Effect of Natural Oral Spray Products against Oral Bacteria and User Satisfaction in the Elderly Compared with Chlorhexidine Mouthwash

Chanon Suwanprapis[1], Thipawan Tharapiwattananon [2], Anjalee Vacharaksa [2]

[1]Master of Science Program in Geriatric Dentistry and Special Patients Care (International Program), Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[2]Department of Microbiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
Corresponding author, E-mail: 6075806632@student.chula.ac.th

Abstract

The natural oral spray is beneficial in elderly patients who become disabled to maintain their oral health because the elderly may have difficulties in using their hands. Chlorhexidine mouthwash retains unfavorable flavor, while the natural oral spray containing essential oil which tastes and smells better. Therefore, the purpose of this study is to investigate the antimicrobial activity of natural oral spray against oral bacteria compared with chlorhexidine mouthwash, and patient satisfaction when using the mouthwash. Participants had used two types of natural oral spray products, including essential oil and essential oil with mangosteen extract, and chlorhexidine mouthwash, with 2 weeks of washout period between each product. Unstimulated saliva samples were collected before and after using each product for microbial culture. Total colony forming units (CFUs) were enumerated and compared. Then, satisfaction score was recorded by questionnaire. The results demonstrated that natural oral spray products reduced the total colony forming units after continuous use for 2 weeks, similar to the antimicrobial effect of chlorhexidine, and most patients expressed better user satisfaction than chlorhexidine mouthwash. This study suggested that the oral spray with essential oil and mangosteen extract demonstrated effective antimicrobial activity and favored patient compliance.

Keywords: Antimicrobial activity, natural oral spray products, total oral bacteria, essential oil, essential oil with mangosteen extract, chlorhexidine mouthwash

1. Introduction

Nowadays, people in Thailand are living longer than ever before. This means a great increase in Thailand aging population. The progression of Thailand aging population has been accompanied by severe problem in oral health such as tooth loss, dental caries, oral candidiasis and severe periodontitis because aging can induce several risk factors. First, there are internal factors such as senescence of tissue, decline of immune functions. Second, there are external factors such as poly-medication due to systemic disease, malnutrition. Both factors which contribute to changes during aging may also disturb oral homeostasis. This can cause oral infectious disease (Bodineau A et al, 2009).

The primary care to prevent oral infectious disease is to alleviate of any predisposing factor and control plaque biofilm by using mechanical methods such as brushing, interdental cleansing or chemical methods such as mouthwash. Barnett (2006) has shown that the daily use of an antimicrobial mouthwash can play a role in controlling plaque biofilm and prevent oral infectious disease including periodontal disease. Moreover, the antimicrobial activity at mucosal sites throughout the mouth can have a significant impact on the supragingival and subgingival colonization of tooth by oral bacteria in six-month clinical study suggesting that effective mouthwash can be a useful component of oral hygiene regimens. The use of antiseptic mouthwash can improve oral hygiene and reduce oral bacterial flora.

For instance, dental caries is the one oral health problems that mostly occurred in the elderly. One of risk factors that can cause dental caries is oral bacteria. For example, *Streptococcus mutans* is the one that is a primary cause of enamel caries in young adults and root surface caries in the elderly. *Streptococcus mutans* can ferment sugar and generate weak acids such as lactic acid as metabolic end-product which can cause the plaque pH changes to below the critical pH for enamel demineralization and attain the critical pH more rapidly

than other common plaque bacteria. Moreover, severe periodontitis is another oral health problem in the elderly. It is also caused by oral bacteria such as *Porphyromonas gingivalis*. The presence of bacterial plaque represents the etiologic factor involved in the initiation and progression of periodontitis. Eliminating biofilm plaque by mechanical method or chemical method is the one that can prevent dental caries and severe periodontitis. Nowadays, a new approach in treating dental caries is the use of antiseptic agents which can control plaque biofilm (Krzyściak et al, 2014).

