Role of chemical and herbal dentifrices against indigenous oral pathogens

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Abstract: The oral cavity has its own significant micro-flora but under unhygienic conditions can cause infections or diseases like gingivitis, caries, plaque and gum bleeding. Out of more than 700 oral microbial species, some opportunistic pathogens such as *Staphylococcus aureus*, *Streptococcus* spp. and *Candida albicans* are more prevalent. In this study, the antimicrobial activities of various toothpastes (dilutions ranging from 1:1-1:128) against above mentioned pathogens were assessed. The pathogens were isolated from clinical samples using various differential and selective media and identified through microscopic examination, cultural characteristics and biochemical tests using both conventional and API kit system (Biomerieux, France). Antimicrobial activities of selected dentifrice formulations against identified microbes were determined using agar well diffusion and Minimum Inhibitory Concentration assays. Statistical analysis of the data on different variables has been performed by Analysis of Variance and Mean ±SD using SPSS software. From the collected samples *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus intermedius* and *Candida albicans* were isolated and identified. All the selected toothpastes showed significant ($p<0.01$) antimicrobial activity against the bacterial and fungal isolates. Variable results (inhibitory zone diameters ranging from 35.10±8.00 to 2.40±5.37) were found when mean of different dilutions were compared. Conventional dentifrices exhibited more inhibition as compared to herbal products.

Keywords: Agar well diffusion assay, antimicrobial, dentifrice, minimum inhibitory concentration, oral pathogens.

INTRODUCTION

Since centuries, dental caries is considered as most common infection around the globe and nowadays its evidence has increased (Okpalugo *et al*., 2009). One of the major adaptable pathogens involved in dental caries is *Streptococcus mutans*, responsible for the demineralization of tooth enamel due to the production of acid during fermentation of carbohydrates (Gamboa *et al*., 2004). The auxiliary structures of the teeth i.e. alveolar bone, cementum, gingival, and periodontal membrane are often affected by periodontal diseases caused by bacteria. The most prevalent type of periodontal infection is an inflammatory condition of gums called gingivitis. If alveolar bone and periodontal membrane affected due to severe condition of periodontal infections, loss of tooth may results. The probable causative agents for the infection were reported as Streptococci, Bacteroids and Spirochetes (Prasanth, 2011). In addition, other micro flora like *Escherichia coli* and *Candida* spp. are also associated with active caries lesions. *Candida albicans* is the most common yeast isolated from the oral cavity. Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities.

To control the growth rate of these microorganisms, many measures have been adopted but most frequent and the simplest method to do so is the use of toothpaste. To inhibit plaque from recurring, various compounds are being used as supplementary ingredients of toothpastes. Traditional oral care formulations have fluoride and triclosan as the main antimicrobial components. These constituents have been evidenced to be greatly effective against caries causing bacteria. Sodium fluoride was used in the earlier toothpastes; however, shortly substituted by other forms of fluoride like amine fluoride, sodium monofluorophosphate and stannous fluoride. The non-ionic, less toxic, phenolic derivative, triclosan is an active agent in several oral hygienic products (Nogueira-Filho *et al*., 2002). Currently, triclosan having broad spectrum of antimicrobial activity has been positively amalgamated in oral hygienic products, leading to moderate but distinguishable optimistic effects on both dental plaque (biofilm) and inflammation of the gums (Jones *et al*., 2000).

Very few products (tooth pastes) however, have undergone advanced and thorough research concerning their affectivity in different parts of globe (Kumari *et al*., 2011). To date no research work on local brands of tooth pastes have been reported in the country, therefore, scientific information on these products is unavailable. The main objective of this study was *in-vitro* evaluation of antimicrobial activity and determination of Minimum Inhibitory Concentration (MIC) of different toothpastes frequently used in Pakistan against common oral pathogens.

MATERIALS AND METHODS

Dentifrice sampling

For *in-vitro* evaluation of antimicrobial activity, 12 commonly used toothpastes were selected and purchased

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from the local market of Faisalabad, Pakistan (table 1). The suspensions of these products were made by mixing 1.0 g of each toothpaste material per ml of distilled water to prepare 1:1 (w/v) dilutions. Furthermore, two fold serial dilutions of each product were also prepared (Adejumo *et al*., 2008).

