Antimicrobial effect against oral microbiota after continuous use of natural oral spray products

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ผลการต้านเชื้อจุลินทรีย์ในช่องปากหลังจากใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ชานน สุวรรณประพิศ : ผลการต้านเชื้อจุลินทรีย์ในช่องปากหลังจากใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง. (Antimicrobial effect against oral microbiota after continuous use of natural oral spray products) อ.ที่ปรึกษาหลัก : ผศ. ทญ. ดร. อัญชลี วัชรักษะ, อ.ที่ปรึกษาร่วม : รศ. ทญ. ดร.ทิพวรรณ ธราภิวัฒนานนท์

สารสกัดจากธรรมชาติหลายชนิดมีฤทธิ์ต้านเชื้อจุลินทรีย์ที่ก่อโรคในช่องปากที่พบได้บ่อยในผู้สูงอายุ อาทิเช่น โรคฟันผุและโรคเชื้อราแคนดิดาในช่องปาก อย่างไรก็ตามยังไม่มีการศึกษาถึงผลของผลิตภัณฑ์ดูแลสุขภาพช่องปากที่มีสารสกัดจากธรรมชาติหลังผ่านกระบวนการทางอุตสาหกรรม และการทดลองที่ผ่านมาส่วนใหญ่เป็นการศึกษาในห้องปฏิบัติการ การศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลการเชื้อจุลินทรีย์ในช่องปากในผู้สูงอายุหลังจากใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติชนิดฉีดพ่นในช่องปากอย่างต่อเนื่อง

วิธีการ คัดเลือกอาสาสมัครจากคลินิกทันตกรรมผู้สูงอายุและการดูแลผู้ป่วยพิเศษจำนวน 21 คน โดยการใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากชนิดฉีดพ่นที่ผลิตจากน้ำมันหอมระเหย และน้ำมันหอมระเหยผสมสารสกัดจากเปลือกมังคุด และน้ำยาบ้วนปากคลอร์เฮ็กซิดีนร้อยละ 0.2 เป็นตัวแปรควบคุมเชิงบวก โดยแต่ละชนิดใช้ระยะเวลาในการใช้ผลิตภัณฑ์เป็นเวลา 2 สัปดาห์ และเว้นช่วงในการใช้ผลิตภัณฑ์ชนิดต่อไปเป็นเวลา (wash-out period) 2 สัปดาห์ วันละ2 ครั้งเช้าเย็น วัดผลจากน้ำลายอาสาสมัครก่อนและหลังการใช้แต่ละผลิตภัณฑ์ โดยการเพาะเชื้อด้วยอาหารเลี้ยงเชื้อและการทำ PCR หลังจากการใช้ผลิตภัณฑ์แต่ละชนิด อาสาสมัครจะได้รับแบบประเมินความพึงพอใจหลังการใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปาก

ผลการศึกษา ผลิตภัณฑ์ดูแลสุขภาพช่องปากชนิดฉีดพ่นที่ผลิตจากน้ำมันหอมระเหย และน้ำมันหอมระเหยผสมสารสกัดจากเปลือกมังคุด สามารถลดเชื้อจุลินทรีย์ได้อย่างมีนัยสำคัญในอาหารเลี้ยงเชื้อชนิด nutrients agar หลังจากใช้เป็นระยะเวลา 2 สัปดาห์ และอาสาสมัครมีความพึงพอใจต่อผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติ เมื่อเปรียบเทียบกับการใช้น้ำยาบ้วนปากคลอร์เฮ็กซิดีนร้อยละ 0.2 ในแง่ของรสชาติ กลิ่น การแสบร้อนในช่องปาก การใช้งาน และโดยรวมอย่างมีนัยสำคัญ

สรุปผลการศึกษา การใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากชนิดฉีดพ่นในช่องปากอย่างต่อเนื่อง อาจเป็นอีกหนึ่งทางเลือกในการใช้เพื่อต้านเชื้อจุลินทรีย์ที่ก่อโรคในช่องปากในผู้สูงอายุได้

สาขาวิชา	ทันตกรรมผู้สูงอายุและการดูแลผู้ป่วยพิเศษ	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORD: ANTIMICROBIAL ACTIVITY / NATURAL ORAL SPRAY / STREPTOCOCCUS MUTANS / CANDIDA ALBICANS

Chanon Suwanprapis : Antimicrobial effect against oral microbiota after continuous use of natural oral spray products . Advisor: Asst. Prof. ANJALEE VACHARAKSA, D.D.S., Ph.D. Co-advisor: Assoc. Prof. THIPAWAN THARAPIWATTANANON, D.D.S., Ph.D.

So far as we know there were several natural extracts possessed antimicrobial activity against oral microbiota. These natural extracts can be used as an alternative to replace chlorhexidine mouthwash which retains unfavorable flavor and also has some adverse effects. However, the antimicrobial activity of the natural extracts in commercial products have never been demonstrated especially in vivo studies. 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. Participants had used two types of commercial 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. To determine the antifungal and antibacterial activity, 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 suggests that the oral spray with essential oil and mangosteen extract demonstrates effective antimicrobial activity and favored patient compliance.

Field of Study:	Geriatric Dentistry and Special Patients Care	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

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Chanon Suwanprapis

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Chapter I

INTRODUCTION

Background and Rationale

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 factor such as senescence of tissue, decline of immune functions. Second, there are external factor 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. (1)

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 method such as mouthwash. Barnett (2006) has shown that the daily use of an antimicrobial mouthwash can play 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 and suggesting that effective mouthwash can be a useful component of oral hygiene regimens. The use of antiseptic mouthwash can improve oral hygiene and reduced oral bacterial flora. (2)

For instance, dental caries is the one oral health problems that mostly occurred in elderly. One of risk factor 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 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 elderly. It 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 (3)

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. (4)

At the present, there are many commercially available oral antiseptic agents that can treat oral infectious disease. Essential oils and mangosteen extract are both two of them which are 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 oils 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 similar to chlorhexidine dihydrochloride. (5) 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. (6) 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. (7) 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 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

Research questions

Do natural oral spray products on the market have an antimicrobial effect in comparison with chlorhexidine oral rinse

Research objectives

1. To investigate the antimicrobial activity of natural oral sprays against oral microbiota.

2. To compare patients' satisfaction of natural oral spays and chlorhexidine

Research hypothesis

Natural oral sprays have antimicrobial effect against oral microbiota.

Scope of Research

This research aims to study an antimicrobial effect against oral microbiota after continuous use of natural oral spray products collected from different sociodemographic characteristics in private dental clinic and dental hospital at the Faculty of Dentistry, Chulalongkorn University.

Limitation

A limitation of this study was that the study lacks information regarding adhesion of each oral spray to mucosal site or how long did oral spray retain 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, the number of subjects based on inclusion criteria and DNA extraction kit are limited.

