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Full Length Research Paper

Antibacterial effects of Oradex, Gengigel and Salviathymol-n mouthwash on dental biofilm bacteria

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Three mouthwashes Gengigel, Oradex and Salviathymol N, were tested using experimental microorganisms included *Fusobacterium nucleatum*, *Streptococcus mitis*, *Streptococcus constellatus*, *Eikenella corrodens* and dental plaque. *Staphylococcus aureus* and *Escherichia coli* were used as internal controls. Antibacterial activity was done by diffusion test. Minimum inhibitory concentration test and assessment of bacterial morphology was carried out using scanning electron microscopy (SEM). Results showed that Oradex had a higher antibacterial effect, followed by Salviathymol N; Gengigel mouthwash have weak antibacterial effects against tested microorganisms and dental plaque. SEM observations demonstrated that chlorhexidine exhibited obvious changes in that most of the bacteria loss their original shape and became irregular. The cell also shrunk, became reduced in size. Salviathymol N showed some significant changes while Gengigel failed to exhibited changes on the bacterial morphology of the tested microorganisms. In conclusion Oradex and Salviathymol N can be used as antibacterial mouthwash for chemical plaque control. Gengigel demonstrated weak antibacterial effects which could not be recommended as anti-dental plaque agent.

Key words: Gengigel, Oradex, Salviathymol N, Dental biofilm.

INTRODUCTION

Dental plaque is a complex biofilm that contains many bacterial species that accumulates on the hard tissues such as teeth in the oral cavity (Rosan and Lamont, 2000). Dental plaque is not a uniform structure. It varies from tooth to tooth and location to location in the oral cavity. Thus supragingival plaque appears to be different both morphologically and bacteriologically from subgingival plaque (Listgarten et al., 1978). The effectiveness of antimicrobial agents in mouthwashes to control bacterial plaque both *in vitro* (Baker et al., 1978; Gjermo et al., 1970) and *in vivo* (Roberts and Addy, 1981); have been proven by several considerable amount of research. Hyaluronic acid is a naturally occurring physiological constituent of the connective tissue, especially in the gingival mucosa. It is a glycosaminoglycan composed of repeating disaccharides, D-glucoronic acid and N-acetylglucosamine (Moseley et al., 2002). High molecular weight hyaluronic acid based mouthwash (Gengigel) can exert an efficient anti-hyaluronidase action, tissue reconstruction and anti-oedematigenous effect (AL- Bayaty et al., 2010; Pagnacco et al., 1997). Hyaluronic acid was used in a clinical study as an adjunct to supragingival scaling in the treatment of patients with gingivitis (Jenstch et al., 2003). The bacteriostatic effects of high molecular weight hyaluronic acid on various bacterial strains have been revealed by AL-Bayaty et al. (2010) and Pirnaza (1999). Chlorhexidine is a dicationic chlorophenyl biguanide and has a broad spectrum antibacterial effect against gram -positive and gram-negative bacteria (Seymour and Heasman, 1992). Low concentration of chlorhexidine is able to inhibit the growth of bacteria (bacteriostatic) while in high concentration it is able to kill the bacteria (bactericidal) (Ribeiro et al., 2007). Oradex,

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an antibacterial mouthwash contains chlorhexidine which is recognized as a primary agent for chemical plaque control (Jones, 1997) . Chlorhexidine has been used to prevent the formation of plaque on teeth, which help to prevent gingivitis (Cervone et al., 1990).

Essential oils and other extracts of plants have evoked interest as source of natural medicinal products. The oils and extracts have been screened for their potential uses as an alternative medicine for the treatment of many infectious diseases (Prabuseenivasan et al., 2006). Essential oils have been proved to exert antibacterial, antifungal, antiviral, and antioxidant properties but the mechanism of action is often not fully elucidated (Schelz et al., 2006). Essential oils based mouthwash (Salviathymol N) has proved its value over years for the treatment of inflammatory conditions affecting the oral cavity and pharynx. This mouthwash mainly contains sage oil (*Oleum salviae*), eucalyptus oil (*Oleum eucalypti*), cinnamon oil (*Oleum cinnamoni*), fennel oil (*Oleum foeniculi*), aniseed oil (*Oleum anisi*), peppermint oil (menthol) and chlorophyllin (Graf, 2006). Recently, bacteriological investigation has been done to determine the spectrum of antibacterial activity of Salviathymol N against bacteria found in the oral cavity (Graf, 2006). The purpose of this study was therefore to evaluate and to compare the antibacterial effects of Gengigel, Oradex and Salviathymol N on certain dental plaque bacteria, on pooled samples of dental plaque (supragingival and subgingival plaque) and to compare some dental plaque bacterial morphology before and after treatment with Gengigel, Oradex and Salviathymol N mouthwash, respectively, by examination under Scanning Electron Microscope.