Microbial isolates

A total of 180 samples were collected from different hospitals of Faisalabad, Pakistan. These were collected by direct swabbing method from various parts of oral cavity and inoculated on Nutrient agar plates (Kateete *et al*. 2010). The isolated colonies were further inoculated on Staph 110 agar and Blood agar media and incubated at 37°C for 24 hours. Bacterial growth was identified on the basis of colonial, microscopic and morphological characteristics (Liberio *et al*., 2011). Biochemical characterization was done using catalase, coagulase, oxidase, mannitol fermentation and API tests. For fungal isolates, samples were inoculated on Sabouraud dextrose agar (Adwan *et al*. 2012). The identification was made on the basis of phenotypic characteristics (Alghalibi *et al*. 2011).

Antimicrobial assay

From pure cultures fresh 24 hour growth was obtained in Nutrient broth and turbidity was set at 0.5 McFarland. The Nutrient agar plates were seeded with 5mL soft agar containing 100 µl (0.1 ml) of the culture (1.5 x 10^8 CFU / ml). Wells of 10 mm in diameter were bored and filled with 100 µl from each dilution of test samples. The plates were incubated at 37°C for 24 hours after pre-incubation at 4°C for 30 min. Sabouraud dextrose agar was used for *C. albicans* and incubated at 28°C for 48 hours. After incubation, the plates were examined and the diameters of zones of inhibition were measured (excluding diameter of the well) with the help of transparent ruler (Iqbal *et al*, 2011).

Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined by micro-broth dilution assay (Riaz *et al*. 2008). Nutrient broth (50 µl) was added in each well of a microtitration plate. In the first well, 50µl of toothpaste suspensions were pipetted and two fold serial dilutions were prepared. The inoculum suspension (50µl) of each isolate was added in each well. For fungal spp., instead of

Table 1: Selected brands of dentifrice formulations and their ingredients

Dentifrice Formulation code	Ingredients as mentioned on packages by manufacturers
1	Potassium nitrate
2	Clove extract
3	Clove, Salt, Eucalyptus oil, Spearmint, Syloblanc
4	Sodium monoflourophosphate
5	Potassium nitrate, Sodium fluoride
6	Sodium monoflourophosphate, Sodium flouride, Dicalcium phosphate dehydrate
7	Potassium citrate, Chlorhexidine digluconate, Allantoin, Sodium benzoate, Sodium chloride, Sodium monoflourophosphate
8	Sodium monoflourophosphate
9	Sodium monoflourophosphate, Flouride, Calcium extracts from Neem, Eucalyptus, Clove, Mint
10	Potassium nitrate
11	Calcium carbonate, Water, Sorbitol, Hydrated silica, Sodium lauryl sulphate, Triclosan, Sodium monoflouro-phosphate, Cellulose Gum, Sodium silicate, Potassium citrate, Phenylcarbinol, Sodium saccharin, Cl 12490
12	Sorbitol, Hydrated silica, Water, PEG-32, Sodium laurylsulphate, Coccamidopropyl, Betabetaine, Flavour, Sodium monoflourophosphate, Cellulose gum, Sodium hydroxide, Sodium saccharin, Zinc Sulphate, Mica, Cl16255, Cl17200, Cl77491, Cl77891

