Antimicrobial activity of different types of mouthwashes against *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans* (In vitro study)

Zainab A. Aldhaher, B.Sc., M.Sc. [(1)]

ABSTARCT

Background: Recently increasing number of people are using mouthwashes for general and oral care while the primary appeal of a mouthwash is an aid to breath freshness and cleansing the mouth, the majority of mouthwashes also claim to have antiseptic properties. The aim of this study is to determine the antimicrobial effectiveness of eight types of mouthwashes against *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans* in vitro.

Materials and methods: Agar diffusion technique was used to evaluate the antimicrobial activity of eight types of mouthwashes against *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans* isolated from the oral cavities of patients attending dental clinics at college of dentistry - Baghdad University.

Results: Al-Mansour mouthwash emerged as the most effective mouthwash giving the maximum mean diameter of inhibition zones against *Staphylococcus aureus (26 mm), Candida albicans* (25 mm) and *Streptococcus mutans* (20 mm) followed by corsodyl mouthwash, emoform mouthwash and zac mouthwash all of them had excellent levels of antimicrobial activity also Breath, close up, sensodyne and aquafresh had good antimicrobial activity

Conclusion: All the mouthwashes used revealed antimicrobial activity against the three microorganisms used even when these mouthwashes were diluted but the concentrated mouthwash had the strongest antimicrobial activity. Al-Mansour mouthwash an Iraqi mouthwash was the best one according to the results of this study.

Key words: Mouthwash, antimicrobial activity, *Streptococcus mutans, Staphylococcus aureus, Candida albicans.* (J Bagh Coll Dentistry 2013; 25(2):185-191).

INTRODUCTION

Many bacterial species have been identified from the human mouth. These microorganisms are easily grown and produce dental plaque in the mouth environment, due to the constant influx of nutrients through; saliva, food intake, warm temperatures and moisture. [(1)]

Plaque associated oral disease affects a considerable portion of the population and is considered one of the major causes of tooth loss. In most cases, the chronic accumulation of dental plaque often leads to caries and periodontal disease (in genetically susceptible individuals), that may not only affect the patient's oral health, but may also contribute to a number of chronic systemic diseases [(2)].

Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases [(3)].The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by *Streptococcus mutans,* a cariogenic bacterium. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases [(4)]. *Streptococcus mutans* and *Candida albicans* are the two microbes often implicated in oral diseases, *Candida albicans* is the most common yeast isolated from the oral cavity and a common cause of oral thrush, endocarditis, septicemia, vaginitis and infection of skin, nails and lungs [(5)].

(1)Lecturer. Department of Basic Sciences. College of Dentistry. University of Baghdad.

It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intercanal medication [(6)]. ***Staphylococcus aureus*** is a major human pathogen, responsible for a number of hospital-acquired infections, initially colonizes several locations in the human body, but the mouth and hands are the main reservoirs for propagation of this pathogen in the hospital environment [(7)]. Recent studies demonstrate that ***Staphylococcus aureus*** is isolated from pre-implant lesions and from the site of surgical implants [(8)], hence eradication of these microorganisms is important for dental treatment.

Prevention of oral diseases is easier than a cure. The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in the developing countries of the world [(9)].

The Mechanical removal of plaque through frequent and efficacious brushing and flossing is the principal means of preventing periodontal diseases and diminishing the risk of caries however, some individuals lack the dexterity, skill or motivation for mechanical plaque removal. Mouth-rinsing is easier to perform and may aid in controlling supragingival plaque and gingivitis, but it should always be used in conjunction with mechanical hygiene. [(10)]

Several studies focusing on the efficacy of mouthwashes with diverse chemical composition demonstrated that these mouthwashes are able to

diminish the metabolic activity of microorganisms present in the oral cavity [11].