Chlorhexidine mouthwash is considered as the gold treatment for controlling the dental biofilm due to its efficacy against different kinds of bacteria, fungi, and viruses. However, it has some adverse effects such as changing in color teeth and mucosa, mucosal desquamation, alteration of taste perception, irritation, dryness of mouth, and side systemic effects as the result of swallowing were reported. Therefore, The World Health Organization (WHO) has recommended on finding the new natural sources such as the herbal extracts for overcoming on side effects of chemical agents (Rezaei et al, 2016).

At the present, there are many commercially available oral antiseptic agents that can treat oral infectious disease. Essential oils and mangosteen extract are both natural extracts. Natural extracts have been widely used in Thai medicine for treatment and for maintaining healthy condition. For instance, Janjić-Pavlović et al. (2017) showed that the use of essential oil mouthwash as an antiseptic solution can be treated denture stomatitis which is caused by *Candida albicans*. Moreover, essential oils also have an antibacterial effect against some cariogenic bacteria including *Streptococcus mutans* and *Lactobacillus casei* with minimal inhibitory concentration (MIC) values ranging from 31.2 to 500 mg/ml. Essential oil that extracts from *Tetradenia riparia* has a bactericidal effect against *S. mutans* for first 12 hours with direct cell contact similarly to chlorhexidine dihydrochloride. Another one is mangosteen extract. Mangosteen extract consisted of alpha-mangostin. Alpha-mangostin has a potential for oral candidiasis therapy. Kaomongkolgit et al. (2009) showed that alpha-mangostin was effective against *C. albicans* and more effective than Clotrimazole and Nystatin. As above, essential oils and mangosteen extract can treat oral candidiasis. Furthermore, alpha-mangostin showed the most potent antibacterial effect by inhibition of tyrosinase enzyme associated with glucan synthesis, against the pathogenic bacteria in the oral cavity including *S. mutans*, *Porphyromonas gingivalis*, and *Streptococcus pyogenes* at minimum inhibitory concentration (MIC) of 0.01 mg/ml, and *Staphylococcus aureus* at MIC of 0.1 mg/ml by agar dilution method (Tadtong et al, 2009). Owing to the strong bactericidal activity of mangosteen pericarp extract, it has been conclusively suggested to add into the composition of oral spray, oral paste and toothpaste for further development as an antibacterial agent.

Beside toothpaste and mouthwash, natural oral spray is another option that can be used for reducing bacterial flora in oral cavity. Oral spray is easy to use and beneficial in the elderly who has difficulties in brushing or become disabled to maintain their oral health. However, most of the studies of natural oral health care products are limited in the in vitro studies, and the antimicrobial activity of the Thai natural oral health care products including oral spray that extract from essential oils and mangosteen extracts in the in vivo studies remains unknown. Therefore, the purpose of this study is to investigate the antimicrobial activity of natural oral spray against oral bacteria and user satisfaction compared with chlorhexidine mouthwash.

2. Objective

The aim of this study is to investigate the antimicrobial activity and user satisfaction of natural oral spray products against oral microbiota compared with chlorhexidine mouthwash.

3. Materials and Methods

Two oral care natural products were selected based on a literature survey:
1. Myherbal mybacin trospray (Greater Pharma Co., Ltd.) – essential oil group
2.Myherbal mybacin trospray with mangosteen extract (Greater Pharma Co., Ltd.) – essential oil with mangosteen extract group. And 0.2% chlorhexidine mouthwash (Faculty of Dentistry, Chulalongkorn University) was used as positive control.

This randomized, double-blind controlled clinical trial and crossover clinical study was conducted at Geriatric Dentistry and Special Patients Care Clinic and the Department of Microbiology, the Faculty of

Dentistry, Chulalongkorn University. Prior to inclusion criteria in this study, subjects were informed about the purpose and the protocol of this study and also provided their consent to participation. This study was conducted on 21 patients who came to visit at Geriatric Dentistry and Special Patients Care clinic. Criteria for inclusion of patients in the study was good general health conditions or well-controlled chronic disease aged up to 50. Exclusion criteria was as follow: the use of any antibiotics or corticosteroids during this study in last 1 month, history of HIV or any immunosuppressive therapy and radiotherapy in the head and neck area, severe periodontitis and high caries risk assessed by oral hygiene caries risk assessment of the Faculty of Dentistry Chulalongkorn University modified from caries risk assessment by American Dental Association. This assessment has been used in operative dentistry and also geriatric dentistry and Special Patients care clinic, Faculty of Dentistry, Chulalongkorn University.