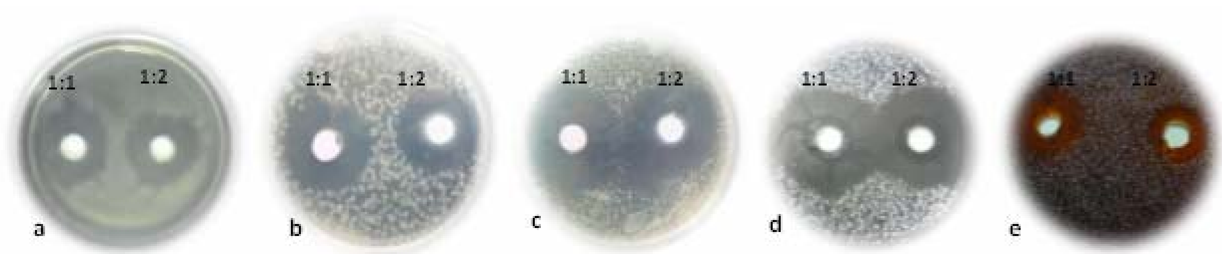

Fig. 1: Inhibitory activity of (a) brand 2 against *Staphylococcus aureus*, (b) brand 6 against *Streptococcus salivarius*, (c) brand 11 against *Streptococcus mutans*, (d) brand 11 against *Streptococcus intermedius*, (e) brand 9 against *Candida albicans* by agar well diffusion assay.

Table 2: Identified microbial isolates from clinical oral samples

S. No.	Microbial isolates	Identified spp.
1.	Bacterial	*Staphylococcus aureus*
2.		*Streptococcus salivarius*
3.		*Streptococcus mutans*
4.		*Streptococcus intermedius*
5.	Fungal	*Candida albicans*

Table 3: Comparison of Mean ± SD of different dilutions of various formulations against *Staph. aureus*

Dentifrice Formulation code	1:1 Mean ± SD	1:2 Mean ± SD	1:4 Mean ± SD	1:8 Mean ± SD	1:16 Mean ± SD	1:32 Mean ± SD	1:64 Mean ± SD	1:128 Mean ± SD
1	17.80± 11.88*	16.00± 11.92	14.20± 10.01	12.80± 8.73**	11.00± 7.07**	00±00	00±00	00±00
2	21.80± 10.16	19.75± 3.30	17.50± 3.42	14.50± 3.42**	13.00± 2.58**	00±00	00±00	00±00
3	28.80± 6.72*	25.60 ± 4.93	23.60 ± 3.85	21.60 ± 3.85	18.20 ± 3.49**	14.20 ± 3.49**	00±00	00±00
4	19.80± 9.96	17.00± 2.58	15.00± 2.58**	10.50± 7.19**	00±00	00±00	00±00	00±00
5	23.00± 10.00	21.50± 3.42	18.50± 3.42**	16.50± 3.42**	00±00	00±00	00±00	00±00
6	27.20± 10.06*	24.00± 5.48	20.40± 4.77	16.00± 3.16	9.00± 6.22**	5.50± 6.40**	00±00	00±00
7	21.20± 8.38	19.00± 2.58	16.00± 3.65	11.50± 8.06**	7.50± 8.70**	00±00	00±00	00±00
8	21.60 ± 3.85	19.40± 6.35	14.20± 8.96	12.20± 7.29	5.40± 7.47**	2.40± 5.37**	00±00	00±00
9	26.60± 6.23*	23.67± 6.98	21.33± 6.65	18.67± 5.16**	14.00± 7.80**	00±00	00±00	00±00
10	18.80± 8.07	15.75± 3.50	10.75± 7.46	6.50± 7.55**	3.00± 6.00**	00±00	00±00	00±00
11	33.20± 6.26*	32.50± 7.00	29.00± 6.83	25.00± 5.29	18.00± 5.16	7.67± 8.62	00±00	00±00
12	24.00 ±3.85	22.60± 4.28	19.40± 4.45	17.40± 4.45	13.20± 7.95**	9.40± 8.65**	00±00	00±00

* P<0.01, ** P<0.05

Nutrient broth, Sabouraud dextrose broth was used. All microtitration plates for bacterial growth were incubated at 37°C for 24 hours and for fungal growth at 28°C for 48 hours. The last well in each dilution series without turbidity / growth was considered as the MIC. The test was performed in triplicate.

STATISTICAL ANALYSIS

The data of all samples (including undiluted and diluted), was statistically analyzed by two –way ANOVA (dentifrice brands and dilutions were taken as independent variables and zone of inhibition as dependent variable), with post-hoc least square differences (LSD) method using the SPSS version 16.0 for windows (Adejumo *et al*. 2008).