The aim of this study was to determine the antimicrobial properties of eight commonly available mouthwashes against three oral pathogens related to caries and to oral infections

MATERIALS AND METHODS

Thirty patients attending dental clinics at college of dentistry Baghdad University with various oral infections such as dental caries, periodontal diseases, thrush, oral abscesses and oral lesions associated with artificial denture were included in this study and samples were taken from these patients to obtain *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*

- *Streptococcus mutans* isolation: - stimulated saliva samples were collected under standard condition from patients with dental caries. The collected saliva was homogenized by vortex mixer for two minutes. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected for each microbial type and inoculated on Mitis-Salivarius Bacitracin Agar (MSB Agar), a selective media for *Streptococcus mutans*: 0.1ml was withdrawn from dilutions 10^{-2} and 10^{-3} using adjustable micropipette with disposable tips and then spread in duplicate by using sterile microbiological glass spreader on the plates of MSB agar, The plates were then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hours at 37°C followed by aerobic incubation for 24hours at 37°C[12,13].
- *Staphylococcus aureus* were isolated from oral cavity of patients with oral abscesses, periodontal disease, gingivitis or other related oral infections. Swab samples taken from abscesses and gum of these patients were first inoculated in nutrient broth for 18 hours at 37°C then The broth was cultured on mannitol salt agar which is a selective medium with 7-9% NaCl and phenol red as pH indicator.[14]
- *Candida albicans* were isolated from patients with denture swab has been taken from the palatal mucosa of the patient and the dorsal surface of the tongue then swab had been cultured on sabaraud Dextrose (SD) agar and incubated at 37°C for 72 hours. A selective media for cultivation and isolation of *Candida albicans*.
- Identification: -

a) Colony morphology: - the colony on MSB agar, mannitol salt agar and SD agar were examined directly under dissecting microscope (magnification ×15).

b) Morphology of the Microbial Cells: - a colony was picked up from MSB agar, mannitol salt agar and SD agar plates separately under sterilized conditions and subjected to gram`s stain.

c) Biochemical Tests: - Bacterial colonies of different morphology were picked up from MSB agar, mannitol salt agar separately under sterilized conditions using inoculating loop and then inoculated in 10 ml of sterilized Brain Heart Infusion Agar and incubated aerobically at 37°C for 18 hrs. The following tests were conducted:-
   1. Catalase production test on both type of *Streptococcus mutans* and *Staphylococcus aureus* separately.
   2. Coagulase this test was conducted on *Staphylococcus aureus*

d) Identification system of API (analytical profile index) strep: - API 20 strep was a standardized system used in the identification of *S. mutans.*API Staphwas used in the identification of *Staphylococcus aureus*, API – YEAST-IDENT candida system was used for the identification of *Candida albicans* the tests done in these API need inoculation with a dense suspension of organisms from a pure culture

v Mouthwashes collection

Eight mouthwashes products (table 1) from Baghdad (Iraq) pharmacies were used in this study

Table 1: Types, compositions and manufacturer of the mouthwashes used in this study

Name	Composition	Manufacture
Breath RX	Sorbitol, propylene glycol, xylitol, zinc glyconate, aroma (mint, thymol, eucalyptus oil) , sodium saccharin, cetylpyridinum chloride	United State of America
Sensodyne pro enamel	Sodium fluoride (450 ppmf)	Germany
Close Up	Sodium benzoate, xylitol , glycerine, flavor, sodium saccharin, cetylpyridinum chloride, zinc glyconate, citric acid, calcium lactate	United Kingdom
Corsodyl	Chlorhexidine gluconate 0.2%	United Kingdom
Aquafresh	Sodium fluoride 0.5%, cetylpyridinum chloride 0.05%	United Kingdom
Emoform-F	Water-propyleneglyclo, Glycerin, Potassium Nitrate 5%, SLS-Sodium Chloride 4%, Levomentholum, Menthoe pip, Gneolum, Anetholum, Sodium fluoride (1400 ppm F), potassium Sulfate 0.25%, Disodium phosphate, Sodium Sulfate, sodium saccharin	Egypt
Zac	Chlorhexidine gluconate 0.12%, Sodium fluoride 0.05%	Syria
Al-Mansour	Chlorhexidine gluconate 0.2%	Iraq

Screening for antimicrobial activity

Antimicrobial activity or effectiveness of the eight mouthwashes was assessed by using Agar diffusion technique.

The mouthwashes were tested at 6 concentrations 1:1, 1:2, 1:4, 1:8, 1:10 and full strength (100%) taking sterile distilled water as the diluent.

