AN IN VITRO ANTIMICROBIAL ACTIVITY OF SIX COMMERCIAL TOOTHPASTES

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ABSTRACTS

The antimicrobial activity of six different toothpastes Colgate Total ,Signal , Crust, Bio fresh, Close up & Miswak were evaluated on some pathogenic oral microorganisms : *Streptococcus* sp. (α- hemolytic), *Staphylococcus aureus* & *Candida albicans*. The preliminary antimicrobial activity evaluation was performed using an agar well diffusion method and distilled water was used as controls. The samples were tested in 0.5, 0.25, 0.125, 0.06, 0.03, 0.01,0.005,0.0025 g/ml. Inhibition zones were measured in millimeter after 24 hr. All tested toothpastes have antimicrobial activity on bacterial & fungal type but antimicrobial activity on bacteria more than activity on *Candida albicans* . Antimicrobial activity of all toothpastes on bacterial types were approach one another . In conclusion the antimicrobial activity of Colgate Total ,Signal & Crust were better than Bio fresh, Close up & Miswak .

INTRODUCTION

Tooth brushing with toothpaste is the most widely practiced form of oral hygiene in most countries (Pannuti *et al*., 2003) . The success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral microflora. Fluoride dentifrices have been widely used all over the world and extensive research has established their abilities in terms of caries resistance (Itthagarum & Wei, 1996). A wide range of chemicals, mainly antimicrobial agents, have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation (Fine *et al* ., 2006;Pannuti *et al*., 2003) ,Clearly, most individuals find it difficult to maintain an effective level of plaque control and this is reflected in the levels of periodontal disease in the population. The addition of antimicrobial agents to toothpaste has been suggested as one possible method to improving the efficacy of mechanical tooth-cleaning procedures (Fine *et al* ., 2006;Moran *et al*,1988) , aiding the control of dental plaque and preventing dental caries and periodontal diseases (Ozaki *et al* .,2006; White *et al* .,2006). When these substances are added to oral products, they kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. They prevent bacterial aggregation, slow multiplication and release endotoxins (Bou-Chacra *et al*.,2005;Ozaki *et al* .,2006).

The aim of this study were isolation and identification some microbial types from infected patients with dental diseases and showed antimicrobial activity of six toothpastes against that microbial types .

MATERIAL & METHODS

To demonstrate antimicrobial activity of commercial toothpastes, locally available toothpastes: Colgate Total ,Crust, Miswak ,Signal ,Close up & Bio fresh, the tested toothpastes and their ingredients in Table (3),Collected samples were transferred in nutrient broth and immediately transported to Microbiology Laboratory /medicine

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college where the study was carried out, the samples were enriched in nutrient broth at 37ºC for 4 hours and streaked on nutrient agar plate. Corresponding pure culture was obtained by streak plate method (Cheesbrough,1984; Collee *et al* ., 1996) .The organisms were identified by standard microbiological techniques including colonial characteristics, morphological characteristics and biochemical characteristics (Holt,2004) ,with using many culture media (Nutrient broth, Nutrient agar ,Blood agar, MacConkey agar, Bile esculin agar ,6.5 NaCl broth, 5% Sucrose broth and 5% sucrose agar and using Sabroud dextrose agar for *Candida albicans* .

The Minimal Inhibitory Concentration of Toothpastes

All toothpastes samples were diluted in sterile water and prepare series dilution 0.5, 2.5, 1.25, 0.06, 0.03, 0.01, 0.005, 0.0025 g/ml ,The different concentration of toothpastes were known as 1,2,3,4,5,6,7,8 and water was employed as the negative control . Test organisms were : *Streptococcus* sp. (α- hemolytic),, *Staphylococcus aureus* and *Candida albicans* . Plates were incubated at 37°C for bacteria strains and 28 ˚C for fungi .

RESULTS

Depending the morphological characterizes and biochemical test were identify these types of bacteria to: *Streptococcus* (α-hemolytic), *Staphylococcus aureus* and Fungi : *Candida albicans* .The microbial inhibition zones of toothpastes are shown in Table 1&2 . The results indicated that all tested toothpastes demonstrated a significant antimicrobial activity against the tested microorganisms, and the negative control showed no activity.

Antimicrobial activity of all toothpastes on *Streptococcus* sp. (α- hemolytic), and *Staphylococcus aureus* were approach one another . Miswak, Close up & Bio fresh have lower activity than Colgate total, Signal & Crest ($P < 0.05$) .