RESULTS

Among the total microbial isolates, 2 bacterial genra viz, *Staphylococcus* (1 spp.) & *Streptococcus* (3 spp.) and 1 fungal genus were identified (table 2). Antagonistic activities in terms of zone of inhibition (fig. 1) and comparative analysis of Mean ±SD of various two fold dilutions of different toothpaste formulations against the selected microbial spp. are exhibited in tables 3-7. Maximum and minimum inhibitory zones were observed as 35.10±8.00 and 2.40±5.37 respectively. For undiluted samples of toothpastes, Analysis of variance (table 8) showed highly significant values (P<0.01). e.g., 1:1 dilution of formulation 1 found significantly different from same dilution of formulations 3, 6, 9 & 11 for the same microorganism (table 3); formulation 11 was significantly different from same dilution (1:1) of rest of the formulations (table 4); formulation 5 exhibited significantly different from same dilution (1:1) of rest of the formulations (table 5); formulation 4 and 11 were significantly different from same dilution (1:1) of rest of the formulations (table 6); formulation 7 and 10 revealed significantly different from same dilution (1:1) of rest of the formulations (table 7). Comparison of Means and Standard deviation was performed between diluted samples of similar brands. The higher dilutions of most of the samples showed significant ($P<0.05$) results as compared to lower dilutions (tables 3-7).

The overall results revealed that toothpaste formulation 11 had maximum zones of inhibition against the test organism, *Strept. intermedius* ($p<0.01$, table 6) compared to all other toothpaste brands. In rest of the strains, antimicrobial activity was found less in comparison to *Strept. intermedius*. Highest observed MIC against each isolate is as follows: *Staph. aureus* 1:32 (formulations 3, 6, 8, 11 & 12), *Strept. salivarius* 1:64 (formulation 2), *Strept. mutans* 1:64 (formulations 2, 7 & 11), *Strept. intermedius* 1:128 (formulation 6) and *C. albicans* 1:16 (formulations 2, 4 & 6) (table 9).

DISCUSSION

The oral micro-flora has high significance for causing bad breath and oral diseases. Therefore, it is stressed that the population of these microbial species should be kept at a healthy level by using antimicrobial agents in toothpastes (Ciancio, 2007). Hence, these substances are incorporated in order to inhibit microbes, prevention of their accumulation and release of toxins. Various studies have revealed the inhibitory effects of antimicrobial agents on oral bacteria (Fine *et al*., 2006; Victorino *et al*., 2009).

The current work is in support of this affirmation as each selected oral product against all the analyzed microbes, presented wide differences in their efficacy; a characteristic that may have been mainly due to their antimicrobial active constituents. Significant differences between the inhibitory activities of the investigated dental care products against oral pathogens were observed (Adejumo *et al*., 2008). On the basis of the mean diameter of the inhibitory zone appeared by the toothpastes in agar well diffusion assay, against all the five investigated pathogens, formulation of brand 11 (containing triclosan as active ingredient) appeared as the most effective amongst all the examined toothpastes. The remarkable ability of this formulation to maintain its antimicrobial activity against all the tested microorganisms is noteworthy (maximum activity especially for two spp. i.e., *Staph. aureus* and *Strept. mutans,* even at higher dilution of 1:64). Related findings were observed by Okpalugo *et al*. (2009), who reported that the triclosan-containing toothpaste formulations exhibited increased inhibitory activity against oral micro-flora as compared to non-triclosan toothpaste brands.

Similarly, different dentifrices exhibited varying results according to their constituents on agar well diffusion assay. For bacterial species, brand 11 showed maximum zone of inhibition against *Strept. intermedius* (35.10± 8.00), *Strept. salivarius* (34.50±2.52), *Staph. aureus* (33.20±6.26), *Strept. mutans* (30.67±3.66) whereas, formulations 3, 6, 9, and 12 also exhibited almost similar inhibitory activities. These results are in agreement with the work done by Ciancio (2007) and the studies reviewed by Iqbal *et al*. (2011), as the similar antimicrobial agents (triclosan and Sodium monoflourophosphate) were present in their products. Similarly, an extensive clinical study over six months by Davies *et al*. (2004) found that formulations including triclosan and copolymer significantly meliorate the periodontal health and oral plaque. Another substance in these products was fluoride and its substitute sodium monofluorophosphate as active ingredients showed larger zones. Sodium mono fluorophosphates acts as source for fluorides through hydrolysis process (Schrödter *et al*., 2008). Fluoride compounds are used in vast majority of toothpastes, as they help to prevent tooth decay. When formulated and used in accordance with other substances in toothpaste, it will help safe and effective prevention of dental caries (Marinho, 2009).