Expected Outcomes

1. Obtain the data that can help deciding what oral natural spray products to be used in elderly patient.

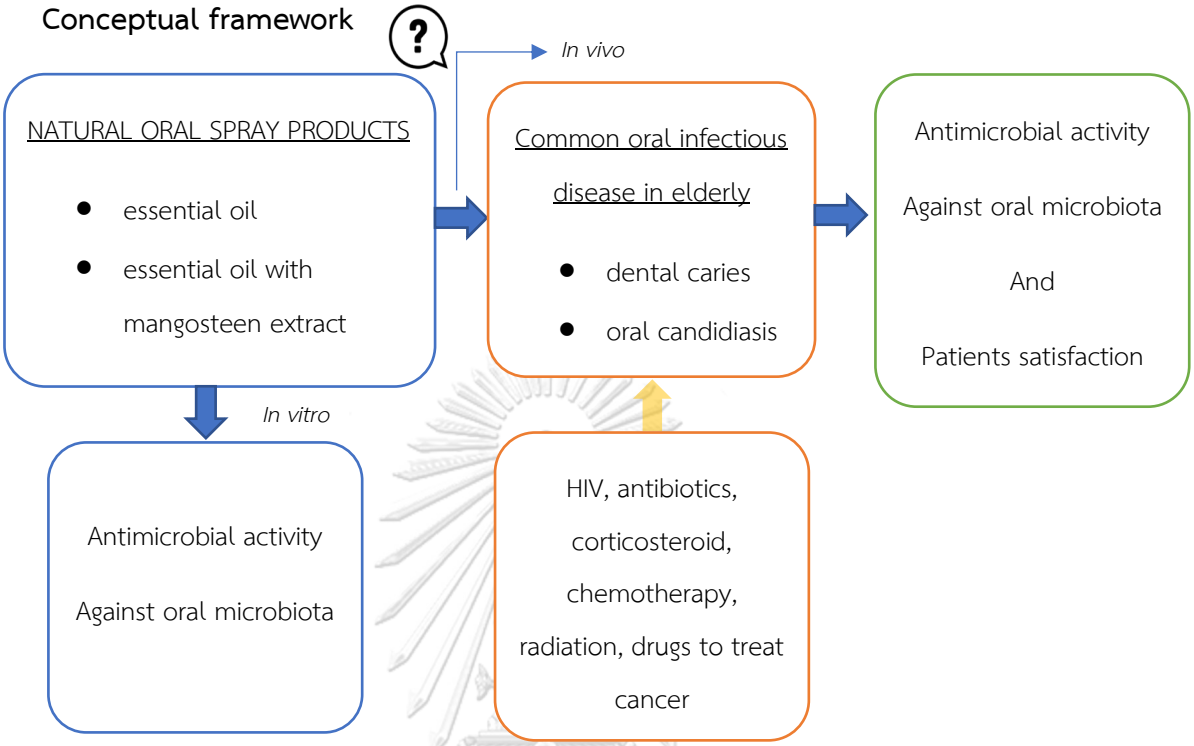
Keyword

ANTIMICROBIAL ACTIVITY / NATURAL ORAL SPRAY / STREPTOCOCCUS MUTANS / CANDIDA ALBICANS

Research design

Clinical and laboratory research

Conceptual framework



Chapter II

LITERATURE REVIEW

Saliva

Saliva is a complex fluid that consists of water, electrolytes, mucus and enzymes. It is secreted into the oral cavity by the parotid, submandibular, sublingual, and minor salivary glands. All of salivary glands have secretory cell units called acini that can produce saliva. Saliva can flow out to oral cavity by collecting duct and alter the composition of electrolytes within the duct. When it came out through the oral cavity, it becomes contaminated with bacteria, food residues, epithelial cells, and exudate from the gingival sulcus. Saliva has many roles that are essential for human species such as digestion, lubrication, protection, and taste perception. (8, 9)

Saliva can be as a diagnostic fluid for detection specific disease such as autoimmune diseases, cardiovascular diseases, diabetes, HIV, oral cancer, caries and periodontal diseases. As above, caries and periodontal diseases are both of all specific disease that saliva can be as a diagnostic marker. Saliva can show the quality and quantity of oral microbiota in order to detect or monitor caries and periodontal disease. (10) Although, L Denepitiya, I Kleinberg revealed that plaque contained more bacteria than salivary sediment per unit mass which was attributed mainly to the presence of more non-viable epithelial cells in salivary sediment than in plaque. (11)

Natural oral spray products

- Essential oils

The use of essential oils as an adjunctive treatment has been used worldwide in medicine similar to dentistry. Nowadays, essential oils have been used in dentistry to many forms of oral health care products including the composition of dentifrices, mouthwash and oral spray. Essential oils have both antifungal and antibacterial effects depending on concentration and duration of time.

Now, essential oils can be used as the treatment of denture stomatitis instead of using nystatin and miconazole due to the fact that these drugs may be less efficient

against biofilm. Ognjenka et al. showed that the use of essential oils mouthwash as antiseptic solution can be treated denture stomatitis. It reduces the inflammation intensity and fungal colony-forming units number on palatal mucosa by use Listerine® cool mint TM (Johnson&Johnson, S.p.A. Rome, Italy). Listerine® acts directly against fungal cell, chemically, causing damage to the cell wall structure and membrane permeability. Also, it disrupts metabolic processes dependent on microorganism membrane enzymes and also has anti-inflammatory effect. It acts against free unbound candida cells as well as against formed biofilm and it is shown to be more efficient than Daktanol® oral gel (2% miconazole, Galenika a.d. Belgrade, Serbia) (5)

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 similar to chlorhexidine dihydrochloride. (12) Isabel Prada-López et al. found that antibacterial effect against oral biofilm of essential oils solution compared with 0.2% chlorhexidine. The oral biofilm was formed in situ on the glass disks in intraoral device overlaid disk holding splint made from vinyl sheet. The intraoral device overlaid disk holding splints were worn intraorally for 4 days, except during meals and oral hygiene maintenance. Essential oils solution showed an effective antibacterial effect against oral biofilm in situ. Although, 0.2% chlorhexidine is more effective than essential oils solution to reduce the thickness and bacterial mass of the biofilm, the number of vital cells remaining in biofilm is similar to essential oils treatment. (13) A meta-analysis of 6 months clinical trials showed clinically significant for the use of essential oil containing mouth rinses as an adjunctive treatment for plaque control in gingivitis cases. (14)

- Mangosteen extract

Mangosteen extract has been used in medicine especially in Southeast Asia because of the antioxidative, antibacterial, antiviral, antifungal, anti-allergic and anti-

inflammatory properties of xanthones, the active ingredients found in many parts of mangosteen pericarp. Xanthones include such as alpha, beta and gamma mangostins. Particularly, 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 met hod. (7) 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. The antibacterial effect varies on the cultivated area of mangosteen, extraction protocol and strain of microbes. Time-kill assay of mangosteen pericarp extract found that at 60 min, *S. mutans* treated with mangosteen extract at the concentrations four times higher than minimum bactericidal concentration , or 2.5 µg/ml, was decreased viable cell count, and completely killed at 90 min. Moreover, the time-kill kinetics which using the crude extract of mangosteen at 160 µg/ml, the crude extract completely killed the bacteria within 15 minutes. (15)

Furthermore, mangosteen extract also has an antifungal effect against candida albicans. The study found that Alpha-mangostin at 4,000 µg/ml, or lower, was demonstrated in cytotoxicity test not to be toxic to human gingival fibroblast. Alpha-mangostin was effective against *C. albicans*, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were 1,000 and 2,000 µg/ml. The antifugal activity of alpha-mangostin was more effective than Clotrimazole and Nystatin (6)

Chlorhexidine

Chlorhexidine is considered as a gold standard antimicrobial agent. Chlorhexidine has been widely used in dentistry as an antiplaque and antigingivitis agent due to high affinity for oral structures. It acts on bacterial cell membrane and increase the permeability resulting to cell lysis. It can be bacteriostatic or bactericidal depending

on dose. It is a broad-spectrum antiseptic agent because it can act against many species of microbiota including not only gram positive and gram-negative bacteria but also dermatophytes, lipolytic viruses, fungi, yeasts and some viruses including Hepatitis B virus and Human Immunodeficiency Virus. It acts against *S. mutans* making it anticariogenic in nature. (16) A meta-analysis of clinical studies reveal that the average caries-inhibiting effect from the chlorhexidine treatment studies in caries prevention was 46% (95% CI = 35% - 57%). (17) Nevertheless, it has many side effects of long term using Chlorhexidine such as brown discolouration of the teeth, restorative materials and dorsum of tongue, alteration of taste perception, oral mucosal erosion due to idiosyncratic reaction and dose dependent, bitter taste. (16)

Streptococcus mutans

Streptococcus mutans is a cocci facultative anaerobic gram-positive bacteria. There are cariogenic mutans groups which is the major agents of dental caries. However, without the predisposing factor such as sucrose, they cannot cause dental caries. (18) *S. mutans* have a characteristic ability to produce soluble and insoluble extracellular polysaccharides from dietary carbohydrates called glucan, mutan and fructan that are related to oral biofilm formation leading to dental caries. *S. mutans* can ferment sugar and generate weak acids including lactic, formic and acetic acid as metabolic end product. Especially, Lactic acid, the strongest acid, can cause the plaque pH changes to below the critical pH for enamel demineralization. *S. mutans* show the ability to attain the critical pH for enamel demineralization more rapidly than other common plaque bacteria. They are able to grow and survive under the acidic conditions they generate, by the induction of a specific molecular stress response. (3) Several epidemiological studies have revealed that *S. mutans* is a primary cause of early childhood caries in infants, enamel caries in children and young adults, and root surface caries in the elderly. (19, 20)

Lactobacillus casei

Lactobacillus is a rod, facultative anaerobic gram-positive bacteria. There are categorized into two main groups; homofermenters which are the major metabolic end product of glucose fermentation is lactic acid including *L. casei***.** They are highly acidogenic and acid-tolerant bacteria. Another one is heterofermenters which produce lactic acid as well as acetate, ethanol, and carbon dioxide. (18) Even though lactobacilli are commonly found from the oral cavity, they are hardly isolated from incipient lesions frequently isolated from deep carious lesions. (21) On the other hand, *L. casei* was shown to inhibit the growth of *S. mutans* and *S. sobrinus*, as well as, periodontal pathogens *P. gingivalis*. Therefore, lactobacilli may have a beneficial role during the initiation of disease through interaction between bacterial species. (22) Moreover, the number of lactobacilli in saliva can be an indication of an individual's caries activity. Although this test is not very reliable, it is useful for monitoring the dietary profile of a patient because the level of lactobacilli significantly associates with the intake of dietary carbohydrate. (18, 21, 22)

