

## Inhibitory Effect of Chlorhexidine and Clove Extract Oral Rinses in Reducing Dental Plaque Microbes

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**Abstract:** Dental plaque is a thin film of microorganisms on the tooth surfaces and contributes 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. This study has been carried out with the aim of comparing the clinical effectiveness of Chlorhexidine (CHX) (Oradex®) and commercialized clove extract (Mustika Ratu®) oral rinses in reducing dental plaque microorganisms. In addition, the aim was also to determine the duration of effects and magnitude of suppression of plaque microbes with the usage of the oral rinses. The antibacterial effects of CHX and clove extract oral rinses of dental plaque microbes were evaluated in healthy adults, which were not on any antibiotic treatment for the past 6 months. The oral rinses were tested on volunteers for a period of 30 min interval for up to 120 min. Volunteers were required to suspend normal oral hygiene habits and on the sampling day, the tooth surfaces of each volunteer were swabbed, followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 min. Swab samples were serially diluted and plated on BHI agar. Phenotypic appearance and the colony forming units (CFU mL<sup>-1</sup>) were obtained. Results indicated that bacterial colony formation was significantly inhibited by both oral rinses and exhibited similar levels of antibacterial activity. However, the oral rinse containing CHX exhibited a longer duration of activity in controlling oral microbes compared to clove extract oral rinse. In conclusion, CHX has a better antimicrobial action to reduce and exhibit longer magnitude of suppression of dental plaque microbes than clove extract oral rinse.

**Key words:** Chlorhexidine, clove extract, oral rinse, dental plaque

### INTRODUCTION

A thin film containing microorganisms that attached on tooth surfaces are known as dental plaque or dental biofilm (Socransky and Haffajee, 2002). Dental plaque is said to contribute to the development of oral diseases such as dental caries and periodontal disease. In the oral cavity, bacteria have the ability to attach to a wide variety of surfaces, including the soft tissues, the pellicle-coated teeth and also to other bacteria.

The importance of effective dental plaque control has been emphasized over the years. As an adjunct to mechanical methods for dental plaque elimination, oral rinse products containing chemotherapeutic agents with a variety of antimicrobial mechanism have been beneficial and desirable (Axelsson and Lindhe, 1987). There are 2 types of oral rinse; that is medicated and non-medicated oral rinses. Medicated oral rinse usually contains chlorhexidine gluconate, having pronounced as antiseptic properties, which could inhibit the formation of dental

plaque (Elworthy *et al.*, 1996). Chlorhexidine has a wide-spectrum of antibacterial activity encompassing Gram positive and Gram negative bacteria, yeasts and other microbes. The non-medicated oral rinse usually contain herbal extracts such as phenol, thymol, eugenol (derived from the buds of cloves) and many others (Elworthy *et al.*, 1996). Such important compounds can be isolated from plant sources and are safe for humans.

Oral rinse products are capable of reducing the rate of dental plaque formation to a statistically significant degree. However, currently the information on the inhibitory duration effect on dental plaque is insufficient (Christine and Eugene, 2000). Therefore, this study has been carried out with the objective of comparing the clinical effectiveness of Chlorhexidine (CHX) (Oradex) and commercialized clove extract (Mustika Ratu) oral rinses in reducing dental plaque microorganisms. In addition, the aim was also to determine the duration of effects and magnitude of suppression of dental plaque microbes with the usage of the oral rinses.

## MATERIALS AND METHODS

**Oral rinses:** Commercialized oral rinses used in the experiment are Oradex containing 0.12% chlorhexidine gluconate as the active compound and Mustika Ratu containing clove extract as its active compound. Distilled water was used in the experiment as a negative control.

**Rinsing procedures:** The antibacterial effects of chlorhexidine and clove extract on dental plaque microbes were evaluated on dentally healthy adults which were not on any antibiotic treatment for the past 6 months. The used rinsing volume of each of the oral rinse was based on the suggested instructions on the manufacturer's product labels. 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.

**Sampling procedures:** On the sampling day, the tooth surfaces of each volunteer were swabbed with sterile cotton swab, followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 min. Swab samples were vortexed for 30 sec to dislodge all microbes. Following this, samples were serially diluted and plated on Brain Heart Infusion (BHI) agar. All plates were incubated at 37°C for 18-24 h.

**Bacterial identification:** Pure colonies were isolated and Gram stained for cell morphology identification. The isolates were then subjected to bacterial identification procedures using the API Identification System (Bio Merieux, France).

## RESULTS AND DISCUSSION

Three different types of bacterial colonies were successfully cultured on the BHI agar plates. Colonies were yellowish, white and cream in colour. The yellowish colonies had diameter approximately ranging between 1-2 mm which were identified as *Staphylococcus* sp. whereas the cream colour colonies with diameter ranging from 1-2 mm were identified as *Streptococcus* sp. and the white colonies which were around 4-5 mm in diameter were identified as *Candida* sp.