Lower antibacterial activity was noted for brands 1, 5 and 7 (for most of the tested microbes) containing potassium nitrate and chlorhexidine gluconate as active ingredients. These toothpastes showed zones of inhibition against *Strept. mutans* (22.00±4.00, 18.67±7.57, 26.33±3.06), *Strept. intermedius* (23.33±8.08, 24.35±5.29, 20.00±8.72), *Strept. salivarius* (21.33±9.15, 21.50±6.40, 22.50±7.72), *Staph. aureus* (17.80 ±11.88, 23.00±10.00, 21.20± 8.38), respectively. These results are supported by Iqbal *et al*. (2011). According to the studies they reviewed toothpastes having potassium nitrate did not show long term antibacterial activity. Potassium nitrate was added in toothpastes for its desensitizing effects (Shih *et al*., 2009). A drawback of chlorhexidine formulations was described by Sheen *et al*. (2001), as found highly effective in mouth rinse formulations due to its prolonged broader activity and plaque inhibitory potential. But its toothpaste formulation did not show good antimicrobial activity due to the additional substances used as shiners. This might be the reason for lower efficacy of these toothpastes.

Herbal toothpastes including brands 2, 3, and 10 showed effective results against *Strept. mutans* (30.33±4.16, 28.60±3.06, 26.33±4.16), *Strept. intermedius* (24.33± 5.13, 26.00 ±5.57, 23.67± 4.73), *Strept. salivarius* (25.25 ±1.893, 24.75±3.10, 21.00±2.58), *Staph. aureus* (21.80± 10.16, 28.80±6.72, 18.80±8.07), respectively. These outcomes are in agreement with the work done by Almas and Al-Zeid (2004) on antimicrobial activity of herbal extracts of miswak (50%), miswak chewing stick and found that *Strept. mutans* showed highest and most significant results as compared to other microorganisms tested.

Highest antifungal activity was shown by herbal toothpastes and fluoride based dentifrice brands 12(24.33 ±10.02), 11(23.00±9.64), 9(22.67±9.45), 4(20.33± 10.97), 3(20.27±9.29) and 2(20.00±10.58).

Table 4: Comparison of Mean ± SD of different dilutions of various formulations against *Strept. salivarius*

Dentifrice Formulation code	1:1 Mean ± SD	1:2 Mean ± SD	1:4 Mean ± SD	1:8 Mean ± SD	1:16 Mean ± SD	1:32 Mean ± SD	1:64 Mean ± SD	1:128 Mean ± SD
1	21.33± 9.15	20.6± 11.12	14.00± 9.66	12.50± 8.70**	11.00± 7.75**	00±00	00±00	00±00
2	25.25± 1.893	23.00± 3.74	19.00± 5.76	17.33± 5.16**	16.17± 5.00**	15.00± 3.74**	12.67± 3.50**	00±00
3	24.75 ± 3.10	23.50± 5.97	20.00± 4.32	17.50± 3.42**	10.00± 7.12**	00±00	00±00	00±00
4	24.50± 3.42	23.21± 5.97	21.00± 5.29	17.50± 3.42**	10.50± 7.19**	00±00	00±00	00±00
5	21.50 ± 6.40	19.33± 3.06	16.00± 2.00	8.67± 7.57**	00±00	00±00	00±00	00±00
6	26.00± 2.83	24.00± 5.16	21.50± 4.43	17.50± 4.43	14.00± 3.65**	00±00	00±00	00±00
7	22.50± 7.72	20.00± 4.00	17.00± 3.6	14.00± 2.00**	00±00	00±00	00±00	00±00
8	24.20± 4.76	22.50± 3.42	20.50± 3.42	18.50± 3.42	15.00± 2.58**	00±00	00±00	00±00
9	25.50± 3.42	23.00± 6.22	20.50± 5.51	16.50± 3.42**	10.50± 7.19**	00±00	00±00	00±00
10	21.00± 2.58	19.20± 4.82	16.40± 3.85	11.60± 7.02**	8.20± 7.69**	5.20± 7.16**	00±00	00±00
11	34.50± 2.52*	30.50± 6.19	27.00± 6.83	23.50± 5.97	17.00± 4.16**	00±00	00±00	00±00
12	25.10± 2.83	22.00± 3.16	19.20± 4.15	17.20± 4.15	12.40± 7.54**	6.40± 8.88**	00±00	00±00