Three different types of commercially available mouthwashes were used in this study. It included Oradex, Gengigel and Salviathymol N mouthwash. The Oradex is manufactured by Fortune Laboratories, Malaysia for Cavico Sdn Bhd Malaysia and contains 0.12%w/v Chlorhexidine. Gengigel is manufactured by Ricefarma Marketing Malaysia Sdn Bhd. It contains 0.025%w/w hyaluronic acid. Salviathymol N mouthwash is manufactured by MADAUS GmbH, Germany and obtained from MADAUS Company. It contains 1% w/w essential oils. All the mouthwashes were kept under room temperature.

EXPERIMENTAL MICROORGANISMS

Six types of microorganisms were used in this study where two microorganisms were used as control strains whereas four microorganisms represented dental plaque bacteria. The control strains were *Staphylococcus aureus* (ATCC 25923) and *Esherichia coli* (ATCC 25922) that were obtained from the Bacteriology Laboratory, Department of Molecular Medicine, University of Malaya. This control strains were used to check the effectiveness of the antibacterial agents. Microorganisms that represented the dental plaque organisms were *Fusobacterium nucleatum*, *Streptococcus mitis, Streptococcus constellatus* and *Eikenella corrodens* which were obtained from Clinical Diagnostic Laboratory (CDL) of University Malaya Medical Center, Kuala Lumpur. The microorganisms were subcultured on Brain Heart Infusion (BHI) agar and also inoculated into BHI broth as stock cultures for use throughout the experiment later on. Apart from these microorganisms, dental plaque pooled samples were also obtained, these were obtained randomly from the oral cavity of some individuals and cultured in BHI broth. The pooled samples of plaque were incubated both aerobically and anaerobically.

Bacterial count

The microorganisms were counted using a hematocytometer to give an actual and precise number of organisms that were used through out the assessment of antibacterial activity which were 1×10 cells/ml. Methylene blue dye was used to differentiate viable cells from dead cells under light microscope prior to cell counting. Viable cells appeared bright color and ring shaped whereas dead cells were stained dark. Concentration of bacteria was calculated according to the following formula:

$$\text{Bacterial concentration (cells/ml)} = \frac{\text{Total viable cells counted in four squares}}{4} \times \text{dilution factor} \times 10000$$

RESULTS

Assessment of antibacterial activity

Disk diffusion test

Screening of antibacterial activity was done by the disk diffusion test, which is normally used as a preliminary check to select mouthwash with higher antibacterial activity. The concentration of each mouthwash used in this study was as recommended by the manufacturer's instruction (Oradex, 0.12% w/v; Gengigel, 0.025% w/w; Salviathymol N, 1%w/w). Under aseptic conditions, empty sterile discs were impregnated with 100 µl of different concentrations of the respective mouthwashes and placed on the surface of the agar using sterile forceps. All Petri dishes containing the microorganisms were sealed and incubated overnight at 37°C except for Petri dishes containing anaerobic bacteria which were placed first in anaerobic jars and incubated in the same condition. After the incubation period, diameter of the inhibition zones were observed where clear zones were seen on the agar and measured using a ruler to the nearest millimeter (mm) readings. The test was performed in triplicates, and mean values of the diameters of the inhibition zones were calculated.

Minimal inhibitory concentration (MIC) test

A minimum inhibitory concentration test was carried out to determine the lowest concentration of mouthwash needed to inhibit visible (99%) bacterial growth of fixed concentration of experimental microorganisms after an

Table 1. The average diameter of inhibition zone (DIZ) for the three independent mouthwashes in the Disk Diffusion Tests for each type of organisms tested.