The highest antimicrobial activity on *Candida albicans* show in Colgate total . While, the activity of Crest and Signal were the same, it showed a weaker activity compared to Miswak, Close up& Bio fresh . All toothpastes have activity on bacterial type more than activity on *Candida albicans* .

Statistical analysis showed that the zones-of-inhibition of toothpastes against the test organism were not differed significantly on repeated attempts ($P<0.05$).

DISCUSSION

Ideating of this research was investigated to choice the best toothpaste , were using every day to reduce proliferation microorganisms in mouth as well as bad smell of mouth because of presenting microorganism in mouth and were found food between teeth .

If that balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes (Lee *et al* ., 2004). The use of a toothpaste as an adjunct to tooth brushing may assist oral hygiene practices in a number of ways. It may prevent plaque formation by interfering with bacterial adherence to the tooth surface and reducing salivary bacterial numbers (Jenkins *et al*., 1990; Fine *et al*.,2006 ; Herrera *et al* ., 2003) .

The diffusion method can be used as a preliminary test for detecting antimicrobial activity in substances or products. Since the diffusion phenomenon depends on each substance’s physical-chemical properties, as for example its diffusion coefficient, as well as the medium where the diffusion occurs (Barry, Thornsberry, 1991).

It is possible to obtain a qualitative indication of antimicrobial activity. The antimicrobial agents in tested toothpastes

include triclosan, bromochlorophene, sodium lauryl sulfate (SLS), sodium mono-fluorophosphate (MPF), and sodium fluoride (SF). Although SLS existing in these toothpastes is a detergent, it is also known to have antibacterial and plaque inhibitory activities (Moran *et al*., 1988) .

The viridian streptococci- *S. mutans, S. sanguis, S. sobrinus* and *S. mitis* are the major pathogens while *S. salivarius* is an initiator of the dental infection. These oral streptococci poses the significant health risks if they enter into bloodstream via. Wounds, oral infection, dental procedures and can cause endocarditis. Followed by primary invaders, oral cavities are vulnerable for secondary invaders like *Candida albicans* and species of *Actinomyces*, *Bacteroids*, *Spirochetes* and *Lactobacillus* etc. inviting severe conditions (Cheesbrough,1984; Collee *et al* ., 1996) .

Therefore, the toothpastes that having the largest microbial inhibition zone and thus, probably the strongest antimicrobial properties may not be necessarily superior to those found to have smaller diameter inhibition zones (Lee *et al*.,2004). A systematic review indicated that a toothpaste containing triclosan/copolymer provides a more effective level on plaque control and periodontal health than conventional fluoride toothpaste (Davies *et al*.,2004) .

The fluoride tooth paste reduces the number of streptococcal colony forming units of dental plaque (Jabbarifar *et al*., 2005) despite the fact that fluoride was added to the toothpastes first with aiming to preserve the product and then to protect the teeth (Bou-Chacra *et al*.,2005) .

Most commonly used and recommend by the WHO, ADA, FDI is the fluoride and triclosan. But the excess use of the fluoride can cause the dental fluorisis so the recommended amount of the fluoride should be used as the ingredients in the toothpaste. And the regular evaluation of the efficacy of the fluoridated toothpaste by the private laboratory have been recommended by the WHO (Peterson , 2003).

In conclusion in this study , Colgate Total ,Signal & Crust were better than other toothpastes and I suggest to use toothpaste manufacturers take into consideration to improving the antimicrobial properties of their products.

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Table 1. The diameter of inhibition zones (mm) in six toothpastes against *C. albicans*

TOOTHPASTES	1	2	3	4	5	٦	7	8
Colgate Total	33	31	25	23	20	17.3	-	-
Crest	35	26	22	21.6	21	13.6	-	-
Miswak	36.6	26	19	9	-	-	-	-
Bio fresh	12	9	-	-	-	-	-	-
Signal	28	26	25	23.6	23	12	-	-
Close up	25,3	24.6	19.6	13.6	-	-	-	-
Control	-	-	-	-	-	-	-	-

L.S.D = 0.0422

Table 2. The diameter of inhibition zones (mm) in six toothpastes against *Staphylococcus aureus* & *Streptococcus* sp. (α- hemolytic)

