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The Effectiveness of Chlorhexidine, Hexetidine and *Eugenia caryophyllus* Extracts in Commercialized Oral Rinses to Reduce Dental Plaque Microbes

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Abstract: A thin film of microorganisms on the tooth surfaces is known as dental plaque, which can contribute to the development of dental caries and periodontal disease. It has been widely known that the usage of antimicrobial oral rinses plays an important role in maintaining oral hygiene mainly by reducing the numbers of dental plaque microbes. The study was carried out with the aim of comparing the effectiveness of antimicrobial compound in commercialized oral rinses, chlorhexidine (Oradex®), hexetidine (Bactidol®) and *Eugenia caryophyllus* (clove) extracts (Mustika Ratu®) in controlling the numbers of dental plaque microbes. In addition, the aim was also to determine the duration of effects in suppressing and reducing plaque microbes with the usage of the selected commercialized oral rinses. The antibacterial effects of all oral rinses were evaluated in healthy adults, which were not on any antibiotic treatment for the past 6 months and were tested on volunteers for a period of 30 min interval for up to 90 min. Volunteers were required to suspend normal oral hygiene habits and on the sampling day, the exposed tooth surfaces of each volunteer were swabbed, followed by rinsing with the specified oral rinse. Swab samples were serially diluted and plated on BHI agar. Phenotypic appearance and the Colony Forming Units (CFU mL^{-1}) were obtained. The study showed that immediately after rinsing with the respective oral rinses, chlorhexidine were effective than hexetidine and *Eugenia caryophyllus* extract in reducing the microbial load and sustaining the longest duration in controlling the build-up of plaque microbes. On the other hand, *Eugenia caryophyllus* extract acts faster than hexetidine in reducing the dental plaque microbial load, but has demonstrated to exhibit the shortest duration effect in controlling plaque build-up.

Key words: Oral rinse, chlorhexidine, hexetidine, *Eugenia caryophyllus*, dental plaque

INTRODUCTION

Dental plaque is a thin film of microorganisms, which adhered to tooth surfaces. This dense concentration of bacteria in dental plaque in organic matrix is also known as dental biofilm (Socransky and Haffajee, 2002). Accumulation of dental plaque on tooth surfaces contributes to the development of various oral diseases including dental caries, gingivitis and periodontal diseases. In order to prevent these oral diseases, it is important to have effective ways and strategies to reduce and control dental plaque accumulation in the oral cavity.

For many years, oral rinse has been formulated as an adjunct to the common mechanical dental plaque removal methods in an attempt to improve plaque control. Oral rinse products containing chemotherapeutic agents with a variety of antimicrobial mechanism have been reported to be beneficial and desirable (Axellson and Lindhe, 1987).

Medicated oral rinse usually contains antimicrobial agents, such as Chlorhexidine Gluconate (CHX), Hexetidine (Hx), phenolics compound such as thymol, eucalyptol, methol and etc. which could prevent dental plaque formation (Elworthy *et al*., 1996). Currently, there are commercialized oral rinses, which contain natural compounds with antimicrobial activity and are known to be safe for human used (Jacobsen *et al*., 2001; Elworthy *et al*., 1996). Natural extracts such as *Punica granatum*, *Eucalyptus globules*, *Salvadora persica*, *Malva sylvestris* and *Eugenia caryophyllus* (clove) are among the extracts added to the formulation of many commercialized oral rinses as antimicrobial agents, due to the broad range of effects towards oral microbes (Watanabe *et al*., 2008). *Eugenia caryophyllus* (clove) extract has been used by Chinese as traditional medicine, including India's traditional Ayurvedic healers. Currently, it has also been widely used in Europe, German, America including Asian countries to treat indigestion, diarrhea,

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toothache, etc. Clove oil has also been used by dentists as an anaesthetic and to disinfect root canals (holistic online). In addition, *Eugenia caryophyllus* extract has also been used as an active ingredient in commercialized herbal oral rinse.

Oral rinses have been reported to be able to influence the reduction of dental plaque bacterial attachment, proliferation or retention on the tooth surfaces and thus, it is able to lower the rate of dental plaque formation to a statistically significant level (Christine and Eugene, 2000). However, the information on the inhibitory duration effect of oral rinses towards dental plaque is currently insufficient (Christine and Eugene, 2000).

Therefore, the objectives of this study involved the determination of the effectiveness of chemically derived antimicrobial agents (chlorhexidine and hexetidine) and natural extracts of *Eugenia caryophyllus* (clove) obtained in the commercially available oral rinses in reducing the numbers of dental plaque microbes and to determine the duration of effect of the agents in reducing and controlling dental plaque microbes.

MATERIALS AND METHODS

Active antimicrobial compounds: In the experiment, the active antimicrobial compounds were obtained from selected commercialized oral rinses of Oradex®, which contains 0.12% chlorhexidine gluconate, Bactidol® containing 0.1% hexetidine and Mustika Ratu®, which contains *Eugenia caryophyllus* (clove) extract as the active compounds. Distilled water was also used in the experiment as a negative control.