Oral candidiasis

Oral candidiasis is the one of oral infectious disease caused by yeast of the genus Candida and mainly *C. albicans*. Candida are true opportunistic pathogens and express oral infection when there is an underlying predisposing condition in the host such as the use of antibiotics and corticosteroids, chemotherapy, malnutrition and aging. Moreover, a greater use of invasive clinical procedures and a more widespread use of immunosuppressive therapies have also contributed to the oral infectious disease. (23, 24)

Oral candidiasis mainly occurred in elderly and very young person. Nowadays, the treatment of oral candidiasis becomes questionable because of the potential toxicity of traditional antifungal agents against host cells. They also have drug resistance against traditional antifungals. Oral candidiasis is most frequently superficial, occurring on moist mucosal surfaces in individuals. Candida express the virulence

factor including the ability to adhere to host mucosal surfaces, producing filamentous growth forms and then releasing hydrolytic enzymes resulting to induce damage to host cells. (25)

Candida infections can be treated by three groups of pharmapheutical agents including the polyenes, the azoles and the DNA analogues based on the type and severity of infection. Superficial infections can be treated topically with a polyene group (nystatin or amphotericin) or an imidazole (miconazole, clotrimazole). Polyenes are very effective for oral candidal infections. Systemic infections and disseminated candidiasis require intravenous amphotericin, either alone or in combination with flucytosine. The triazole agent fluconazole, effective for both superficial and systemic mycoses, is the drug of choice in treating Candida infections in HIV patients. The use of newer agents such as echinocandins and terbinafine in dentistry are as yet ill defined. (26)

Candida albicans

Candida albicans is the species most commonly found in humans such as to the oral cavity, gastrointestinal tract, female genital tract and sometimes the skin. Moreover, *C. albicans* occurred in the mouths of up to 80% of healthy individuals. *C. albicans* typically grows as spherical to oval budding yeast cells. They can grow on Sabouraud medium as creamy-white colonies, flat or hemispherical in shape with a beer-like aroma. *C. albicans* may be differentiated from other candida species by their ability to produce germ tubes and chlamydospores whereas other candida species do not. However, definitive identification of the species is made on the basis of carbohydrate assimilation (aerobic metabolism) and fermentation (anaerobic metabolism) reactions and other biochemical tests such as polymerase chain reaction (PCR). (18)

C. albicans presence rarely causes disease in healthy individuals. The most prevalence induced by *C. albicans* is found in immunocompromised person. Moreover, Daniluk et al. showed that *C. albicans* is found more often in denture-wearing person comparing to non-denture-wearing persons. The inflammation intensity degree is based on how contaminate the denture base is. (27)

Oral microbiota in aging

Microbiota in aging varies in individuals. It depends on the immunological condition of the host and also the therapeutic procedures such as the use of antibiotics and corticosteroids, chemotherapy.

Compare between adults and older adults in Poland, pawel j. zaewadski et al. showed that potentially pathogenic microbiota identified in oral cavities (see in table-1) is clear differences in the prevalence of particular strains. Protista is rarely in children, but more frequent in older people. Next, Fungi can isolate and identify from the oral cavities of all age group. *C. albicans* were the species most frequently associated with normal oral human flora. Lastly, among gram-positive bacteria strains, *Enterococcus faecalis* were detected in all age groups. (28)

Table 1 Potentially pathogenic microbiota identified in oral cavities of patients of particular age groups. (28)

Age of patients	Microbiota identified in oral cavities		
	Protista	Fungi	Bacteria
41-50 yr.	*Trichomonas.tenax* *Entamoeba.gingivalis*	*Candida albicans* *Candida glabrata* *Candida spp.*	Gram-positive bacteria *Enterococcus faecalis* *Enterococcus faecium* *Staphylococcus epidermidis* *Staphylococcus aureus* *Micrococcus luteus* *Gram-negative bacteria* *Enterobacteriaceae:* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Non-Enterobacteriaceae:* *Acinetobacter baumannii* *Pseudomonas aeruginosa*
51-60 yr.	*Trichomonas.tenax* *Entamoeba.gingivalis* *Acanthamoebasp.*	*Candida albicans* *Candida spp.*	Gram-positive bacteria *Enterococcus faecalis* *Staphylococcus epidermidis* *Staphylococcus aureus* *Gram-negative bacteria* *Enterobacteriaceae:* *Pantoea agglomerans* *Escherichia coli* *Non-Enterobacteriaceae* *Pseudomonas aeruginosa*
61-70 yr.	*Trichomonas.tenax* *Entamoeba.gingivalis*	*Candida albicans* *Candida spp.*	Gram-positive bacteria *Enterococcus faecalis* *Staphylococcus epidermidis* *Gram-negative bacteria* *Enterobacteriaceae:* *Escherichia coli*

In healthy adults, R. S. Percival et al. 1991 showed that the effect of age on quantitative or qualitative differences in selected bacteria of dental plaque and whole saliva, *S.mutans* and *spirochetes* were similar in all age groups. In contrast, viable counts and proportions of *lactobacilli* and *actinomyces* shifted with age. (29)

In hypertensive adults, the microbial load of streptococci and staphylococci were significantly higher than normal blood pressure and candida yeasts were detected in the saliva culture of most samples. In addition, Arthur leibovitz et al. showed that the prevalence of pathologic bacteria was found in frail elderly patients who fed by nasogastric tube or percutaneous enterogastric tube was high. *Pseudomonas aeruginosa* has been cultured exclusively in tube-fed patients. (30)

Interactions between candida and bacteria forming biofilm

As the dental biofilm, candida shares their environment with many bacterial species. Each of bacterial species has different interaction with candida species. For example, *C. albicans* can bind to *Streptococcus sanguinis* in co-aggregation by using cell-wall polysaccharides and cell surface proteins. Not only cell to cell interactions can affect biofilm formation, but also extracellular signaling molecules such as quorum sensing molecules. It is an autoinducer which act as mediator to communicate among cells and to respond to environment condition such as express virulence gene.

For instance, farnesol, one of quorum sensing molecules that candida has used to survive in biofilm. Farnesol can interact with the cell membranes of several bacteria species such as *P. aeruginosa* and inhibit its biofilm. Moreover, it can reduce the production of the pseudomonas signaling molecule. (31)

As the table below, it has been shown that each bacterial species has different effect on candida biofilm.

Table 2 Bacterial species effect on growth of candida albicans biofilms (37-44)

Bacteria species	Inhibitory effect	Stimulatory effect	Reference
Actinomyces	+	+	Gutierrez dA. Et al.2004 (32)
Lactobacilli	+		Collins EB et al. 1980, Fitzsimmons N et al. 1994 (33) (34)
P.intermedia	+		Nair RG et al. 2001 (35)
P.gingivalis	+		Nair RG et al. 2001, Teanpaisan R et al. 1995 (35) (36)
P.aeruginosa	+		Hogan DA et al. 2004 (37)
E.coli	+		Samaranayake LP et al. 1989 (38)
S.sanguis	+	+	Nair RG et al. 2001 (35)
S.salivarius	+	+	Nair RG et al. 2001 (35)
S.oralis	+	+	O'Sullivan et al., 2000 (39)
S.gordonii	+	+	O'Sullivan et al., 2000 (39)

Not only bacterial species that affect candida biofilm, but also its numbers. El azizi et al. showed that the large number of bacteria affect candida biofilm by competing colonization sites and nutrition. (40)

Oral health care for biofilm removal

Oral biofilm control plays as a key role in prevention, treatment, and decrease of recurrence of oral infectious disease. Interventions for removing oral biofilm include mechanical approaches and pharmaceutical approaches. First, mechanical approaches include tooth brushing with fluoride toothpaste. Many countries suggested that good oral hygiene cares may provide tooth brushing twice a day, using soft toothbrush, flossing on the adjacent area that tooth brushing cannot clean. (41) Next one is pharmaceutical approaches such as rinsing with mouthwash. 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, salivary stones creation, 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. (16, 42)

HIV, antibiotics, corticosteroid, chemotherapy, radiation, drugs to treat cancer

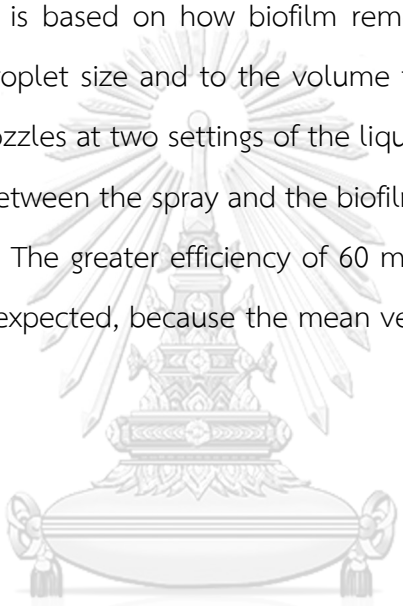
The oral healthy state is optimum balance between the oral microbiota in oral cavity and the host immune system. The healthy state maintains the diversity of the oral microbiota. A reduction in diversity may respond to host immune system. Finally, it shifts into a disease state. HIV, antibiotics, corticosteroid, chemotherapy, radiation, drugs to treat cancer are the predisposing factors that makes changes in oral microbiota diversity and host immune system. (23)