Staphylococcus aureus, *Streptococcus mutans* cultures of 18 hours at 37°C in Brain heart broth and *Candida albicans* culture of 18 hours at 37°C in sabaraud broth were used. The cultures were adjusted to approximately 10^5 CFU/ml with sterile saline solution then each one of the three inoculum was inoculated on Brain heart in fusion agar separately and plugs were removed from each agar plate producing holes. To each hole 100 µl of each concentration of mouthwash was added directly on the inoculated media agar plates for each test organisms. The plates were allowed to stand for 10 minutes for diffusion of the mouthwash to take place and incubated at 37°Cfor 24 hours. The antimicrobial activity is indicated by an inhibition zone surrounding the holes containing the mouthwash. The experiments were performed in triplicates (8 isolates were used for each test microorganisms) and the mean values of inhibition zones were calculated.

RESULTS

1. Identification of *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans* was carried out by:-

a) Colony morphology.
   (1) On the selective MSB agar plates, *Streptococcus mutans* colonies appeared light blue in color about 1-2mm in diameter as spherical or ovoid in shape with raised or convex surface.
   (2) On mannitol salt agar plates *Staphylococcus aureus* colonies appeared small and surrounded by a yellow zone.
   (3) Colonies of *Candida albicans* appeared smooth, creamy in color with a yeast odor and typically medium sized 1.5-2mm diameter which later develop into high convex, off-white larger colonies after 2 days.

b) Morphological test of bacterial cells.
   (1) *Streptococcus mutans* cells were gram positive, spherical or ovoid in shape, arranged in short or medium length non spore forming chains.
   (2) *Staphylococcus aureus* cells were Gram positive cocci arranged in clusters.
   (3) *Candida albicans* under light microscope are rounded or oval yeast cells which stained Gram positive.

c) Biochemical test: - All colonies of *Streptococcus mutans* were catalase negative
   All colonies of *Staphylococcus aureus* were catalase positive, coagulase positive.

d) Identification system of API strep: - The reaction read according to the reading table and the identification was obtained by referring to the analytical profile index.

The antimicrobial activity of the mouthwashes were evaluated against *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans* and the results were recorded as the mean diameter of inhibition zones and this was summarized in the following tables:-

As shown in table (2) all the mouthwashes used inhibit the growth of *Streptococcus mutans* at all the concentration used but the concentrated mouthwash (full strength without dilution 100%) give the highest inhibition zones and the diameter of inhibition zones decreased slightly in all the

mouthwashes were moving from 100% (full strength) to 1:10. Statistical analysis using t-test demonstrated that there are significant differences when the effect of each one of the concentration used was compared separately to the effect of the full strength 100% and this was for all the mouthwashes.

Table 2: Effect of various mouthwashes on the growth of *Streptococcus mutans*

Mouthwashes	Mean diameter of inhibition zone (mm) of different concentrations of mouthwashes					
	100%	1:1	1:2	1:4	1:8	1:10
Breath RX	15	13*	12*	11*	10**	9**
Sensodyne proenamel	17	15*	15*	13*	10**	8**
Close Up	10	8.5*	8*	7*	0**	0**
Corsodyl	18	15*	14*	12**	9**	9**
Aquafresh	11	9*	9*	8*	0**	0**
Emoform-F	17	15*	15*	12.5**	10**	9**
Zac	16	14*	12.5*	10**	8**	7**
Al-Mansour	20	17*	16*	14.5**	12**	10**

***: significant at P<0.05, ** : highly significant at P<0.01**

Table 3 revealed that all the mouthwashes used have antimicrobial activities against *Staphylococcus aureus* at all the concentrations used but the concentrated mouthwashes (without any dilution 100%) give the highest inhibition zone.

Statistical analysis using t-test demonstrated that there are significant differences when every concentration (dilution) is compared separately with the concentrated mouthwash (100%)

Table 3: Effect of various mouthwashes on the growth of *Staphylococcus aureus*

Mouthwashes	Mean diameter of inhibition zone (mm) of different concentrations of mouthwashes					
	100%	1:1	1:2	1:4	1:8	1:10
Breath RX	16	14*	13*	13*	12*	10.5**
Sensodyne proenamel	13	11*	10*	9*	8**	8**
Close Up	15	13*	12*	12*	11*	10**
Corsodyl	26	23*	22*	20**	18**	16**
Aquafresh	12	9.5*	9.5*	8*	0**	0**
Emoform-F	24	21*	20*	15**	12**	11**
Zac	23	21*	20*	17**	15**	14**
Al-Mansour	26	24*	22*	20**	17**	16**

***: significant at P<0.05, ** : highly significant at P<0.01**

As shown in table 4, all the mouthwash used have anti-fungal activities against *Candida albicans* in all the concentration (dilution) used and the diameter of inhibition zones decreased when these mouthwashes were diluted with distilled water. Statistical analysis using t-test demonstrated that significant differences are shown when every one of the concentration (dilution) used is compared separately with 100% full strength.