Organisms	Oradex 0.12% w/v (chlorhexidine)	Gengigel 0.025% w/w (Hyaluronic acid)	Salviathymoln 1.0% w/w (Essential oils)	sdH_2O (-ve control)
	Diameter of inhibition zone (DIZ) mm			
F. nucleatum	52	8	22	-
E. corrodens	52	7	20	-
S. constellatus	53	7	23	-
S. mitis	55	8	24	-
S. aureus (Internal control)	52	7	24	-
E. coli (Internal control)	50	7	23	-
Dental Plaque (aerobic)	52	7	30	-
Dental Plaque (anaerobic)	50	7	24	-

overnight incubation. The test was performed using the concentration of mouthwash as recommended by the manufacturers. The MIC value was confirmed based on the inhibition and growth observed on the agar plate which had been spot inoculated. The test was carried out in triplicate and the mean value of MIC was calculated.

Assessment of bacteria morphology

Scanning electron microscopy

This assessment was carried out to evaluate the changes occurring on the dental biofilm bacteria in terms of shape and structure before and after treatment with Salviathymol N mouthwash, Gengigel and Oradex independently. All the samples were examined under scanning electron microscope (JEOL SM-6400 Scanning Microscope) at 10 kV. Micrographs were taken at 3500 X magnification to investigate the general pattern of bacteria and up to 10000 X and 20000 X magnifications for specific morphological features. A final digital image was produced on screen (Bogner et al., 2007). The above procedure was repeated for the bacterial sample after treatment with Salviathymol N mouthwash, Gengigel and Oradex.

Table 1 shows the diameter of inhibition zones seen on agar plates according to each of the experimental organisms. The results revealed that the selected mouthwashes showed antibacterial activity with varying magnitudes. The zone of inhibition above 6 mm in diameter was taken as a positive result. Generally most of the experimental organisms were sensitive to the mouthwashes. Oradex showed maximum activity against all the bacterial species tested and also dental plaque pool samples. In other words, all the experimental organisms were sensitive towards Oradex. On the other hand, Gengigel showed minimal activity against all the tested strains and also the pool sample of dental plaque. All the tested organisms were less sensitive to Gengigel. Experimental organisms were seen moderately sensitive towards Salviathymol N. No obvious differences in susceptibility were found between the gram-positive and gram -negative bacteria. The largest diameter of inhibition zone which was 55 mm was seen around the disc impregnated with Oradex on the plate containing *S. mitis*. The smallest diameter of inhibition zones (7 to 8 mm) were observed around the discs containing Gengigel for all the tested organisms. The negative control discs, inoculated with sterile distilled water did not inhibit the tested organisms; therefore no inhibition zones could be seen around all the negative control discs used.

Minimum inhibitory concentration (MIC) test

The antimicrobial activity of the selected mouthwashes against the experimental organisms is shown in Table 2. It illustrates the bacterial growth seen on agar plates after overnight incubation in the confirmation step (spot inoculation) . The lowest mouthwash concentration that inhibits the growth of these organisms is regarded as the Minimum Inhibitory Concentration (MIC) value.

Table 3 shows the MIC value for each mouthwash against each tested organism. This study indicated that Oradex showed maximum activity with a MIC value 0.12% w/v which the concentration is recommended by the manufacturer against all tested organisms. Furthermore, these values are the lowest MIC values obtained in this study. Salviathymol N seems to have moderate MIC value which was 1% against all the bacteria and pooled dental plaque samples. On the other hand, the MIC value of Gengigel could not be determined because at 0.025% there was still the presence of bacterial growth on agar plates for all the tested organisms. From this study, the MIC value of Gengigel was expected to be higher than 0.025% .There were no significant differences in MIC values seen between

Table 2. The presence or absence of bacterial growth in the various mouthwashes with different concentrations (minimum inhibitory concentration (MIC)) for each of the organisms tested.

Organisms	Oradex (Chlorhexidine)		Gengigel (Hyaluronic acid)		Salviathymol N (Essential oils)		sdH_2O (-ve Cntrl)
	0.120% w/v	0.060% w/v	0.0250% w/w	0.0125% w/w	1.000% w/w	0.500% w/w	
F. nucleatum	-	+	+	+	-	+	+
E. corrodens	-	+	+	+	-	+	+
S. constellatus	-	+	+	+	-	+	+
S. mitis (Internal control)	-	+	+	+	-	+	+
S. aureus (Internal control)	-	+	+	+	-	+	+
E. coli	-	+	+	+	-	+	+
Dental plaque (aerobic)	-	+	+	+	-	+	+
Dental plaque (anaerobic)	-	+	+	+	-	+	+

+ = Bacteria growth, - = No bacteria growth.

Table 3. Investigation of the lowest mouthwash concentrations to inhibit the visible growth of tested organisms after overnight incubation (MIC).