According to K.G. vargas et al. revealed that the rate of oral *C. albicans* carriage in HIV-positive subjects is higher than in control subjects, and patients with CD4+-cell counts of 200–400/microliter had a significantly higher level of yeast carriage. Therefore, oral candidiasis is mostly found in HIV patients. Similar to the use of antibiotics, corticosteroid, chemotherapy, radiation, drugs to treat cancer as a medical treatment, it disturbs the oral homeostasis between bacteria and fungi. Resulting to that, it may cause oral infectious disease. (43)

Oral spray

Nowadays, Oral spray is the one of oral cleansing approaches for oral hygiene care. Oral spray can be defined as fine liquid droplets that move through a gas. Natural sprays can be found as drizzle, mist and clouds. Spray can be characterized by the droplet size distribution, the velocity distribution, the liquid flow rate and the patternation (spatial distribution of liquid that is deposited on a surface)

Arjen Cense et al. (2005) have reported that the removal process of plaque by means of water sprays is based on how biofilm removal efficacy is related to the droplet velocity, the droplet size and to the volume flux. Biofilms were exposed to sprays from different nozzles at two settings of the liquid flow rate, 30 ml/min and 60 ml/min. The distance between the spray and the biofilm remained constant at 6 mm. The result showed that The greater efficiency of 60 ml/min compared to 30 ml/min for diferrent nozzles is expected, because the mean velocity of the droplets is higher at 60 ml/min. (44)



Chapter III

RESEARCH METHODOLOGY

Test solution preparation

two oral care natural products were selected based on a literature survey:

1. Myherbal mybacin trospray (Greater Pharma Co., Ltd.)

2. Myherbal mybacin trospray with mangosteen extract (Greater Pharma Co., Ltd.)

Composition of each oral care natural products are in table-3. And 0.2% chlorhexidine mouthwash (Faculty of Dentistry, Chulalongkorn university) was used as positive control. Chlorhexidine mouthwash was used as a spray with the same container as oral spray natural products

Table 3 Two oral care natural products ingredients

Oral care Natural Product	Ingredients (mg. per ml.)	Uses	administration
Myherbal mybacin trospray (15ml.)	Menthol 1.2 mg., thymol 0.3 mg, eucalyptol 5 ml., chamomile 35.014 mg, peppermint oil 13.808 mg., anise oil 1.26 mg., spearmint oil 0.25 mg., pine oil 0.014 mg, basil oil 0.0532 mg., bergamot oil 0.028 mg , aloe vera 10 mg., witch hazel 10 mg., sage 6.084 mg. and methyl salicylate 1 mg. xylitiol 6.5 mg.	Reduce bad breath and treat oral ulcer	Spray into your mouth 1-2 times after meal or when needed
Myherbal mybacin trospray with mangosteen extract (15ml.)	Mangosteen extract 10mg., Menthol 1.2 mg., thymol 0.3 mg, eucalyptol 5 ml., chamomile 35.014 mg, peppermint oil 12 mg., anise oil 1.26 mg., spearmint oil 0.25 mg., pine oil 0.014 mg, basil oil 0.0532 mg., bergamot oil 0.028 mg , aloe vera 10 mg., witch hazel 10 mg., sage 6.084 mg. and methyl salicylate 1 mg. xylitiol 6.5 mg.	reduce bad breath and treat oral ulcer	Spray into your mouth 1-2 times after meal or when needed

Study population

This clinical study was conducted at the department of Geriatric Dentistry and Patients Special Care and the microbiological laboratory of the faculty of dentistry Chulalongkorn University. Prior to inclusion in the study, subjects were informed the purpose and the protocol of this research and also provided their consent to participation. A randomized, double-blind controlled clinical trial was conducted on 21 patients who came to visit at geriatric dentistry and patients special care clinic. Criteria for inclusion of patients in the study was good general health or well-controlled chronic disease. Exclusion criteria were 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.

Sociodemographic data was obtained by filling out the questionnaire as the checklist. The data included age, gender, occupation, medical conditions, marital family situation, oral hygiene care, dietary habits, smoking habits, presence of prosthesis. After collecting sociodemographic data, saliva samples were collected as described (baseline#1) before routine scale and tooth polish was given to all participants to remove all dental deposits.

Two weeks after routine scaling, participants were appointed to collect saliva sample. 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 sequences use 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. 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). As you see below (Figure1).

Patient satisfaction

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, easy-to-use, and overall of oral care products. Using a ruler for measurement, the score is determined by measuring the distance on the 10-cm line between “no satisfaction” and the participant’s mark, providing a range of scores from 0-10. A higher score indicates greater patient satisfaction.

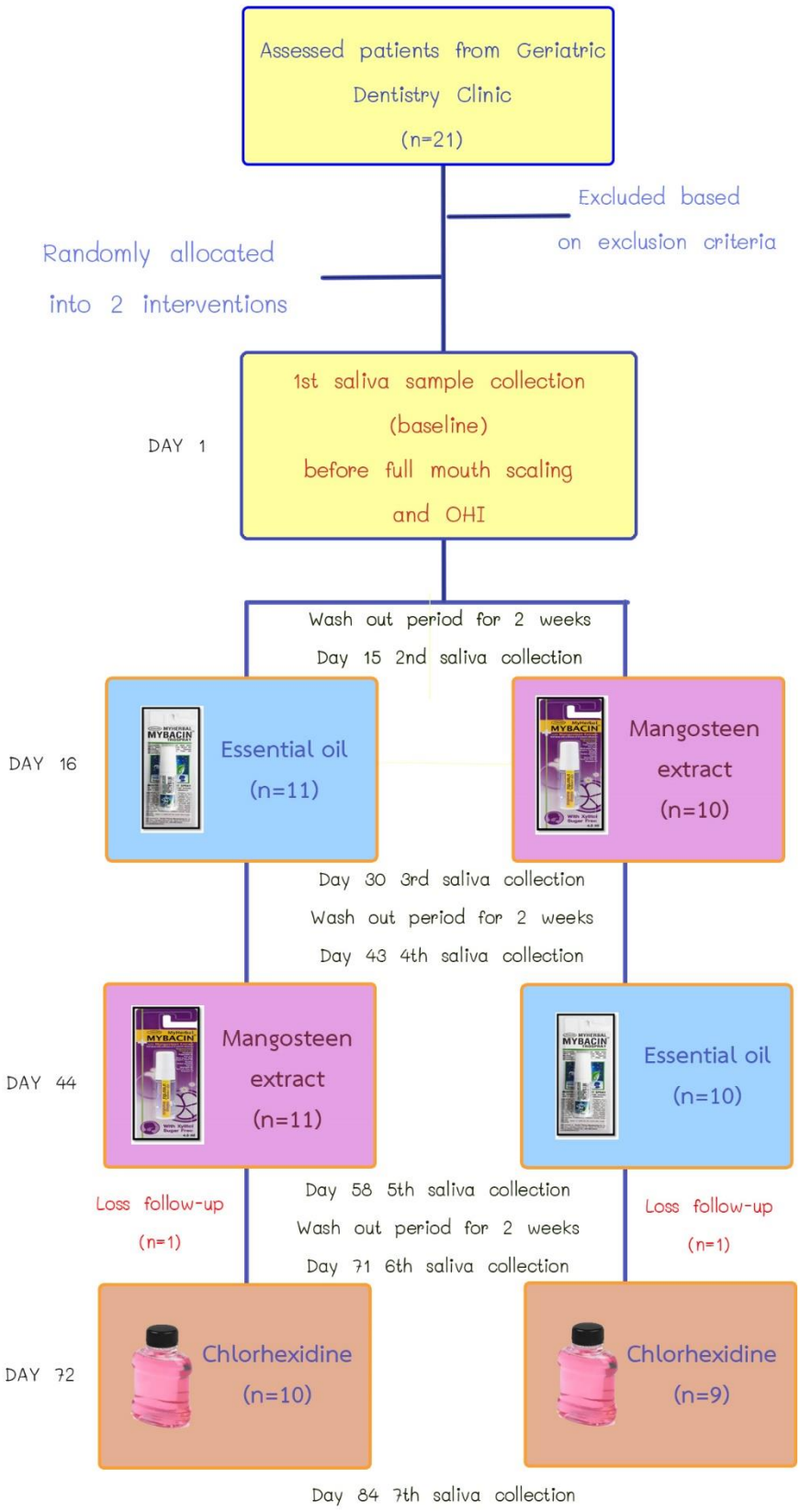


Figure 1 Methods of this research

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 and Mitis-salivarius Bacitracin (MSB) agar to grow *Streptococcus mutans*. 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 was registered to calculate the number of CFU per milliliter (ml) of sample and converted into log CFU per milliliter (ml) as you see in Figure2

