has been used for more than 30 years

as a general antibacterial and antifungal agent, which is found in formulations such as dentifrices and mouthrinses. Triclosan is suggested to block lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR) [13]. Systematic reviews of six-month clinical studies have concluded that formulations containing Triclosan and copolymer significantly improve plaque control and periodontal health [14]. Investigation of dentifrices containing Triclosan on resistant oral Streptococci measured in-vitro sensitivity of Streptococci strains against Triclosan [15]. This become more plausible as the utility and effectiveness of a 1% Triclosan formulation in health care industry has been reviewed [16]. On *Streptococcus mutans*, the antimicrobial efficacy of IC was observed to be significant with that of IIA (p 0.05) but their antimicrobial potential was still comparable (Table 5).

Conclusion

Dentifrices containing herbal combinations, in comparison to chemical agents based dentifrices, have no significant difference in their antimicrobial efficacy and also negligible side effects are observed with their usage. A dentifrice has to be evaluated in all dimensions, the added advantage of being natural as compared to chemical dentifrices tip the scales in favor of herbal combination based dentifrices.

The selection of dentifrice depends upon the oral hygiene status and caries index of the individual. Amongst the low caries index individuals, herbal dentifrice containing Clove, Pudina, Tomar and Ginger can be used as a potential measure for prevention of caries initiation as it is found to be most efficacious against *Streptococcus mutans*.

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