Table 4: Effect of various mouthwashes on the growth of *Candida albicans*

mouthwashes	Mean diameter of inhibition zone (mm) of different concentrations of mouthwashes					
	100%	1:1	1:2	1:4	1:8	1:10
Breath RX	13.5	11.5*	10*	9*	8.5**	8**
Sensodyne proenamel	12.5	11*	11*	10*	9.5*	8*
Close Up	15	11.5*	11.5*	9.5**	8**	7**
Corsodyl	20	17*	15**	12**	10**	9**
Aquafresh	12	10*	10*	9.5*	9*	8*
Emoform-F	19	16.5*	15*	14**	10**	8**
Zac	16	13*	13*	11**	9**	8**
Al-Mansour	25	22*	20.5**	18**	15**	13**

***: significant at P<0.05, ** : highly significant at P<0.01**

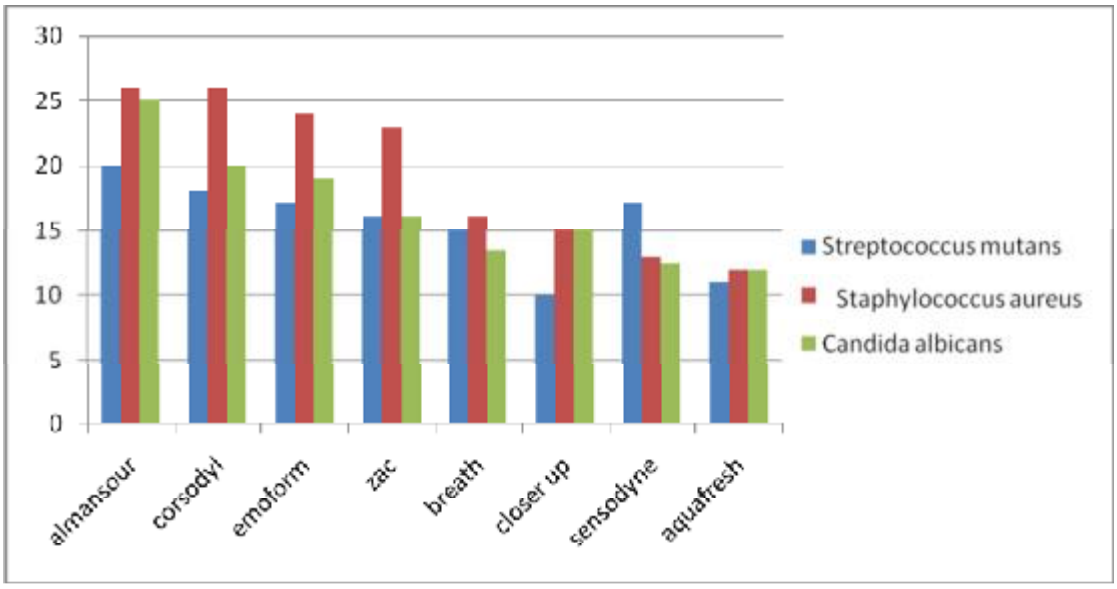

Figure 1: Mean diameter of zones of microbial inhibition exhibited by eight mouthwashes after 24hours at full strength (100%concentration) against three microorganisms

Figure 1 illustrated the antimicrobial activity of eight mouthwashes (100 %) without dilution against *Streptococcus mutans, Staphylococcus aureus* and *Candida albicans* and it is clear that all the mouthwashes used in this study have antimicrobial activity against the three selected organisms with wide variation and Al-Mansour mouthwash was the most effective giving the maximum inhibition zones against the three studied microorganisms followed by corsodyl, emoform, Zac, Breath, Close up, sensodyne and Aquafresh.

Results were analyzed by using SPSS 15 statistical package (spss LTD working UK).T-test showed differences between the concentrations when each concentration is compared with concentrated mouthwash 100%.

DISCUSSION

The results of this study revealed that all the mouthwashes used give antimicrobial activities against the three tested microorganisms with wide variation in their effectiveness, and these results also demonstrate that the concentrated mouthwashes (100%) full strength gives the strongest antimicrobial activities and when the mouthwashes are diluted they don't lost their antimicrobial activities but their effects decrease with dilution.