Organisms	Oradex	Gengigel	Salviathymol N
	Minimum inhibitory concentration		
	% w/v	% w/w	% w/w
F. nucleatum	0.120	>0.025	1.000
E. corrodens	0.120	>0.025	1.000
S. constellatus	0.120	>0.025	1.000
S. mitis	0.120	>0.025	1.000
S. aureus (Internal control)	0.120	>0.025	1.000
E. coli (Internal control)	0.120	>0.025	1.000
Dental plaque (aerobic)	0.120	>0.025	1.000
Dental plaque (anaerobic)	0.120	>0.025	1.000

gram-positive and gram-negative bacteria. Both groups of bacteria were equally inhibited with the same MIC values. In addition, there was no inhibition seen by the negative controls, therefore bacteria were seen growing on the plates.

Assessment of bacteria morphology

Assessment of antibacterial activity was performed to study the changes occurring on the bacterial morphology after being treated with Oradex, Gengigel and Salviathymol N individually. Before treatment with mouthwashes, the entire bacteria were in their original shape. The surfaces of the bacteria were smooth and most of them are viable cells. However the bacteria morphology obviously changed after treated with Oradex. Most of the bacteria became dead cells with the loss of their original shape, thus becoming irregular. The cell also shrunk and thus became reduced in size. This was observed with *F. nucleatum, E. corrodens, S. constellatus* and *S. mitis*. In addition, the surfaces of bacterial cells showed a wrinkled appearance. Some of the bacterial cells exhibited severe damage with the rupture of the cell wall, and with bursting of the cells.

The presences of folded membranes were seen in *F. nucleatum.* The same changes of antibacterial effect was also observed with the bacterial morphology after treatment with Salviathymol N, although the difference was that the change that occurred in the bacterial morphology was not as obvious as after treatment with Oradex. None of the cells showed any severe damage such as rupturing of cell walls and bursting of the cells. Generally, the bacterial cells appeared shrunken and the surface of the bacteria showed a wrinkled or furrowed appearance. On the other hand, the bacterial morphology did not change at all after treatment with Gengigel. The bacterial structure and shape remained and were maintained in the original shape (Figures 1 to 10).

DISCUSSION

In this study, Oradex exhibited a strong antibacterial

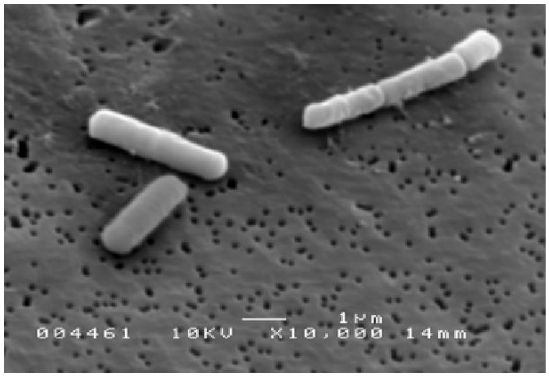

Figure 1. A scanning electron micrograph showing the *F. nucleatum* before treatment (x10000, 10 KV).

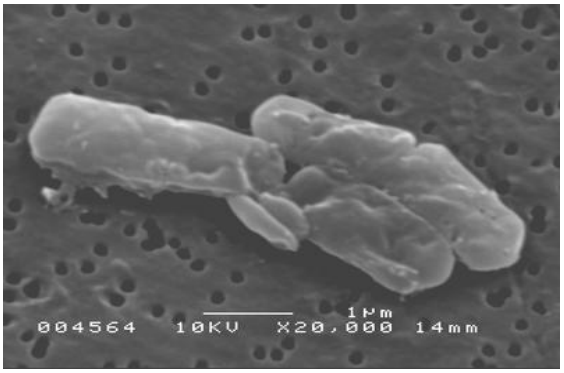

Figure 2. A scanning electron micrograph showing presences of folded membranes of *F. nucleatum* after treatment with Oradex (20000X, 10 KV).

Figure 3. A scanning electron micrograph showing presences of moderate folded membranes of *F. nucleatum* after treatment with Salviathymol N (10000X, 10 KV).