Inclusion and exclusion criteria: The antibacterial effects of chlorhexidine, hexetidine and *Eugenia caryophyllus* (clove) extract on dental plaque microbes were evaluated on adults with healthy oral cavity and were not on any antibiotic treatment for the past 6 months.

Sampling procedures: Volunteers were required to suspend normal oral hygiene habits daily, which include the routine toothbrushing every morning after getting up from bed and before bedtime. The used rinsing volume of each of the oral rinse was based on the suggested instructions on the manufacturer's product labels.

On the sampling day, the tooth surfaces of the buccal, occlusal and lingual sides of each volunteer were swabbed with sterile cotton swab, followed by rinsing with the specified oral rinse. The selected tooth surfaces were swabbed after 30, 60 and 90 min. Swab samples were vortexed for 30 sec to dislodge all microbes. Samples were then diluted serially and plated on Brain Heart Infusion (BHI) agar. All plates were incubated at 37°C for 18-24 h.

Bacterial identification: Bacterial colonies from the dental plaque were isolated and purified. Gram stained for cell morphology identification was also carried out and results were recorded. Finally, the pure bacterial isolates were subjected to bacterial identification procedures using the API identification system (Bio Merieux, France).

RESULTS AND DISCUSSION

Dental plaque comprised of a diverse microbial community that is embedded in a matrix of host and bacterial polymers and grows as a biofilm (Marsh, 2003). Matured dental plaque, which comprised of a high microbial density community with a variety of different microorganisms (Socransky and Haffajee, 2002) is considered to contribute as a major aetiology factor in the formation, development and progression of gingivitis and periodontitis (Kornmann, 1986). A number of studies have showed high positive correlation between the amount of supragingival plaque and the development of gingivitis including between removal of bacterial plaque and the resolution of gingival inflammation. Similarly in our study, we have identified various bacterial species including Candida yeast inhabiting the supragingival plaque. Among the highly encountered bacterial species in our study were *Streptococcus* sp., *Lactobacillus* sp. and *Staphylococcus* sp., which is in agreement to other reported findings elsewhere (Socransky and Haffajee, 2002).

In dental practice, mechanical removal procedures are used to remove dental plaque. However, it has been reported that chemotherapeutic oral rinses could act as an adjuvant to mechanical removing of supragingival plaque (Roberts and Addy, 1981). Antimicrobial properties in oral rinses are considered effective, safe and important in maintaining oral health, as it could kill cariogenic organisms and therefore, prevent dental plaque accumulation, gingivitis and halitosis (Wade and Addy, 1989).

In the study, it has been found that Chlorhexidine (CHX), Hexetidine (Hx) and *Eugenia caryophyllus* (clove) Extract (EC) could significantly reduced the dental plaque population (Table 1). The first 30 min after rinsing, chlorhexidine and *Eugenia caryophyllus* (clove) extract were able to drastically reduced the bacterial population (Fig. 1) compared to hexetidine. The reduction trend continued until an hour, where dental plaque bacteria started to accumulate indicating the increase in bacterial population (Fig. 2). In contrast, hexetidine was less

Table 1: Colony Forming Units (CFU) of microbial population in dental plaque after rinsing with oral rinses containing chlorhexidine, hexetidine and *Eugenia caryophyllus* extracts

	0.12% Chlorhexidine		0.1% Hexetidine		*Eugenia caryophyllus* (clove) extract		Distilled water	
Oral rinse/time	CFU mL^{-1} ($\times 10^3$)	Reduction (%)	CFU mL^{-1} ($\times 10^3$)	Reduction (%)	CFU mL^{-1} ($\times 10^3$)	Reduction (%)	CFU mL^{-1} ($\times 10^3$)	Reduction (%)
Before rinsing	183	-	160.6	-	173	-	96.7	-
30 min	37	79.78	121.1	24.56	48	72.25	74.2	23.27
60 min	38	79.23	128.9	19.73	59	65.90	99.2	-2.56
90 min	40	78.14	128.1	20.20	134	22.54	101.3	-4.76

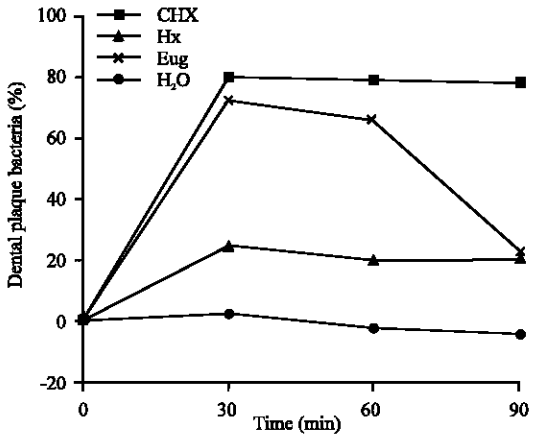

Fig. 1: Percentage of microbial load reduction in dental plaque after rinsing with oral rinses containing chlorhexidine, hexetidine and *Eugenia caryophyllus* extracts