Several studies have demonstrated the effectiveness of rinsing with an antimicrobial mouth rinse in significantly reducing both salivary and mucosal levels of bacteria [(15)]. The result of present study shown that Al-Mansour mouthwash was the best one or the most effective one against the three microorganisms tested based on the mean diameter of the zone of microbial inhibition produced by the mouthwashes in agar diffusion technique followed by Corsodyl, emofarmand zac all of which showed excellent level of antimicrobial activities following by breath, close up, sensodyne and aquafresh that exhibit good antimicrobial activities.

Three of the four mouthwashes that exhibit excellent antimicrobial activities have Chlorhexidine gluconate as the active ingredient. Chlorhexidine gluconate is a cationic biguanide with broad spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental plaque and gingivitis has been demonstrated in several clinical studies [(16)].

Its mechanisms of action are that the cationic molecule binds to the negatively-charged cell walls of the microbes, destabilising their osmotic balance. Its substantivity, the ability of an agent to be retained in particular surroundings, is due to its ability to bind to the carboxyl groups of the mucin that covers the oral mucus and be steadily released from these areas in an active form, displaced by the calcium ions segregated by the salivary glands. Chlorhexidine formulations are considered to be the "gold standard" antiplaque mouth rinses due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential [(17)].

Emofarm is the only one from the four mouthwashes that give excellent antimicrobial activity that didn't have Chlorhexidine but it has sodium fluoride on its composition as the active ingredient. Fluoride mouthwashes have been used for many years to prevent caries by promoting re-mineralisation with fluorapatite and fluoro-

hydroxyapatite, thereby increasing enamel resistance to acid attack [18]. Fluoride is available in different concentrations as either acidulated phosphate fluoride or sodium fluoride. Fluoride mouthwashes reduce dental caries and they are recommended for patients at high risk of dental caries including those with xerostomia after irradiation and chemotherapy, those who have difficulty with oral hygiene procedures and those undergoing fixed orthodontic treatment. It's a well-known fact that fluoride rinses will product the permanent dentition against caries. Many antibacterial rinses now contain fluoride [19].

Breath mouthwash contains essential oil like eucalyptus oil, thymol and mint mouthwashes containing essential oils (thymol, eucalyptol, menthol) claim to penetrate the plaque biofilm and thus kill micro-organisms that cause gingivitis also These mouthwashes display broad spectrum antimicrobial activity, prevent bacterial aggregation, slow bacterial multiplication, retard plaque maturation and decrease plaque mass and pathogenicity. Their mechanism of action is thought to involve bacterial cell destruction, bacterial enzyme inhibition. They also have anti-inflammatory and prostaglandin synthetase inhibitory activity and act as antioxidants by scavenging free oxygen radicals. Clinical studies have concluded that essential oils are effective in reducing plaque, gingivitis and halitosis due to their bactericidal and plaque-permeating abilities [20].

Breath also contains Cetylpyridinium chloride is a quaternary ammonium compound with antiseptic and antimicrobial properties. It is cationic and thus binds to bacterial surfaces causing disruption of the cell membrane, leakage of intracellular components and disruption of metabolism. Mouthwashes containing cetylpyridinium chloride inhibit and reduce plaque build-up. [21]

Close up and aquafresh also contain cetylpyridinium chloride as active ingredient in their composition, while sensodyne contain only sodium fluoride.

All the mouthwashes used revealed antimicrobial activity against the three microorganisms used even when these mouthwashes were diluted but the concentrated mouthwash had the strongest antimicrobial activity. Al-Mansour mouthwash an Iraqi mouthwash was the best one according to the results of this study**.**

A mouthwash may be recommended to treat infection, reduce inflammation, relieve pain, and reduce halitosis or to deliver fluoride locally for caries prevention. Mouth-rinsing is easier to perform and may aid in controlling supragingival plaque and gingivitis, but it should always be used in conjunction with mechanical hygiene