Sociodemographic data was obtained by filling out the questionnaire. The data consisted of age, gender, occupation, medical conditions, marital family situation, oral hygiene care, dietary habits, smoking habits, alcohol drinking, and presence of prosthesis. After collecting the sociodemographic data, saliva samples will be collected as described as baseline before routine scaling and oral hygiene instructions were given to all participants to remove all dental deposits and tried to set the same mechanical approach of oral care by the researcher.

Two weeks after routine scaling, participants were appointed to collect saliva samples. For collecting the saliva, the participant was instructed to avoid food intake for 2 hours before saliva collection. Unstimulated saliva was collected from all subjects by spitting method into a sterile container (50 ml. collection tube) during a 20-min period. Then, participants were randomly allocated into 2 interventions with different sequence uses of oral spray natural products. All interventions had the same duration of time included 14 days for the first oral spray product, 14 days for wash out period, then 14 days for the second oral spray product, 14 days for second wash out period and 14 days for chlorhexidine mouthwash as a positive control. Chlorhexidine mouthwash was used as a spray with the same container of oral spray natural products in order to mimic the same situation as natural oral spray and maintain the same volume flux. Participants were instructed to point the spray nostril towards buccal mucosa of both sides in the mouth and spray 2 times on each side and used 2 times a day. The researcher also emphasized all participants to maintain the volume flux and the distance between oral spray and oral cavity through the study.

One day after each period of time, participants were appointed for saliva collection with the same protocol of the first visit. Consequently, each participant was appointed for saliva collection 7 times (baseline before scaling, before and after using the first oral spray, before and after using the second spray, before and after chlorhexidine mouthwash). And after use of each oral care product for 14 days, all participants were informed their use as a satisfaction score as an analogue scale categorized including taste, smell, burning sensation, ease of use, and overall of oral care product. As you see below (Figure1).

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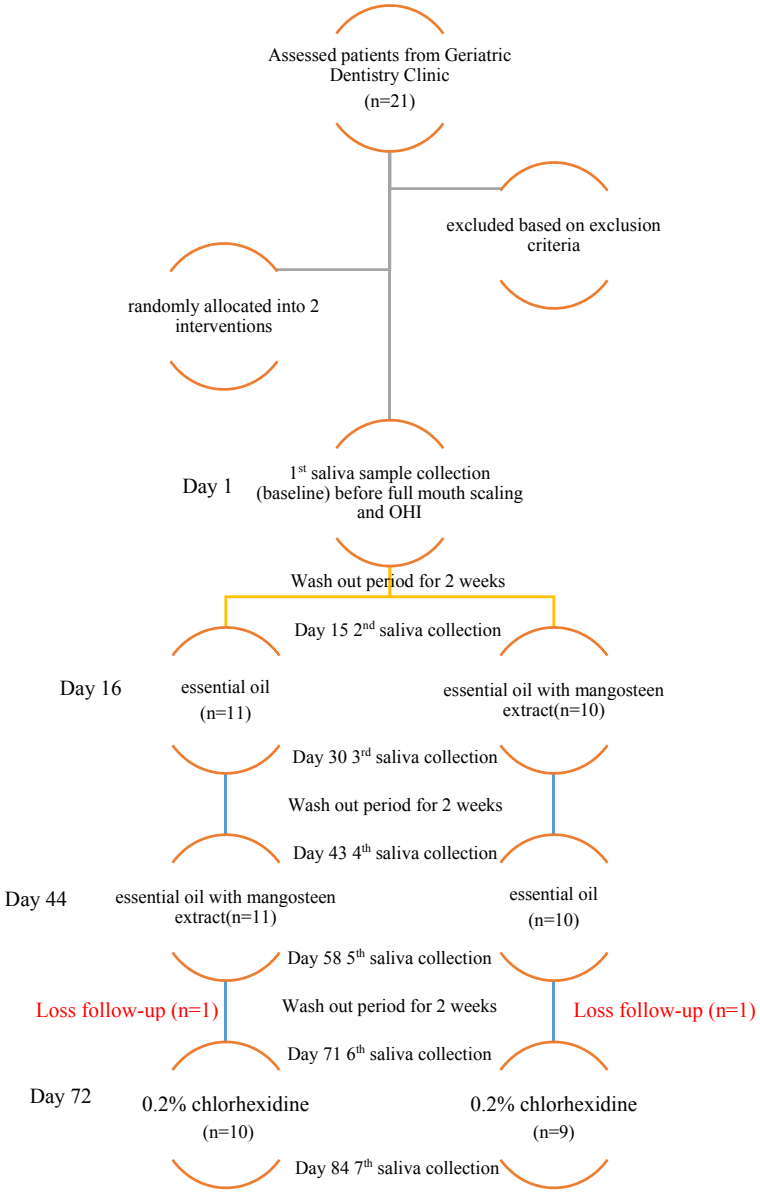