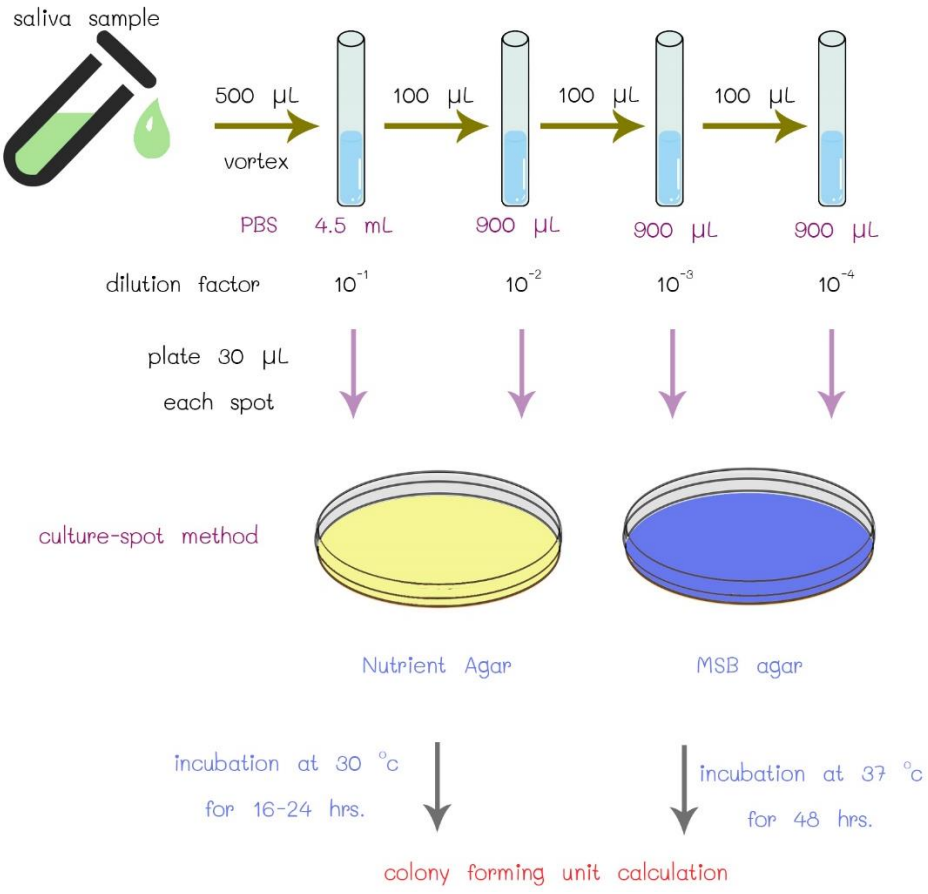


Figure 2 Oral microbiota culture method

Calculating percent reduction

The number of CFU per milliliter (ml) of samples of each oral spray products before and after using were calculated into percent reduction in both Nutrient agar and Mitis-salivarius Bacitracin (MSB) agar as formula below.

$$\text{Percent reduction } (\Delta\%)\ (\text{CFU/ml}) = \frac{\Delta \text{ colony forming unit count (before}-\text{after)(CFU/ml)}}{\textit{baseline colony forming unit (before)}} \times 100$$

PCR detection

DNA had been extracted from the sample saliva using the Power Biofilm DNA Isolation Kit (MO BIO, Carlsbad, CA, USA). The quantity and quality of DNA was measured by using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Species-specific and universal primers of the bacterial 16S rRNA gene for species-specific estimation and estimation of total bacteria were shown in Table 4. The qPCR reaction tube contained 1 µl of DNA template (25 ng), 10 µl of Kapa SYBR FAST qPCR Kit Master Mix (Kapa Biosystems, Foster City, CA, USA), 1 µl forward and reverse primers, and PCR water, which was added to a total volume of 20 µl. The PCR mixtures were analyzed using the Rotor-Gene Q Realtime PCR system (Qiagen, Valencia, CA, USA) which programs were shown in Table-5. Data will be verified using 1% agarose gel electrophoresis and SYBR safe green staining.

Table 4 Primer sequences of the bacterial 16S rRNA gene

Primers	Sequences (5′–3′)	References
Streptococcus mutans (Sm479F and Sm479R)	For: TCGCGAAAAAGATAAACAAACA Rev: GCCCCTTCACAGTTGGTTAG	(45)
Candida albicans (SC5314)	For: TCCGTAGGTGAACCTGCGG Rev: TCCTCCGCTTATTGATATGC	(46)

Primers	Sequences (5′–3′)	References
Universal Lactobacillus	For: TGGAAACAGRTGCTAATACCG Rev: GTCCATTGTGGAAGATTCCC	(47)

For, forward primer; Rev, reverse primer.

Table 5 PCR Cycling Conditions

Steps	**Temp.(°C)**	**Time**	**Cycles**
Initial denaturation	95	3 min	1
Denaturation	95	30 sec	
Annealing	55	30 sec	34
Extension	72	1 min	
Final extension	72	5 min	1
Hold	12	∞	

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 median colony forming units. 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 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. Statistical significance was defined as $P < 0.05$.

Chapter IV

RESULTS

Characteristics of the population

Table 6 summarizes the subject characteristics. The mean years of age was 60 (standard deviation= 11.12; range= 50-91). 52.38% were male and also 52.38% had 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, 61.90% using interdental cleansing and 52.38% having snacks 1-2 times a day). 71.43% were presence of prosthesis.

Table 6 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)

Characteristic of the population	
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)

The effect of 14 days wash-out period against oral microbiota before use of natural oral sprays and chlorhexidine

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-days use of each natural oral spray or chlorhexidine. To determine the therapeutic outcome of each natural oral sprays and ensure the stability of oral sprays products being worn off, comparing baseline of colony forming units before use of natural oral sprays and chlorhexidine had been demonstrated.

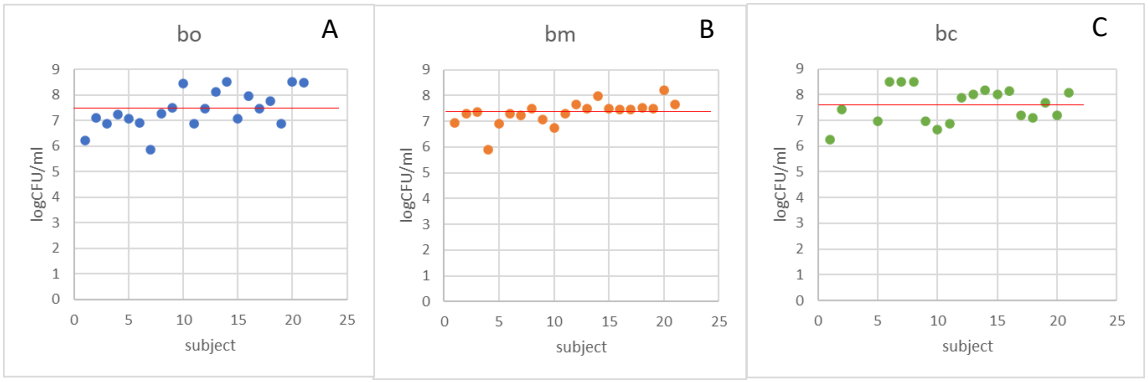


Figure 3 ***The total bacterial counts of saliva samples in nutrients agar.***
(A) The number of colony forming units (log CFU/ml) *before use of essential oil. (B) the number of colony forming units before use of essential oil with mangosteen extract. (C) the number of colony forming units before use of chlorhexidine. Each dot represents the mean from three replicates. Red line referred as the mean of colony forming units (mean log CFU/ml).*

To determine the differences of each baseline in nutrients agar, Data of before use of natural oral sprays and chlorhexidine was converted into log CFU and normally distributed. Data was analyzed using Repeated ANOVA. Figure 3 unveiled that 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 in nutrients agar. Results showed that oral bacteria colony forming units were statistically no differences among natural oral sprays and chlorhexidine mouthwash in nutrients agar. (*P* -value=0.54)