Table 5: Comparison of Mean ± SD of different dilutions of various formulations against *Strept. mutans*

Dentifrice Formulation code	1:1 Mean ± SD	1:2 Mean ± SD	1:4 Mean ± SD	1:8 Mean ± SD	1:16 Mean ± SD	1:32 Mean ± SD	1:64 Mean ± SD	1:128 Mean ± SD
1	22.00± 4.00	19.00± 6.22	16.50± 6.19	15.00± 5.29	11.50± 8.39**	10.00± 7.48**	00±00	00±00
2	30.33± 4.16	28.67± 4.32	22.67± 5.89	21.67± 5.72	20.00± 6.81	18.67± 5.61	15.33± 4.32**	00±00
3	28.60± 3.06	25.00± 2.58	20.50± 4.43**	19.00± 4.16**	18.00± 3.65**	00±00	00±00	00±00
4	24.67± 3.40	22.75± 3.77	20.50± 3.42	18.50± 3.42	15.00± 2.58**	00±00	00±00	00±00
5	18.67 ±7.57*	16.00± 2.0	14.00± 2.00	12.00± 2.00	00±00	00±00	00±00	00±00
6	28.70± 3.16	26.00± 2.00	24.00± 2.00	22.00± 2.00	18.67± 3.06**	15.33± 3.06**	00±00	00±00
7	26.33± 3.06	25.50± 3.42	23.00± 2.58	21.00± 2.58	18.00± 3.65	16.00± 3.65	11.50± 8.06**	00±00
8	25.80± 3.26	22.75± 2.99	20.50± 3.42	18.50± 3.42	15.00± 3.37**	00±00	00±00	00±00
9	29.67± 4.10	27.33± 3.06	24.67± 4.16	21.33± 3.06	17.33± 3.06**	15.33± 3.06**	00±00	00±00
10	26.33± 4.16	23.60± 4.16	20.40± 3.85	18.40± 3.85	16.60± 4.22	11.60± 6.73**	00±00	00±00
11	30.67± 3.66	27.00± 2.58	23.50± 3.42	21.50± 3.42	19.50± 3.42	17.50± 4.43	12.50± 8.70**	00±00
12	28.33± 6.11	25.00± 2.58	21.50± 3.42	18.00± 5.16	13.00± 9.59**	00±00	00±00	00±00

* P<0.01, ** P<0.05

Table 6: Comparison of Mean ± SD of different dilutions of various formulations against *Strept. intermedius*