Figure1 Chart of the method of this research

Oral microbiota culture

The oral microbiota was characterized through a culture of saliva samples for microbiological analysis and determination of the microbial load.

The sample's saliva was used to culture with Nutrient agar to grow total bacteria. The number of total bacterial colonies (colony forming units-CFU) were counted. The CFU was counted in the appropriate dilution (dilution factor) with the help of a stereoscopic microscope and the quantities were registered to calculate the number of CFU per milliliter (ml) of sample as you see in Figure 2.

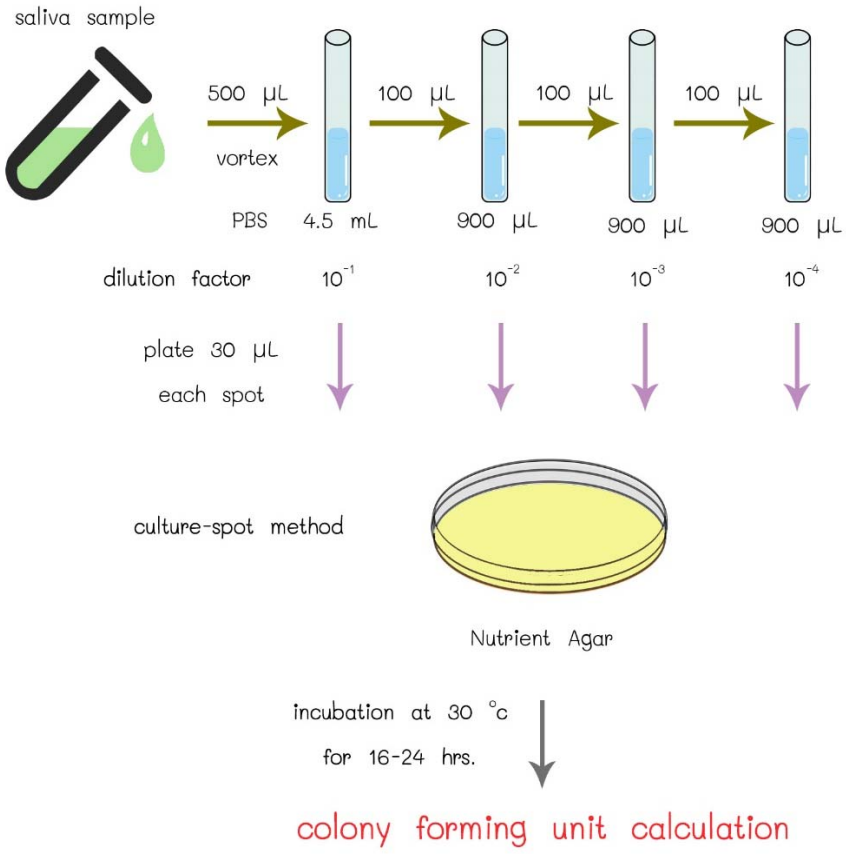

Figure 2 Oral microbiota culture method

Statistical analysis

All statistical computations were performed by SPSS for Windows (version 22.0; SPSS, Inc., Chicago, IL, USA). Description of the sample was carried out by descriptive statistics methods. With all statistical data, Shapiro-Wilk test was used to test for normality. Data from microbiological culture were presented as mean colony forming units with its standard deviation (mean log CFU ± SD). To compare the differences of baseline among natural oral sprays and chlorhexidine mouthwash, Repeated ANOVA was used. Differences in colony forming unit count within a group were analyzed by Wilcoxon's test. To compare the differences in reduced mean colony forming unit count (Δ)(CFU/ml) and satisfaction score including taste, smell, burning sensation, function, and overall between groups, Friedman's test was used. The statistical significance was defined as $P < 0.05$.

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4. Results and Discussion

4.1 Characteristics of the population

Table 1 summarizes the subject characteristics. Shortly, the mean years of age was 60 (standard deviation= 11.12; range= 50-91). 52.38% were male and also 52.38% had been still working their careers. 66.67% was married. The subjects were relatively healthy with 80.95% reporting no medical conditions and no smoking history and 76.19% no alcohol drinking. Most subjects showed good oral hygiene practices (85.71% brushing > once a day and 61.90% using interdental cleansing).