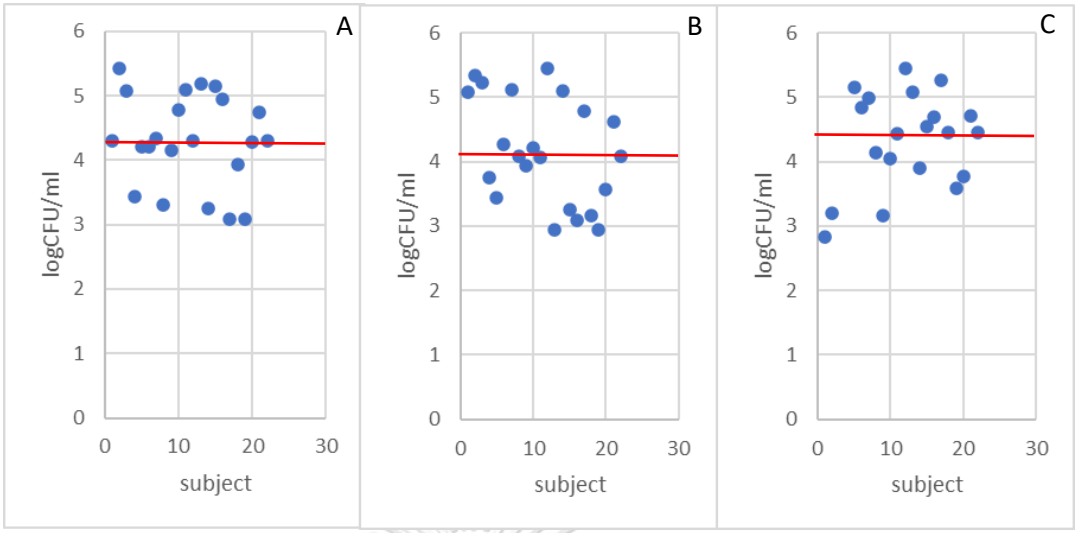


Figure 4 ***The total bacterial counts of saliva samples in MSB agar.***
(A) The number of colony forming units before use of essential oil. (B) the number of colony forming units before use of essential oil with mangosteen extract. (C) the number of colony forming units before use of chlorhexidine. Each dot represents the mean from three replicates Red line referred as the mean of colony forming units (mean log CFU/ml).

Moreover, Data of before use of natural oral sprays and chlorhexidine in MSB agar was also converted into log CFU and normally distributed. The mean log colony forming units as baseline of essential oil, essential oil with mangosteen extract and chlorhexidine group were 4.30,4.13 and 4.33 in MSB agar. Results showed that oral bacteria colony forming units were statistically no differences among natural oral sprays and chlorhexidine mouthwash in MSB agar. (*P* -value=0.70)

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

Table 7 Total bacterial counts in saliva sample before and after using natural oral spray or chlorhexidine in nutrients agar

Oral care product group	Colony forming unit Median Log CFU/ml (IQR)		*P* -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.*

To determine the differences between before and after 14-days continuous use of each natural oral spray or chlorhexidine, Normality test was performed. Data of log CFU before and after intervention in both nutrients agar and MSB agar was not normally distributed and analyzed using Wilcoxon Signed Rank test. The result showed that natural oral sprays including Essential oil and Essential oil with mangosteen extract and chlorhexidine statistically reduced the total colony forming unit in nutrients agar. The median log of colony forming units were changed including 7.48 to 7.46 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 7)

Table 8 Total bacterial counts in saliva sample before and after using natural oral spray or chlorhexidine in Mitis-salivarius Bacitracin (MSB) agar

Oral care product group	Colony forming unit Median Log CFU/ml (IQR)		*P* -value
	Before intervention	After intervention	
Essential oil group	4.29 (1.01)	4.05 (0.92)	0.149
Essential oil with mangosteen extract group	4.09 (1.82)	4.32 (0.69)	0.794
Chlorhexidine group	4.46 (1.22)	3.95 (1.46)	0.022*

IQR = interquartile range

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

Respectively, Table 8 showed that only chlorhexidine group statistically reduced the median log total colony forming unit from 4.46 to 3.95 in MSB agar. However, the median log of colony forming units were reduced from 4.29 to 4.05 in essential oil group but not statistically significant. Lastly, Essential oil with mangosteen extract group were not statistically reduced the median log total colony forming unit after 14-days continuous use.

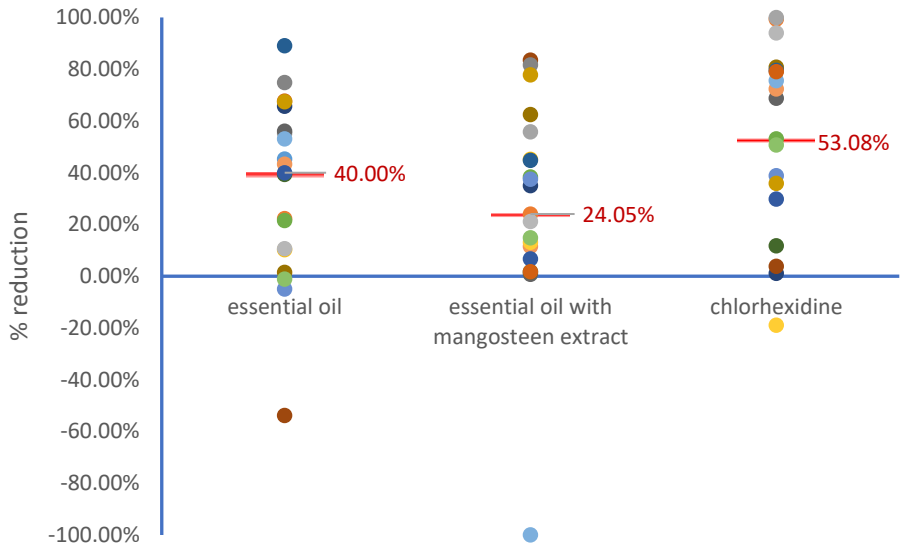


Figure 5 ***The reduction of total oral bacteria after use in Nutrients agar***

Percent Reduction (%Δ) of colony forming unit after the 2-week intervention in nutrients agar (natural oral sprays or chlorhexidine) was demonstrated.

To determine the differences of the therapeutic outcomes among natural oral sprays and chlorhexidine, the percent reduction was performed by calculating the reduced total colony forming units before and after 2-week use of each natural oral sprays or chlorhexidine. The Data was not normally distributed in both nutrients agar and MSB agar. Differences of the intervention were analyzed by Friedman's Rank test. Figure 5 showed that chlorhexidine reduced the median total oral bacteria cell counts the most in nutrients agar (53.08%). The median total oral bacteria cell counts of essential oil was 40%. And the median total oral bacteria cell counts of essential oil with mangosteen extract was 24.05%. Although chlorhexidine possessed the antimicrobial activity against nonselective oral bacteria 1.32 and 2.2 times more than essential oil and essential oil with mangosteen extract, the differences of each intervention were not statistically significant. (*P* -value = 0.76)

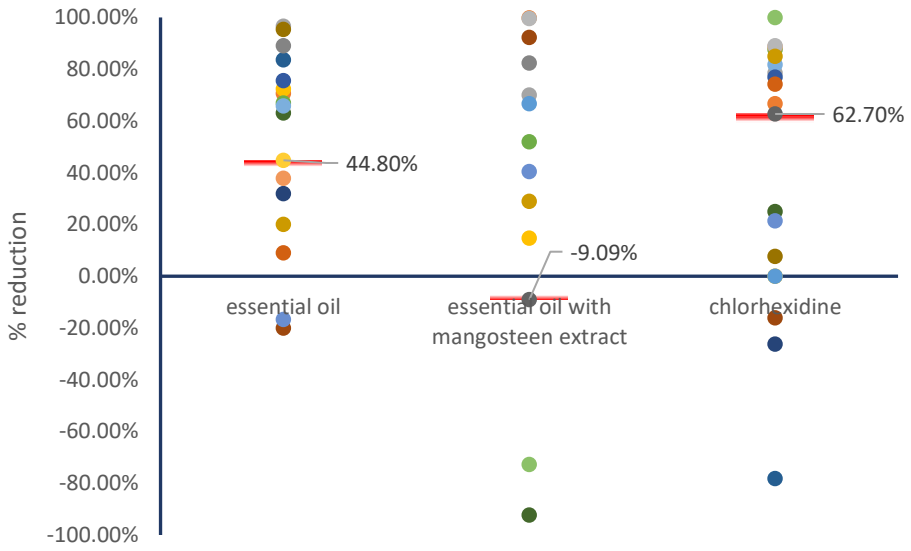


Figure 6 ***The reduction of oral bacteria after use in Mitis-salivarius Bacitracin (MSB) agar.***

Percent Reduction (%Δ) of colony forming unit after the 2-week intervention in MSB agar (natural oral sprays or chlorhexidine) is demonstrated.

Similar to MSB agar which is a selective media for *Streptococcus species,* chlorhexidine reduced the median total colony forming unit the most with statistically 62.60 % reduction. The median oral bacteria cell counts of essential oil was 44.80% reduction but not statistically significant. Lastly, essential oil with mangosteen extract could not reduce the median total colony forming unit in MSB agar after 2-week use (-9.09%). Result investigated that chlorhexidine possessed the antibacterial activity against *Streptococcus species*. *(P -value = 0.368)*

Chlorhexidine mouthwash can reduce oral bacterial number slightly more than others, however, the reduction of colony forming units were not statistically significant.