TOOTHPASTES		*STREPTOCOCCUS* (A-HEMOLYTIC)								*STAPHYLOCOCCUS AUREUS*							
		Concentration								Concentration							
		8	7	6	5	4	3	2	1	8	7	6	5	4	3	2	1
Colgate Total	33	31	30.3	30	30	19	15	14.6		38.3	33.6	33.3	30.6	25.3	20	15.6	13.3
Crest	31	30.3	29.3	29	26	21	16	13		30.6	30	28	26.3	23	21	17	15
Miswak	26	26	22.3	16.3	15.3	14	12	0		26.6	26	16.6	16	13.6	11	9.6	0
Bio fresh	15	15	12	11.3	10	0	0	0		15	14	11.6	9.6	8	0	0	0
Signal	36	35.6	31.3	27.3	23	20	18	12		35	33	31.6	27	26.3	25	19	12
Close up	25	23.3	23.3	22	12.6	10.6	0	0		20.6	20.3	20	20	12	9	0	0
Control	0	0	0	0	0	0	0	0		0	0	0	0	0	0		0
L.S.D		0.0088								0.0323							

Table 3 The tested toothpastes and their ingredients

TOOTHPASTES	MANUFACTURER	INGREDIENTS AS LISTED ON PACKAGES
Colgate Total	Colgate- palmolive Company	**Water, hydrate silica, glycerin, sorbitol, PVM/MA, copolymer, Sodium lauryl sulfate, , Cellulose Gum, flavor, sodium hydroxide, propylene glycol, carrageenan, sodium saccharin, titanium dioxide**
Crest	Procter & Gamble Germany	**Aqua, Sorbitol, Hydrated silica, PEG-6, Sodium lauryl sulfate, Tetrapotassium Pyrophosphate, Disodium Pyrophosphate , Tetrasodium Pyrophosphate ,Aroma,Cellulose Gum,Xanthan Gum, Sodium Floride, Carbomer, Sodium saccharin, Cellulose gum, Aroma, Carbomer, Triclosan, CI 77891, Glycerin , limonene, CI 7416 , Contains 0.321% sodium fluoride**
Miswak	Naturelle LLC,Al Hamra Al Jazera Ind. Area	**Calcium carbonate, sorbitol, treated water, silica, Sodium lauryl sulfate, sulphate, flavour, Miswak extract, sodium carboxy methyl cellulose, and or sodium carrageenate, sodium silicate, sodium benzoate, glycerine.**
Bio fresh	Camiflor Company	**Potassium Nitrate 5%, sorbitol, Aqua hydrate silica,PEG 8, , Aroma, Sodium lauryl sulfate, Cellulose gum,polyglyceryl-3 hydroxylauryl ether, sodium fluoride, Sodium saccharin, methylparaben, propylparaben.**
Signal	Unilever Mashreq-perrsonal Care (S.A.E) 6th of October city	**calcium carbonate, Aqua, sorbitol, hydrate silica, Sodium lauryl sulfate, sodium monofluorophosphate, aroma, Cellulose Gum, triclosan , potassium citrate, Trisodium phosphate,sodium saccharin, calcium glycerophosphate, phenylcarbinol, formaldehyde, glycerin, CI 74160 .**
Close up	**Unilever Mashreq-perrsonal Care company (S.A.E) 6th of October city**	**Sorbitol, water , hydrate silica, Sodium lauryl sulfate,PEG-32, flavour, cellulose gum, sodium saccharin, eugenol, CI 16255, CI 17200**

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الفعالية المضادة للجراثيم لست أنواع من معاجين الأسنان التجارية

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المستخلص :

قيمت الفعالية المضادة للجراثيم لستة أنواع مختلفة من معاجين الأسنان : Colgate total وSignal وCrust و Miswak وClose up و Bio fresh على بعض أحياء الفم المجهرية الممرضة :(α-hemolytic) *Strptococcus* و*Staphylococcus aureus* و*Candida albicans* . وتم التعرف على فعالية هذه المواد باستخدام طريقة الانتشار بالاكار وباستخدام الماء المقطر كمحلول سيطرة . استخدمت تراكيز مختلفة من معاجين الأسنان ٠,٥ ، ٠,٢٥ ،٠,١٢٥ ، ٠,٠٦ ،٠,٠٣ ، ٠,٠١ ،٠,٠٠٥ ، ٠,٠٠٢٥ غم / مل . وبعد ٢٤ ساعة يتم قياس أقطار التثبيط ، أظهرت النتائج أن فعالية كل معاجين الاسنان كانت اكبر على الانواع البكتيرية من *Candida albicans* . كانت الفعالية المضادة للجراثيم لمعاجين الاسنان متقاربه على الأنواع البكتيرية . أن الفعالية المضادة للجراثيم لـ Colgate total وSignal وCrust أكثر من Miswak وBio fresh وClose up .

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*-قسم الأحياء المجهرية- كلية الطب- جامعة ذي قار