Table 1 characteristic of the population

Characteristic of the population	
Number of subjects (n)	21
Male, n (%)	11(52.38)
Age, mean (SD)	60(11.12)
Systemic health condition, n (%)	
Healthy	17(80.95)
Controlled	4(19.05)
Occupation, n (%)	
Formal/informal work/still working	11(52.38)
Retired	6(28.57)
Housewife	4(19.05)
Marital status, n (%)	
Single/separated/divorced	7(33.33)
Married with partner	14(67.67)
Smoking status, n (%)	
Current	1(4.76)
Former	3(14.29)
Never	17(80.95)
Alcohol drinking, n (%)	
Current	1(4.76)
Former	4(19.05)
Never	16(76.19)
Frequency of brushing, n (%)	
< once a day	1(4.76)
Once a day	2(9.52)
> once a day	18(85.72)
Frequency of having snacks, n (%)	
More than 3 times a day	1(4.76)
1-2 times a day	11(52.38)
Eat only between meals	9(42.86)
Presence of prosthesis, n (%)	
No	6(28.57)
Yes	15(71.43)
Only removable	2(13.33)
Only fixed	8(53.33)
Both	5(33.33)

4.2 Similar oral bacteria counts before use of each natural oral sprays

The experimental periods of this study extended up to 84 days. Two participants were lost after collecting salivary baseline before chlorhexidine treatment, and those were excluded. All samples (n=21) were collected after 14 days for the wash-out period, and followed by 14-day use of each natural oral spray or chlorhexidine. The mean log colony forming units as baseline of essential oil, essential oil with mangosteen extract and chlorhexidine group were 7.41, 7.33 and 7.58. The results showed that oral bacteria colony forming units were statistically no differences among natural oral sprays and chlorhexidine mouthwash (ρ-value=0.541). According to the before use data (Figure 3), the prior 2-week washout period is appropriate for the recovery of total bacteria regrowth measures that ensure the least possible previous oral care product