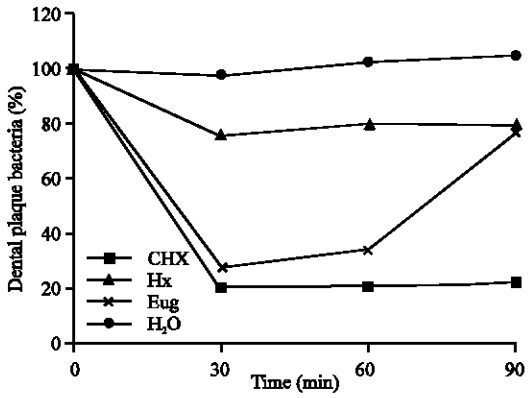

Fig. 2: Percentage of microbial load in dental plaque after rinsing with oral rinses containing chlorhexidine, hexetidine and *Eugenia caryophyllus* extracts

effective in reducing the bacterial population. Differently for rinsing with water, there was no significant falls observed towards bacterial population. The little reduction of bacterial population was probably due to the rinsing force itself, which only allow very low percentage of plaque bacterial removal.

Giuliana *et al.* (1997) has reported similar findings, which mentioned that active agents in oral rinses exert beneficial effects in reducing dental plaque. Chlorhexidine has immediate bactericidal action on plaque bacteria and plaque fungi and is among the most effective active agents to reduce and inhibit plaque accumulation (Jenkins *et al*., 1988; Bergenholtz and Hanstrom, 1974). It is able to kill both gram-positive and gram-negative microbes. This could possibly due to the mechanism of action of chlorhexidine on bacteria, which involves the disruption of bacterial cell membrane.

Chlorhexidine has also been suggested to exhibit antifungal activity and could penetrate the region of the glycerol moieties, which subsequently breakdown the permeability barrier and lead to leakage of the cytoplamic contents, causing cell death. Thus, it is often used as an active agent in various oral rinses to reduce dental plaque.

Natural extracts of *Eugenia caryophyllus* (clove) exhibits broad antimicrobial properties against bacteria and fungi. Thus, the drastic drop in dental plaque population was possibly due to the ability to kill dental plaque bacteria (*Streptococcus* sp., *Lactobacillus* sp. and *Staphylococcus* sp.) and also *Candida* sp. as these were among the majority of plaque microbes isolated in our study.

Hexetidine is a broad spectrum antiseptic, which act on gram-negative and gram-positive bacteria (Roberts and Addy, 1981). Hexetidine is less effective in inhibiting the development and reducing the growth of supragingival plaque compared to chlorhexidine (Bergenholtz and Honstrom, 1974), which is similar to our finding (Table 1).

Our study has also shown that chlorhexidine has a longer suppression effect compared to hexetidine and *Eugenia caryophyllus* extract. This is in agreement with other reported results that chlorhexidine is the best acting antimicrobial agent in reducing and allowing the slowest build-up of dental plaque bacteria. This could be due to chlorhexidine having a prolonged bacteriostatic action and ability to adsorb onto the pellicle coated enamel surface and dental plaque (Jenkins *et al.*, 1988) during rinsing (Bergenholtz and Hanstrom, 1974). The adsorbed chlorhexidine has been reported to be able to be retained in the oral cavity and thus, exerted a prolonged bactericidal effect (Gjermo *et al.*, 1974). Retention of

chlorhexidine in human oral cavity may be based on an interaction with protein. Gjermo *et al*. (1974) has suggested that salivary sulphated glycoproteins and phosphate groups are presumably responsible for a major part of chlorhexidine binding to bacteria and plaque.

Nevertheless, although, hexetidine was found to be less effective than chlorhexidine and *Eugenia caryophyllus* extracts in allowing immediate reduction of dental plaque after rinsing, it has shown to exhibit longer duration of controlling dental plaque buid-up compared to *Eugenia caryophyllus* extracts (Fig. 2). This could possibly due to the adsorbed hexetidine to the dental plaque (Bergenholtz and Hanstrom, 1974). The adsorbed hexetidine has also been reported to be able to remains in the plaque matrix for 7 h (Bergenholtz and Hanstrom, 1974), which answers the higher ability to control plaque buid-up compared to *Eugenia caryophyllus* extracts. In contrast, although *Eugenia caryophyllus* extracts has high ability to reduce dental plaque immediately after rinse, it could not sustain the control effect and has shown a very short duration in controlling plaque build-up (Fig. 2). The weak control effect exhibited by this extracts could be due to the low adsorption and weak retention ability in plaque matrix.

CONCLUSION

The result of this study has demonstrated that supragingival microflora can be successfully suppressed by the use of oral rinses. Judged by the reduction in Colony Forming Units (CFU) of the supragingival microflora, it was clearly shown that all three agents were effective in reducing dental plaque microbes.

Chlorhexidine was the most effective agent in reducing dental plaque compared to *Eugenia caryophyllus* extracts and hexetidine. In addition, chlorhexidine was found to have a longer suppression effect on controlling dental plaque microbes compared to *Eugenia caryophyllus* extracts and hexetidine.

Eugenia caryophyllus extract acts faster than hexetidine in reducing the dental plaque microbial load, but has demonstrated to exhibit the shortest duration effect in controlling plaque build-up.

Nevertheless, daily used of oral rinse is important in maintaining oral hygiene and controlling the accumulation of dental plaque.

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