PCR detection

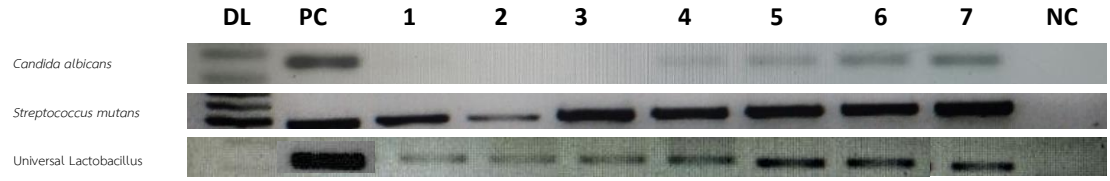


Figure 7 ***PCR gel electrophoresis using 1% agarose gel electrophoresis and SYBR safe green staining***

Lane DL: 100 bp DNA ladder. Lane PC: Positive control [DNA of C. albicans wild-t0ype strains SC5314, DNA of S. mutans wild-type strains, DNA of Universal Lactobacillus wild-type strains]. Subject(S): S1 saliva sample with all intervention Lane NC: Negative control [PCR Master Mix]. Conc. Of DNA template = 50 ng/µL

Table 9 ***PCR detection result and DNA concentration of sample***

Subject (S)	Intervention	DNA conc. (ng/µL)	Universal Lactobacillus	*Streptococcus mutans*	*Candida albicans*
S1	Baseline	81.9	+	+	+
Essential oil (Ess)					
S1	Before Ess	258.2	+	+	+
	After Ess	183.7	+	+	+
S2	Before Ess	9	+	+	+
	After Ess	46.7	+	-	+
Essential oil with mangosteen extract (Man)					
S1	Before Man	10.4	+	+	-
	After Man	83.4	+	+	-
S4	Before Man	107.4	+	-	-
	After Man	150.0	+	-	-
Chlorhexidine (CHX)					
S1	Before CHX	169.6	+	+	+
S1	After CHX	114.1	+	+	+
S5	Before CHX	215.1	+	+	-
S5	After CHX	147.5	+	+	-

From the saliva samples, *Candida albicans*, *streptococcus mutans* and Universal Lactobacillus were identified in saliva samples from some subjects by using specific and universal primers. Despite that most samples were positive for universal Lactobacillus, the samples of each individual were differentially detected positive. Table 9 showed the PCR detection of some saliva samples before and after 14 days of using natural oral sprays or chlorhexidine oral rinse. We found that Universal Lactobacillus were detected in all samples. *Streptococcus mutans* was detected in most saliva samples regardless of any types of oral rinse have been used, except in the samples from one subject (S4). *Streptococcus mutans* was reduced in one subject (S2) after using the essential oil oral rinse. However, the other subjects (S4 and S5) showed no change in PCR detection after continuous use of natural oral sprays products including essential oil and essential oil with mangosteen extract or chlorhexidine. *Candida albicans* could be detected by PCR in some subjects, S1 and S2, but not in S4 or S5. In one subject (S1), *Candida albicans* was negative in the beginning of the first intervention, but became positive while using the second intervention (Figure 7). The presence of *candida albicans* in the samples from the other subject (S2) is regardless to the given intervention.

Due to COVID-19 pandemic, every saliva samples were not identified by PCR. Some participants were loss of follow up. We investigated in only random subjects which had all the interventions and DNA of saliva sample had been extracted for detection in both species-specific and universal primers.

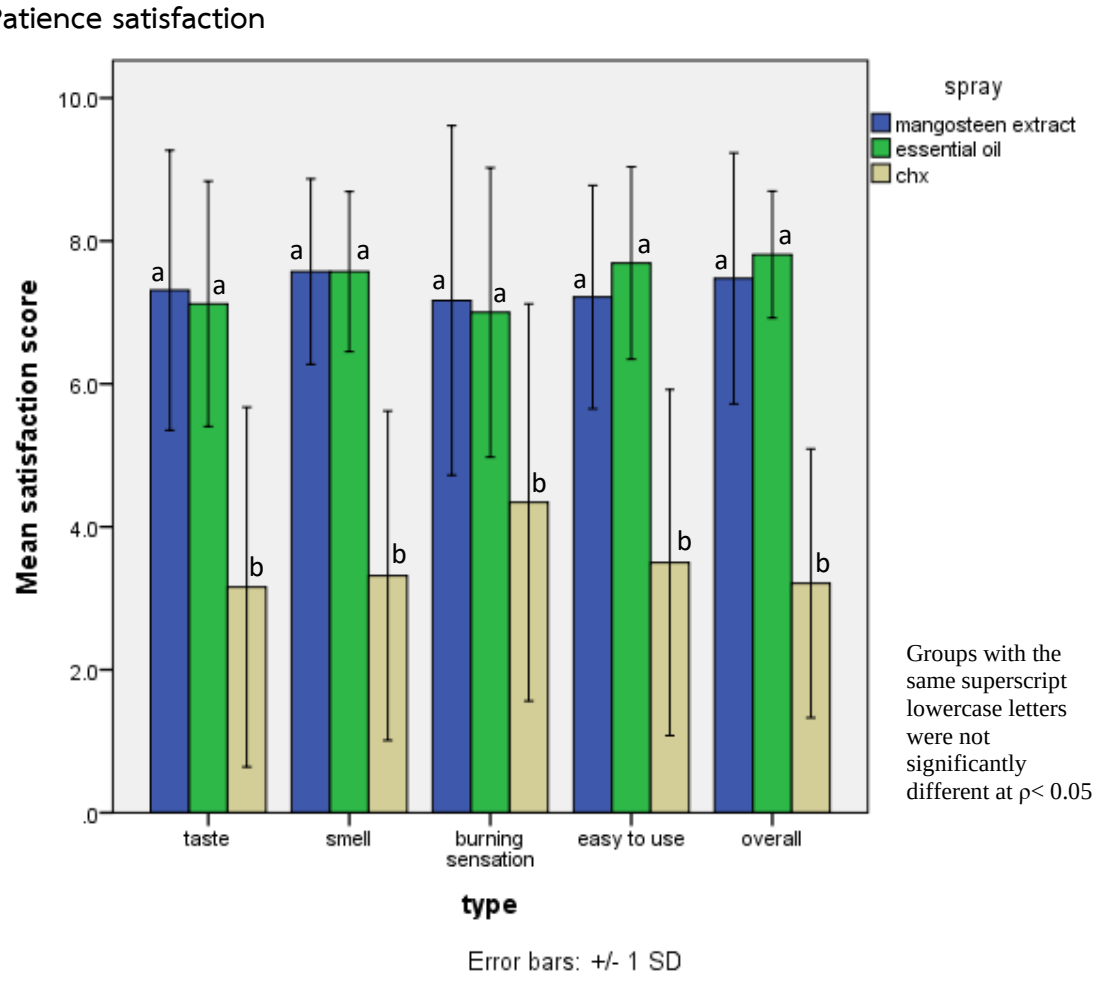


Figure 8 ***Satisfaction score of natural oral sprays as compared to chlorhexidine mouthwash.***

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. Groups with the same superscript lowercase letters were not significantly different at $P < 0.05$

Satisfaction scores of oral care products based on all categories are shown in Figure 8. In all categories, especially taste, smell, easy-to-use product, and overall, essential oil and essential oil with mangosteen extract showed significantly higher satisfaction score than chlorhexidine mouthwash. We concluded that most participants expressed better user satisfaction than chlorhexidine mouthwash.

CHAPTER V

DISCUSSION

This study investigated that essential oil and essential oil with mangosteen extract possessed antifungal and antibacterial activity against oral microbiota in elderly after continuous use for 2 weeks. Before intervention according to the before use data (Figure 3 and 4), 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 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. (48)

Chlorhexidine has been considered as a broad-spectrum antimicrobial agent. Its antimicrobial action is due to the disruption of the bacterial cell membrane by dicationic positively charged chlorhexidine with specific and strong adsorption, inducing bacterial cell lysis. Similar to essential oil which are natural extract. Essential oil also acts on bacterial membrane by the essential oil molecule such as terpene and terpenoids. Due to lipophilic property, essential oil can integrate into the phospholipid bilayer of cell membranes causing increased cell permeability, leaching of intracellular components and inactivation of enzymes involved in cell wall synthesis. Essential oil mainly composed polyenes and echinocandins. Polyenes can act against Candida by inhibiting ergosterol synthesis which is found specifically in fungal cell membrane to form channels through which vital cytoplasmic components leak from the inside of the fungal cell to the outside leading to death of the organism. Also, Echinocandins inhibit 1,3-b-D-glucan synthase and thereby disrupt biosynthesis of 1,3-b-D-glucan, a key component of the fungal cell wall which was a different mechanism of actions to those of azoles and other antifungal drugs today available. (16, 49)

Consistent with previous study, 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 improve oral hygiene in individuals with lead to prevention of oral infectious disease progression. (14)

Moreover, Fine et al.,2000 demonstrated that essential oil mouthwash use for 11 days produced antibacterial activity against *Streptococcus mutans* by reduction of 39.2% in saliva. (50)

In contrast with previous study of McKenzie et al.,1992 using essential oil mouthwash for 2 weeks in mentally handicapped adults are not significantly reduce plaque index score and no clinically significant improvements in periodontal status compared with 0.12% chlorhexidine mouthwash. (51)

In addition to Ibrahim et al., 2016 found that mangosteen extract consist 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. (52)

In contrast with this study, natural oral spray including essential oil with and without mangosteen extract was compared, no additional antifungal and antibacterial effect was detected in the product with mangosteens extracts. Whether mangosteen extract has the synergistic antibacterial effect when combined with essential oil in oral spray cannot be concluded from this study. Moreover, this study found that essential oil with mangosteen extract showed the least potent antifungal and antibacterial activity against oral microbiota.