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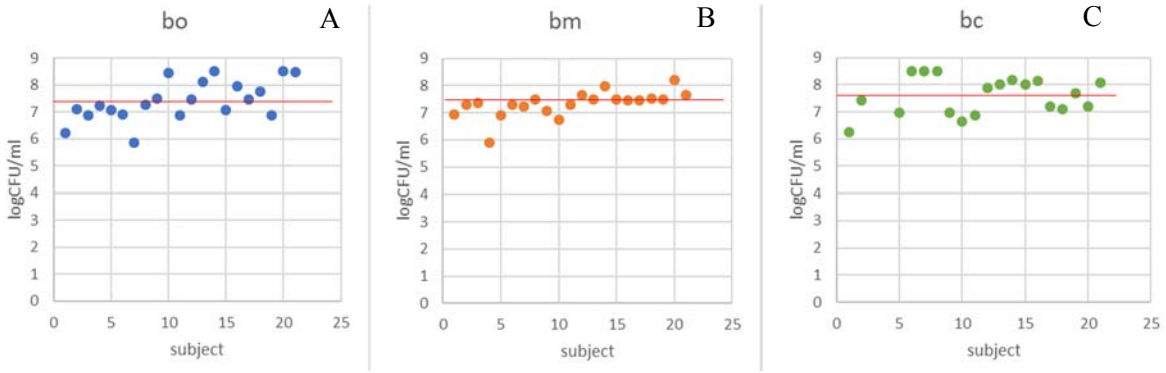
effect were worn off. Therefore, the results were consistent to a study by Bascones et al. (2005) that 2-week washout period is adequate for the recovery of natural oral bacteria level.



bo = baseline of essential oil group
bm= baseline of essential oil with mangosteen extract group
bc = baseline of chlorhexidine group
red line referred as the mean of colony forming units (mean log CFU/ml)

Figure 3 The total bacterial counts in saliva samples. (A) The number of colony forming units before use of essential oil. (B) the number of colony forming units before use of essential oil with mangosteen extract. (C) the number of colony forming units before use of chlorhexidine. Each dot represents the mean from three replicates. Data was analyzed using Repeated ANOVA (ρ-value = 0.541).

4.3 Reduction of total bacterial counts after using natural oral spray or chlorhexidine

Table 2 Total bacterial counts in saliva sample before and after using natural oral spray or chlorhexidine

Oral care product group	Colony forming unit Median Log CFU/ml (IQR)		ρ-value
	Before intervention	After intervention	
Essential oil group	7.48 (1.20)	7.46 (1.16)	0.004*
Essential oil with mangosteen extract group	7.47 (0.28)	7.40 (0.67)	0.01*
Chlorhexidine group	7.68 (1.17)	7.00 (1.29)	0.003*

IQR = interquartile range
* Paired sample, Wilcoxon Signed Rank test demonstrates significance of differences between before and after intervention.

After 14-day continuous use of each natural oral spray or chlorhexidine, the result showed that natural oral sprays and chlorhexidine statistically reduced the total colony forming unit. The median of colony forming units were changed including 7.46 to 7.48 in essential oil group, 7.47 to 7.40 in essential oil with mangosteen extract group and 7.68 to 7.00 in chlorhexidine group. (Table 2) Chlorhexidine mouthwash can reduce oral bacterial number slightly more than other mouthwash, however, the reduction of colony forming units were not statistically significant (Figure 4).

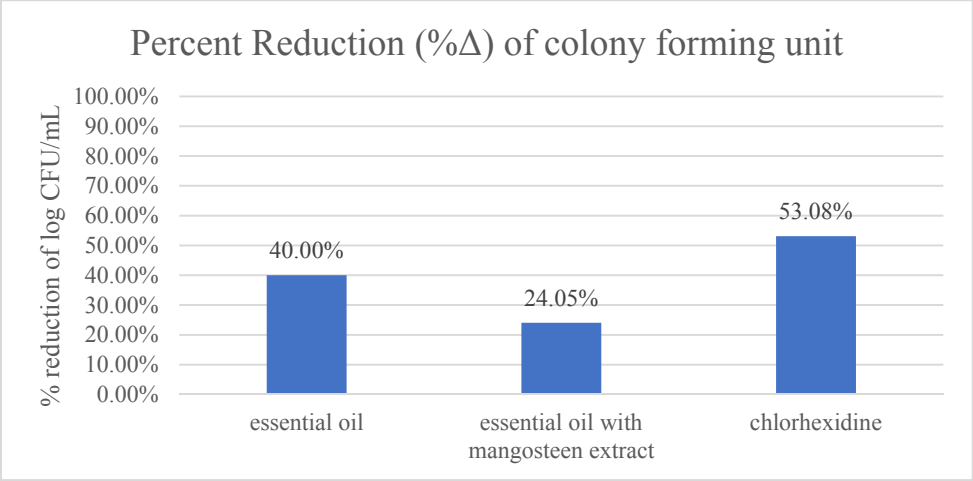


Figure 4 the reduction of total oral bacteria after using the mouthwashes. Percent Reduction (%Δ) of colony forming unit after the 2-week intervention (natural oral sprays or chlorhexidine) is demonstrated. Differences of the intervention is analyzed by Friedman's test Rank test (ρ-value = 0.76).