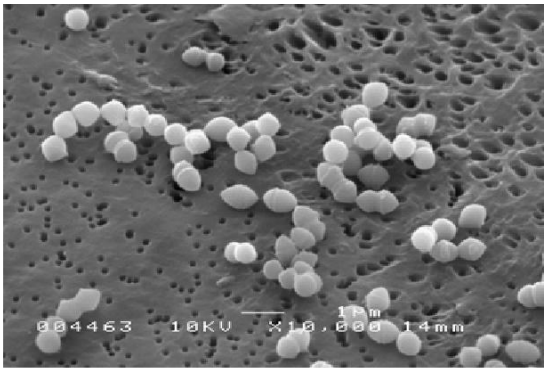

Figure 4. A scanning electron showing *E. corrodens* before treatment (10000X, 10 KV).

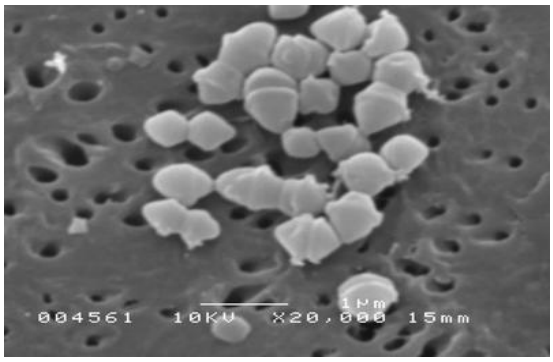

Figure 5. A scanning electron micrograph showing *E. corrodens* shrunk and reduced in size after treatment with Oradex (20000X, 10 KV).

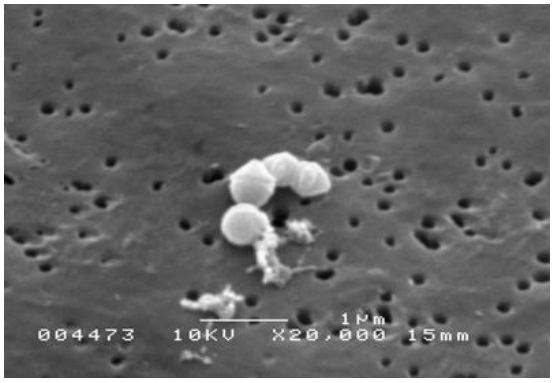

Figure 6. A scanning electron micrograph of *E. corrodens* showing shrunk and reduced in size with the rupture of the cell wall after treatment with Salviathylmol N (20000X, 10 KV).

activity against all tested strains including pooled samples of dental plaque. This was expected because the major chemical constituent in Oradex was 0.12% w/v chlorhexidine which possess antibacterial properties. The results obtained in this study were consistent with previous findings which proved the antibacterial effects of chlorhexidine is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering bacterial osmotic equilibrium (Greenstein et al., 1986).

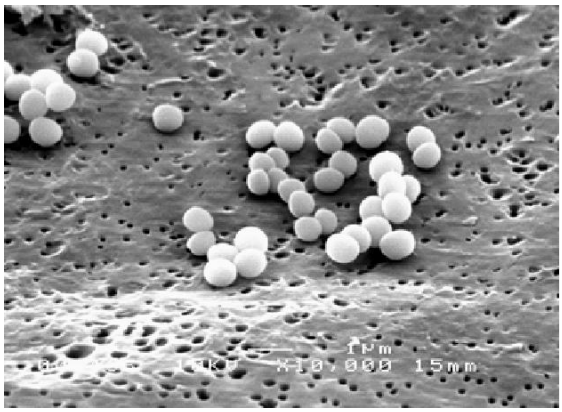

Figure 7. A scanning electron micrograph showing *S. constellatus* before treatment (10000X, 10 KV).

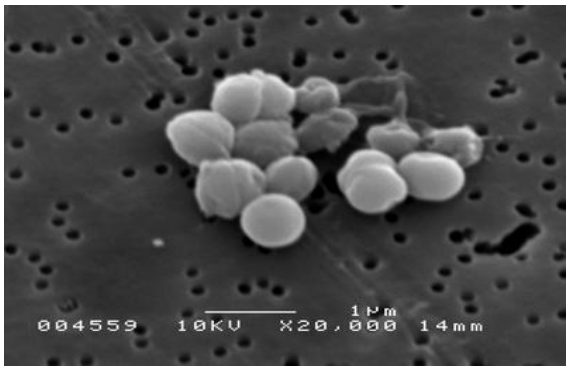

Figure 8. A scanning electron micrograph showing *S. constellatus* shrunk and reduced in size, bacterial cells showed a wrinkled appearance after treatment with Oradex (20000X, 10 KV).