REFERENCES

1. Prashant GM**,** Chandu GN**,** MurulikrishnaKS, Shafiulla MD. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivavius, Streptococcus mitis*, and *Streptococcus sanguis*: An *in vitro* study. Indian J Dent Res 2007; 18(4): 148-151. **(IVSL)**.
2. Mogammad TP, Charlene WJ, Lawrence XG, Johan M, Abdul Majeed N. An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers. Int J Clini Dent Sci 2011; 2(3): 28-32.
3. Van Gemert-Schricks MCM, van Amerongen WE, ten Cate JM, Aartman IHA. The effect of different treatment strategies on the oral health of children: a longitudinal randomized controlled trial. Clin Oral Invest 2008; 12: 361-8.
4. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986; 50: 353-80.
5. Agbelusi GA, Odukoya OA, Otegbeye AF. In vitro screening of chewing stick extracts and sap on oral pathogens: immune compromised infections. Biotechnology 2007; 6(1): 97-100.
6. Oztan MD, Kiyan M, Gerceker D. Antimicrobial effect, in vitro, of gutta-percha points containing root canal medications against yeasts and *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radio Endod 2006; 102: 410-6.
7. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998; 339(8): 520-32.
8. Zmantar T, Kouidhi B, Hentati H, Bakhrouf A. Detection of disinfectant and antibiotic resistance genes in *Staphylococcus aureus* isolated from the oral cavity of Tunisian children. Annals of Microbiology 2012; 62(1): 123-8.
9. Rodis OM, Shimono T, Matsumura S, Hatomoto K, Matsuo K, Kariya N. Cariogenic bacteria and caries risk in elderly Japanese aged 80 and older with at least 20 teeth. J Am Geriatr Soc 2006; 54: 1573-7.
10. Camile SF, Lidija MI, Michael JM. Mouthwashes Aust Prescr 2009; 32:162–4.
11. Taciano RC, Alexandre SC, Marcelo E B, Marcelo H N and Geraldo T J. Metabolic activity of *Streptococcus mutans* biofilms after treatment with different mouthwash formulations. Braz J Oral Sci 10(1): 74-78.
12. Dasanyake AP, Rosemaa JM, Caufield PW. Distribution and determinants of Mutans *Streptococci* among African American children and association with selected variables. Pediatr Dent 1995; 17(3): 192-6.
13. Qanbar FH, Al-Mizraqchi AS. The antimicrobial effect of aqueous and alcoholic extracts of eucalyptus leaves on oral *Mutans streptococci*, *Lactobacilli* and *Candida albicans* (an in vitro study). J Bagh College Dentistry 2009; 21(4): 109-112.
14. Ohara-Nemoto Y, Haraga H, Kimura S, Kemoto TK. Occurrence of staphylococci in the oral cavities of healthy adults and nasal–oral trafficking of the bacteria. J Med Microbiol 2008; 57: 95–99 **(IVSL)**.

15. Mat Ludin CM, MdRadzi J. The antimicrobial activity of different mouthwashes in Malaysia. Malay J Med Sci 2001; 8: 14-8.
16. Lorenz K, Bruhn G, Heumann C, Netuschil L, Brecx M, Hoffmann T. Effect of two new chlorhexidine mouth rinses on the development of dental plaque, gingivitis and discoloration. A randomized, investigator-blind, placebo-controlled, 3- week experimental gingivitis study. J Clin Periodontol 2006; 33: 561-7.
17. Amornchat C, Kraivaphan P, Dhanabhumi C, Tandhachoon K, Trirattana T, Choonhareongdej S. Effect of Cha-em Thai mouthwash on salivary levels of mutans streptococci and total IgA. Southeast Asian J Trop Med Pub Hlth 2006; 37: 528-31.
18. Marinho VCC, Higgins JPT, Logan S, Sheiham A. Fluoride mouth rinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003; 3: CD002284.
19. Jeevarathan J, Deepti A, Muthu MS, RathnaPrabhu V, Chamundeeswari GS. Effect of fluoride varnish on *Streptococcus mutans* counts in plaque of caries-free children using dentocult SM strip mutans test: A randomizedcontrolled triple blind study. J Indian Soci Pedodontics Preventive Dent 2007; 25(4): 157-163 **(IVSL)**.
20. Fine DH, Furgang D, Sinatra K, Charles C, McGuire A, Kumar LD. In vivo antimicrobial effectiveness of an essential oil-containing mouth rinse 12 h after a single use and 14 days' use. J Clin Periodontol 2005; 32: 335-40.
21. Witt J, Ramji N, Gibb R, Dunavent J, Flood J, Barnes J. Antibacterial and antiplaque effects of a novel, alcohol-free oral rinse with cetylpyridinium chloride. J Contemp Dent Pract 2005; 6: 1-9.