Dentifrice Formulation code	1:1 Mean ± SD	1:2 Mean ± SD	1:4 Mean ± SD	1:8 Mean ± SD	1:16 Mean ± SD	1:32 Mean ± SD	1:64 Mean ± SD	1:128 Mean ± SD
1	23.33± 8.08	21.50± 8.54	19.00± 6.83	17.00± 6.22	13.00± 9.02**	11.00± 7.57**	00±00	00±00
2	24.33± 5.13	22.00± 3.65	21.00± 2.58	17.00± 2.58	15.00± 2.58	9.00± 6.22**	00±00	00±00
3	26.00± 5.57	24.00± 7.48	20.00± 7.48	18.33± 6.38	16.33± 4.63	11.67± 6.38**	6.00± 6.69	00±00
4	19.00± 10.58*	17.50± 3.42	15.00± 2.58	13.00± 2.58	9.00± 6.22**	00±00	00±00	00±00
5	24.35± 5.29	22.50± 3.42	20.50± 3.42	18.50± 3.42	15.00± 2.58**	00±00	00±00	00±00
6	27.00± 6.00	25.00± 2.58	23.00± 2.58	21.10± 2.50	21.00± 2.58	19.00± 2.58	12.00± 8.16**	10.50± 7.19**
7	20.00± 8.72	18.00± 4.00	15.33± 3.06	8.67± 7.57**	00±00	00±00	00±00	00±00
8	21.67± 9.29	19.33± 3.06	16.00± 4.00	10.00± 8.72**	00±00	00±00	00±00	00±00
9	25.33± 4.16	22.67± 2.52	20.67± 2.52	18.00± 2.00	14.33± 2.52	8.67± 7.57**	00±00	00±00
10	23.67± 4.73	21.50± 4.43	18.50± 4.43	16.00± 3.65	13.25± 2.22**	00±00	00±00	00±00
11	35.10± 8.00*	33.33± 3.06	30.00± 4.00	26.67± 3.06	24.67 ± 3.06	20.00± 4.00	16.67± 3.06	00±00
12	26.67± 5.5	24.00± 4.00	20.67± 3.06	18.00± 2.00	10.00± 8.72**	00±00	00±00	00±00

Table 7: Comparison of Mean ± SD of different dilutions of various formulations against *C. albicans*

Dentifrice Formulation code	1:1 Mean ± SD	1:2 Mean ± SD	1:4 Mean ± SD	1:8 Mean ± SD	1:16 Mean ± SD	1:32 Mean ± SD	1:64 Mean ± SD	1:128 Mean ± SD
1	18.33± 6.43	16.00± 2.00	12.67± 3.06**	00±00	00±00	00±00	00±00	00±00
2	20.00± 10.58	18.25± 4.03	16.50± 3.42	13.00± 2.58	9.00± 6.22**	00±00	00±00	00±00
3	20.27± 9.29	18.67± 3.06	15.33± 3.06	13.33± 3.06**	00±00	00±00	00±00	00±00
4	20.33± 10.97	18.50± 5.97	13.00± 9.02	10.50± 7.19**	3.00± 6.00**	00±00	00±00	00±00
5	18.67± 5.86	17.00± 1.41	13.50± 2.12**	00±00	00±00	00±00	00±00	00±00
6	19.60± 10.41	17.33± 3.06	15.33± 3.06	8.67± 7.57**	5.82± 9.53**	00±00	00±00	00±00
7	15.67± 4.93*	13.50± 2.12	6.00± 8.49**	00±00	00±00	00±00	00±00	00±00
8	19.67± 8.50	18.00± 4.00	15.33± 3.06**	8.67± 7.57**	00±00	00±00	00±00	00±00
9	22.67± 9.45	19.67± 3.51	16.67± 4.16	9.33± 8.33**	00±00	00±00	00±00	00±00
10	16.67± 6.81*	14.00± 2.00	8.67± 7.57**	4.00± 6.93**	00±00	00±00	00±00	00±00
11	23.00± 9.64	20.00± 4.00	16.00± 4.00	10.00± 8.72**	00±00	00±00	00±00	00±00
12	24.33± 10.02	21.33± 3.06	18.67± 4.16	15.33± 3.06**	00±00	00±00	00±00	00±00