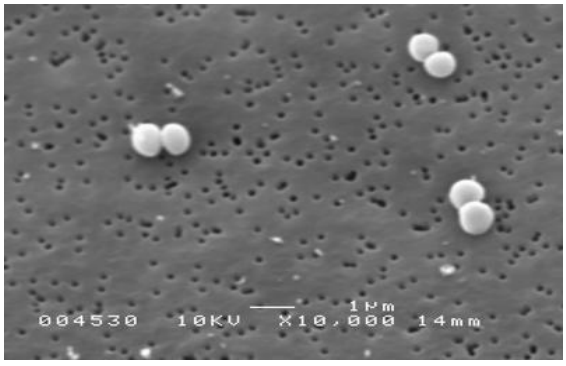

Figure 9. A scanning electron micrograph of *S. mitis* before treatment (10000X, 10 KV).

Chlorhexidine was proven to disrupt sugar transportation in acidogenic organisms of oral streptococci (Keevil et al., 1983), and this enables Chlorhexidine to control plaque organisms that causes dental caries. Numerous clinical studies have confirmed this plaque inhibitory property of

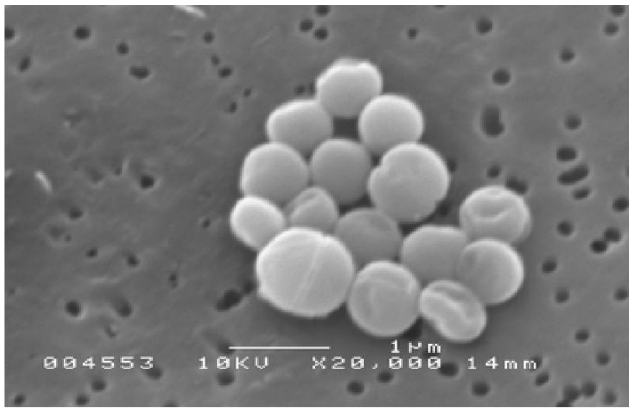

Figure 10. A scanning electron micrograph of S.mitis bacterial cells showed a wrinkled appearance after treatment with Oradex (20000X, 10 KV).

chlorhexidine. Based on the results obtained, Salviathymol N possesses moderate antibacterial activity against tested organisms. Salviathymol N was made up of a mixture of various plant essential oils. The finding of this study is parallel with earlier findings by Zaika (1988). *In vitro* studies of some plant essential oils inhibited bacterial growth (Graf, 2006). Graf claimed the wide spectrum of antibacterial activity of Salviathymol N against gram positive and also gram negative bacteria (2006). An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of bacterial cell membranes, thus disturbing cell structures and rendering them more permeable. Extensive leakages from bacterial cells cause loss of intracellular constituents which will then lead to the death of the bacteria (Prabuseenivasan et al., 2006).

In comparison, Gengigel seem to have weak antibacterial activity against all the tested bacteria including pooled dental plaque samples. This study has been unable to demonstrate the lowest concentration of mouthwash required to inhibit the visible growth of bacteria, since at 0.025% w/w of hyaluronic acid, there was still presence of bacterial growth. In contrast, earlier findings demon-strated that 1.0 mg/ml hyaluronic acid had bacteriostatic effects on different oral bacteria, including *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (Jenstch et al., 2003). However, the clinical study done by Xu et al. (2004) showed that hyaluronic acid in the gel form does not have any antimicrobial effects against oral bacteria (AL-Bayaty et al., 2010; Schelz et al., 2006). The mechanism of action of how hyaluronic acid inactivates bacteria is still unclear. Hyaluronic acid probably inactivates the hyaluronidase enzyme produced by plaque bacteria and thus assists in preventing infestation and further destruction of gingival tissue (Zaika, 1988). Further study needed to test the effectiveness of these mouthwashes clinically (*in vivo*) and to evaluate their cytotoxicity on gingival and periodontal fibroblasts.

Conclusion

This study showed that 0.12% Oradex had a higher antibacterial effect against the tested organisms, followed by 1% Salviathymol which showed moderate antibacterial effects. On the other hand, 0.025% Gengigel seemed to have weak antibacterial effects against tested organisms. Oradex and Salviathymol N can be used as antibacterial mouthwash for chemical plaque control. Gengigel could not be recommended as anti-dental plaque agent. This study was financially supported by the University of Malaya through a grant Vote F0178/2007a.

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