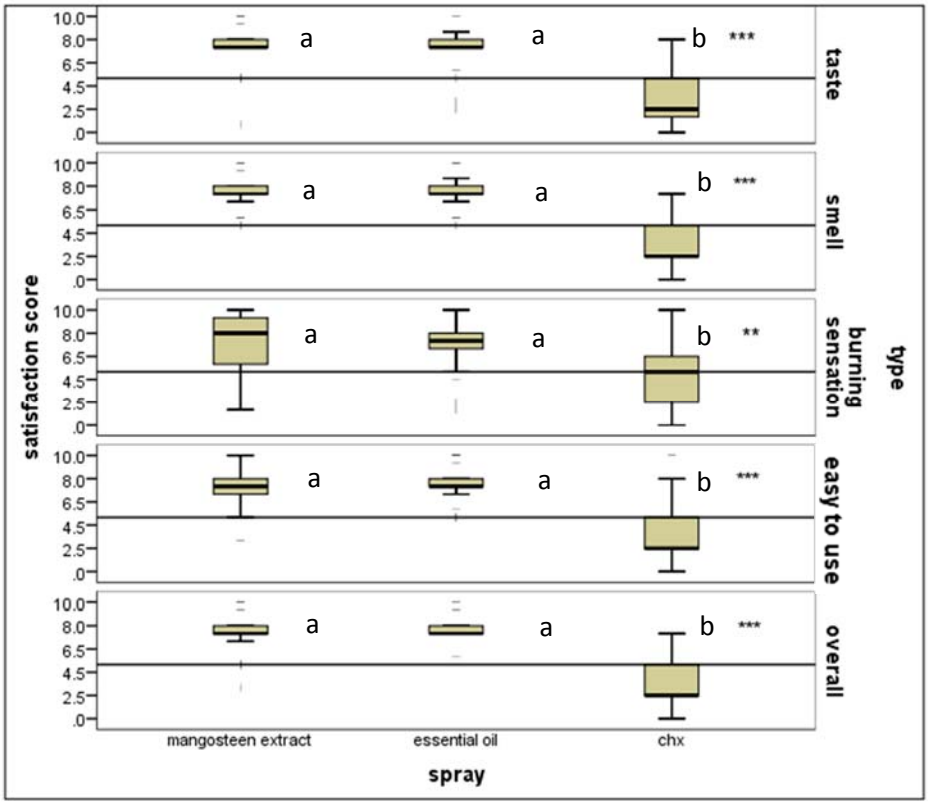
Consistent with previous study (Araujo et al, 2015), essential oil possessed antimicrobial activity against oral bacteria associated with gingivitis and plaque in meta-analysis of 6 months clinical trials supported that daily use of essential oil-containing mouthwash as adjunctive to mechanical cleansing approach statistically improved oral hygiene in individuals with lead to prevention of oral infectious disease progression. Moreover, Fine et al. (2000) demonstrated that essential oil mouthwash use for 11 days produced antimicrobial activity against *S. mutans* by reduction of 39.2% in saliva.

In addition to Ibrahim et al. (2016), the study found that mangosteen extract consists of alpha-mangostin possessed antioxidative, antibacterial, antiviral, antifungal, anti-allergic and anti-inflammatory properties against oral microbiota. For example, alpha-mangostin has antimicrobial activity against pathogenic bacteria in the oral cavity including *S. mutans, Porphyromonas gingivalis*, and *Streptococcus pyogenes* in *in vitro* study. In addition, it revealed that alpha-mangostin is a reliable agent that can be used in the prevention and treatment of a wide array of human pathological conditions especially inflammatory-based processed. This study supported previous studies that essential oil and essential oil with mangosteen extract have antimicrobial activity against oral microbiota compared with chlorhexidine mouthwash. However, this study was not demonstrated that combination with mangosteen extract has more synergistic effect against oral bacteria compared with essential oil only.