Results from the study have also indicated that rinsing with the oral rinses showed reduction in the dental plaque population (Table 1). A 0.12% chlorhexidine showed a significant reduction in the bacterial population of the dental plaque microbes. For the first 30 min after rinsing, 79.8% of the dental plaque microbes were reduced by chlorhexidine. Rinsing with clove extract showed a weaker effect compared to chlorhexidine, when about 72.3% of the dental plaque microbes were killed. In contrast, rinsing with distilled water did not produce a significant falls to the plaque microbes and had given the least reduction when only 21.52% were reduced compared to the bacterial total count at 0 min.

Chlorhexidine has shown to exhibit longer suppression effect towards dental plaque microbes up to the tested duration period of 120 min (Fig. 1). In contrast, the clove extract exhibited shorter duration effect and was only able to control the suppressive effect for 60 min, after which the plaque microbes had started to increase and less reduction percentage observed. Distilled water however, was not able to reduce the plaque microbes and thus there were no changes observed during the 120 min.

Dental plaque is considered the major aetiological factor in the development of gingivitis and periodontitis (Kornman, 1986). This study has shown that dental plaque community was composed of a variety of different microorganisms which is in agreement to other reported results elsewhere (Socransky and Haffajee, 2002). Daily usage of oral rinse is important to maintain the oral hygiene and prevent dental plaque accumulation. The effective control of dental plaque is very important so as to prevent disease progression such as gingivitis and periodontitis.

Oral rinse containing antimicrobial properties play an important role in maintaining oral health by killing cariogenic organisms and preventing plaque, gingivitis and bad breath. In order to reduce and control dental plaque microbes, antimicrobial agents have been employed to help to prevent the development of these diseases (Wade and Addy, 1989) and are considered effective and safe. In the study, the two antimicrobial agents had been proven to reduce the dental plaque microbes significantly compared to distilled water. However, comparison between the 2 agents have revealed that chlorhexidine was significantly more effective in

Table 1: Inhibition effects of rinsing with the oral rinses on dental plaque population

Oral rinse/Time	0.12% Chlorhexidine		Clove extract		Distilled water	
	CFU mL <sup>-1</sup>	Reduction (%)	CFU mL <sup>-1</sup>	Reduction (%)	CFU mL <sup>-1</sup>	Reduction (%)
0 min	1.83×10 <sup>5</sup>	-	1.73×10 <sup>5</sup>	-	1.58×10 <sup>5</sup>	-
30 min	0.37×10 <sup>5</sup>	79.8	0.48×10 <sup>5</sup>	72.3	1.24×10 <sup>5</sup>	21.52
60 min	0.38×10 <sup>5</sup>	79.2	0.59×10 <sup>5</sup>	65.9	1.42×10 <sup>5</sup>	10.13
120 min	0.40×10 <sup>5</sup>	78.1	1.34×10 <sup>5</sup>	22.5	1.33×10 <sup>5</sup>	15.82

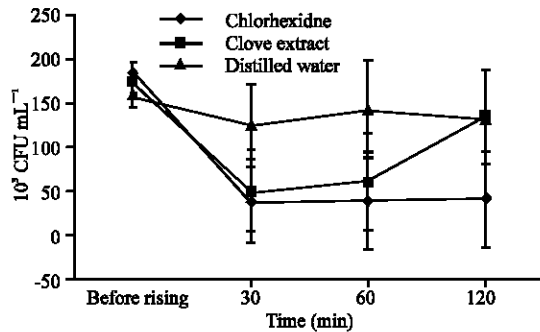


Fig. 1: CFU of dental plaque microbes after rinsing with chlorhexidine, clove extract and distilled water

reducing the plaque microbes than herbal extract (clove extract). A number of common dental plaque microbes such as staphylococci and streptococci including *Candida* sp. have been reported to be highly susceptible to chlorhexidine (Addy, 1986). It is because this active antimicrobial agent could destabilize and penetrate bacterial cell membranes and could interfere with membrane function by inhibiting oxygen utilization that leads to cell death.

This study has also demonstrated that chlorhexidine exhibits a longer suppression effect compared to the clove extract active compound. As for eugenol, which is the antimicrobial compound for clove extract also possesses activity against both Gram positive and Gram negative bacteria, but it is less effective and has shorter duration period in controlling the dental plaque microbes. This is in agreement with other reported results that chlorhexidine performed almost equal killing effect to other antimicrobial agents in most oral rinses for up to 30 min. After rinsing, but continued its microbicidal effect for a longer duration period.

## CONCLUSION

The result of this study has demonstrated that dental plaque microbes can be successfully suppressed by the use of oral rinse. Chlorhexidine and clove extract oral rinse can significantly reduce the microbial load of the dental plaque, with chlorhexidine provides higher antimicrobial activity in reducing and controlling the dental plaque microbes compared to clove extracts.

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