* $P<0.01$, ** $P<0.05$

Table 8: Analysis of variance for selected oral pathogens

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Staphylococcus aureus				
Toothpaste	11	1159.0	105.36	4.61*
Dilution	7	15919.8	2274.26	99.43*
Error	77	1761.2	22.87	
Total	95	18840.0		
Streptococcus salivarius				
Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Toothpaste	11	920.8	83.71	4.44*
Dilution	7	13135.3	1876.48	99.53*
Error	77	1451.7	18.85	
Total	95	15507.8		
Streptococcus mutans				
Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Toothpaste	11	1692.8	153.89	8.13*
Dilution	7	10095.1	1442.15	76.15*
Error	77	1458.3	18.94	
Total	95	13246.2		
Streptococcus intermedius				
Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Toothpaste	11	2123.8	193.07	12.67*
Dilution	7	10510.8	1501.55	98.54*
Error	77	1173.3	15.24	
Total	95	13807.9		
Candida albicans				
Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Toothpaste	11	480.83	43.71	4.29*
Dilution	7	8679.50	1239.93	121.62*
Error	77	785.00	10.19	
Total	95	9945.33		
* = Highly significant (P<0.01)				

Table 9: Minimum inhibitory concentration of various dentifrice formulations

Dentifrice Formulation code	MIC				
	Staph. aureus	*Strept. salivarius*	*Strept. mutans*	*Strept. intermedius*	*C. albicans*
1	1:8	1:16	1:8	1:4	1:8
2	1:8	1:64	1:64	1:32	1:16
3	1:32	1:8	1:2	1:32	1:2
4	1:16	1:8	1:4	1:32	1:16
5	1:16	1:16	1:4	1:4	1:8
6	1:32	1:8	1:8	1:128	1:16
7	1:16	1:32	1:64	1:16	1:4
8	1:32	1:8	1:32	1:16	1:2
9	1:16	1:2	1:4	1:4	1:8
10	1:8	1:2	1:8	1:32	1:8
11	1:32	1:16	1:64	1:8	1:8
12	1:32	1:4	1:4	1:4	1:2

These findings were in accordance with the studies by Adwan *et al*. (2012). According to their results, the herbal toothpastes containing various combinations of herbal extracts showed better results. Whereas, Ellepola *et al*. (2011) suggested that the antifungal activity of miswak extract was inconsistent and depended upon other active ingredients present in the toothpaste.

For the determination of effective concentration (of the selected brands of various tooth pastes) Minimum Inhibitory Concentration (MIC) was performed. Highest recorded MIC against *Strept. intermedius, Strept. salivarius*, *Strept. mutans*, *Staph. aureus* and *C. albicans* were 1:128, 1:64, 1:64, 1:32, 1:16, respectively. These results revealed similarity with the study of Imran *et al*,

(2013) as both studies exhibited variable results among MIC of toothpastes for various oral pathogens.

The data was analyzed statistically using the two-way Analysis of Variance (ANOVA) and comparison of Mean ±SD. All the toothpastes showed highly significant antimicrobial activity against each other and the results vary from specie to specie. The undiluted (1:1) samples of various formulations were found highly significantly different (P<0.01) from other formulations for the same microorganism analyzed. This variation is also supported by Victorino *et al*. (2009) and Prasanth (2011), who described similar features that might be largely due to different antimicrobial active ingredients of various dental care products. The comparison of Mean ±SD showed that there was difference among the means of zones of inhibition for each toothpaste sample against individual microbial spp. whereas, significant values were found (P<0.05; among diluted samples of each dentifrice). Each toothpaste formulation exhibited some level of inhibition against each organism, but inhibition is dependent on product and spp. tested, resulted in highly significant P-value (between undiluted samples). These results are in agreement with Adejumo *et al*. (2008) and Kumari *et al*. (2011). They reported that *in vitro* growth inhibition was dependent on both the sample used and the concentrations tested. There were significant differences (P<0.05) between the exhibited *in vitro* antimicrobial activities of the investigated mouth care products.

CONCLUSION

From the foregoing discussion, it is concluded that all the examined brands of dentifrices showed variable results with respect to antimicrobial activity for each microbial spp. Toothpastes containing triclosan and fluoride exhibited more inhibition of bacterial pathogens as compared to those having chlorhexidine. Whereas, for fungal spp., the outcomes revealed that antagonistic activity of herbal toothpastes was comparable to conventional dentifrices. In order to assess the exact efficacy of toothpaste formulations in human oral cavity, an *in vivo* analysis is recommended. Since, in the oral environment due to the addition of tap water and saliva, the antagonistic activity of these antimicrobial agents may alter.

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