In contrast with previous study of McKenzie et al. (1992), using essential oil mouthwash for 2 weeks in mentally handicapped adults did not significantly reduce plaque index score and have no clinically significant improvements in periodontal status compared with 0.12% chlorhexidine mouthwash.

An oral spray product may be used as adjunctive treatment to conventional therapy. There are many natural oral spray products available in the commercial market. This study demonstrated that natural oral spray products containing essential oil and essential oil with mangosteen extract possessed antimicrobial activity, and decreased salivary bacteria. A decline in the number of oral microbiota suggests a decrease of risk in oral infectious diseases (Axelsson et al, 1991).

4.4 Satisfaction score of each natural oral spray



Groups with the same superscript lowercase letters were not significantly different at ρ< 0.05

Figure5 Satisfaction score of natural oral spray as compared to chlorhexidine mouthwash. After using the oral spray, participants rated satisfaction scores based on 5 different categories including taste, smell, burning sensation, easy-to-use product, and overall rating. (*** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$)

Satisfaction scores of oral care products based on all categories are shown in Figure 5. In all categories, especially taste, smell, easy-to-use product, and overall, essential oil and essential oil with mangosteen extract show a significantly higher satisfaction score than chlorhexidine mouthwash. We concluded that most participants expressed better user satisfaction than chlorhexidine mouthwash.

In addition, the prolonged use of chlorhexidine mouthwash may cause adverse effect including brown discoloration of the teeth, which is the most common adverse effect, yellow-brown-colored stains that turn black on the dorsum and lateral surface of tongue, alteration of taste perception and oral mucous membrane irritation and desquamation due to idiosyncratic reaction (Richards D., 2017). However, participants in this study reported no unfavorable effect of natural spray products. Most participants preferred using essential oil and essential oil with mangosteen extract more than chlorhexidine mouthwash because of taste, smell, burning sensation and function. Therefore, it is possible that essential oil and essential oil with mangosteen extract are more favorable than chlorhexidine mouthwash suggesting higher patient compliance in elderly patients.

A limitation of this study was that the study lacks information regarding adhesion of each oral spray to mucosal site or how long oral spray retains to oral cavity. Future studies should be performed as randomized controlled trials in vivo that control other environment factors such as pH, saliva, dietary habits and especially oral hygiene care in individuals that may influence the effect of oral spray products. Moreover, future studies should include clinical isolates of specific microbiota such as *Candida albicans*, *Streptococcus mutans* and *Porphyromonas gingivalis* which are the key pathogens associated with these common oral

infectious diseases that mostly occurred in the elderly. Investigating the activities on multispecies of dental biofilm should be carried out in order to mimic the real situation of oral cavity. Moreover, future studies with increased sample size, sensitivity, and higher reliability microbiological techniques such as Quantitative PCR analysis and DNA sequencing of specific species should be performed. Finally, the result showed that essential oil and essential oil with mangosteen extract possessed antimicrobial activity which are not significant from chlorhexidine mouthwash. Further in vivo investigations are needed to show more therapeutic outcomes of these oral spray products as herbal extracts or find more new natural resources against oral pathogens for overcoming the side effect of chlorhexidine mouthwash such as tooth discoloration and desquamation of oral mucosa.

5. Conclusion

Therapeutic outcomes after continuous use of natural oral sprays are not significant to the therapeutic outcomes obtained by chlorhexidine mouthwash in antimicrobial activity against oral microbiota. Most elderly patients expressed better user satisfaction than chlorhexidine mouthwash in all categories involved with the side effects of using chlorhexidine mouthwash. This study supported the use of natural products for overcoming side effects of chlorhexidine mouthwash as a chemical agent and more favored patient compliance especially in the elderly who become disabled to maintain their oral health or has difficulties in using their hands for mechanical approach. Further research with higher sensitivity and higher reliability technique may be needed.

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