Regarding oral microbiota culture in MSB agar, this study found that natural oral spray including essential oil and essential oil with mangosteen extract statistically possessed less potent antibacterial effect against *streptococcus* species than chlorhexidine mouthwash. We suggested that if the purpose of use is to reduce cariogenic bacteria in oral cavity. Chlorhexidine would be primary of choice of antibacterial agent. Essential oil and essential oil with mangosteen extract were still inconclusive. Further studies may be needed.

Respectively, this study found that neither essential oil nor essential oil with mangosteen extract could reduce the bacterial counts in some participants. Associated with sociodemographic data, it might be possible that oral hygiene care or dietary habits as a contributing factor in individuals may influence the antimicrobial activity against oral microbiota.

PCR detection result, this study found that presence of *Candida albicans*, *Streptococcus mutans* and Universal Lactobacillus in sample saliva were investigated. This study found that before 14 days use of natural oral spray, some participants did not show the presence of *candida albicans* in saliva, but after 14 days use, we found it detected by PCR. It might be possible that the reduction of total oral bacteria in oral cavity may be interact with candida albicans growth. Like Lucja M. Jarosz et al. (2009) found that total oral bacteria growth can affect GT formation of *Candida albicans* in cocultures even if the bacteria and the fungi are physically separated. Quantitative and qualitative nature of the bacteria modify the physiology of *Candida albicans* biofilm formation on polystyrene substrates. This relationship is complex depending on the bacterial species and its numbers, and may affect the morphogenesis of the yeast. Therefore, we suggested that prolonged use of natural oral sprays or chlorhexidine could make total bacteria in oral cavity affect *candida albicans* growth and survival. (53, 54)

This study suggested that Future studies should include clinical isolates of specific microbiota. 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. Moreover, to confirm the culture identification of oral bacteria and candida, every subject's saliva should be identified by PCR. The limitation of this study was limited DNA extraction kit and loss follow up of subjects due to COVID-19 outbreak. This study was not confirming the culture identification of oral bacteria and candida by PCR detection in every sample's saliva.

In term of patient satisfaction, most participants preferred using essential oil and essential oil with mangosteen extract more than chlorhexidine mouthwash

because of its 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. Like Beverly J. Tepper (2002) found that flavor, texture and other sensory perceptions play as a critical role in food preferences which finally guide dietary behavior and also apply into daily lifestyle oral hygiene and diet planning. (55) Similar to Malhotra et al. (2011), herbal mouthrinse was preferred by patients for its taste, its easy to use and taste alteration compared with chlorhexidine gluconate, though less effective than chlorhexidine gluconate in reducing plaque. (56)

This study supported previous studies that essential oil and essential oil with mangosteen extract has antifungal and antibacterial activity against oral microbiota compared with 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. (57)

CHAPTER VI

CONCLUSION

Natural oral sprays product including essential oil and essential oil with mangosteen extract show therapeutic outcomes of antimicrobial activities against oral microbiota and not significant to the therapeutic outcomes obtained by chlorhexidine mouthwash. However, synergistic effect of mangosteen extract when combined to essential oil oral spray was inconclusive. 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 as an alternative to Chlorhexidine mouthwash for avoiding unsatisfactory effects of chlorhexidine but prolonged use is not recommended and to enhance patient compliance especially in elderly. Further research with higher sensitivity and higher reliability technique may be needed.

APPENDIX

Colony Forming Units of samples in nutrients agar

($x10^4$ CFU/ml)

Sample	Baseline	Before Essential Oil	After Essential Oil	Before mangosteen Extract	After Mangosteen Extract	Before CHX	After CHX
1	3,280	164	128	878	667	177	1.22
2	3,320	1,290	578	2,010	8.89	2,560	0.444
3	1,640	756	678	2,290	1,260	-	-
4	2,640	1,740	956	77.8	533	-	-
5	922	1,190	933	811	500	900	422
6	14.9	833	286	2,030	1,320	30,080	30,040
7	20.0	72.2	111	1,710	282	32,200	31,000
8	7.78	1,840	811	3,100	3,080	31,300	9,780
9	633	3,080	3,030	1,160	433	922	178
10	600	28,200	3,070	556	307	433	88.9
11	1,000	733	444	1,920	1,890	756	667
12	1,710	2,890	3,030	4,440	2,780	7,440	4,560
13	1,800	13,300	7,560	3,100	2,740	10,400	2,890
14	3,070	31,400	28,100	9,440	7,440	10,500	889
15	2,780	1,200	6,440	3,140	2,730	10,000	11,900
16	656	9,000	4,222	2,840	3,110	13,700	33,300
17	3,270	2,930	2,970	3,000	2,560	1,530	756
18	3,140	5,560	3,330	3,310	3,090	1,270	889
19	1,780	756	244	3,200	3,140	4,780	1,000
20	32,000	32,200	8,110	15,800	2,890	1,530	8,220
21	29,600	30,700	10,000	4,560	1,010	11,800	7,560

Colony Forming Units of samples in MSB agar

(x10^4 CFU/ml)

Sample	Baseline	Before Essential Oil	After Essential Oil	Before Mangosteen Extract	After Mangosteen Extract	Before CHX	After CHX
1	20.01	1.97	0.578	10.19	0.022	0.067	0.022
2	7.33	20.61	0.89	20.19	6.56	0.156	0.033
3	20.14	10.17	3.22	10.66	10.41	-	-
4	2.00	0.28	1.22	0.0567	0.0189	-	-
5	0.122	1.61	0.533	0.278	0.133	10.43	1.78
6	0.800	1.63	1.11	1.88	5.00	6.78	8.56
7	5.56	2.22	2.67	10.30	1.00	9.67	10.12
8	0.0444	0.200	1.00	1.22	1.33	1.40	0.522
9	0.411	1.44	0.0667	0.856	2	0.144	0.133
10	1.32	6.11	1.00	1.61	7.00	1.12	2.00
11	0.600	10.23	4.56	1.16	2.22	2.67	2.00
12	2.33	2.00	2.33	20.86	10.70	20.86	20.24
13	0.444	10.56	9.67	0.0889	2.11	10.18	1.33
14	0.567	0.178	7.56	10.24	0.0444	0.811	0.0889
15	20.28	10.39	7.67	0.178	1.00	3.56	20.50
16	10.62	8.78	3.00	0.122	1.44	4.89	0.889
17	0.844	0.122	2.78	6.11	10.06	10.87	0.0111
18	0.133	0.867	0.211	0.144	2.33	2.89	0.667
19	0.344	0.122	0.111	0.0889	4.89	0.389	0.100
20	1.13	1.92	0.211	0.378	0.0667	0.589	2.89
21	1.69	5.56	4.44	4.22	3.00	5.11	0.767

Colony Forming Units of samples in nutrients agar as logCFU

Sample	Baseline	Before Essential Oil	After Essential Oil	Before mangosteen Extract	After Mangosteen Extract	Before CHX	After CHX
1	7.516	6.216	6.106	6.943	6.824	6.247	4.087
2	7.521	7.110	6.762	7.303	6.949	7.407	3.648
3	7.216	6.878	6.831	7.360	7.010	-	-
4	7.422	7.242	6.980	5.891	6.727	-	-
5	6.965	7.075	6.970	6.909	6.699	6.954	6.626
6	5.173	6.921	6.456	7.308	7.121	8.488	8.484
7	5.301	5.859	6.046	7.233	6.450	8.508	8.491
8	4.891	7.266	6.909	7.491	7.488	8.496	7.990
9	6.801	7.488	7.482	7.063	6.637	6.965	6.250
10	6.778	8.451	7.487	6.745	6.487	6.637	5.949
11	7.000	6.865	6.648	7.284	7.276	6.878	6.824
12	7.233	7.461	7.482	7.648	7.444	7.872	7.659
13	7.255	8.125	7.878	7.491	7.438	8.019	7.461
14	7.487	8.498	8.449	7.975	7.872	8.176	6.949
15	7.444	7.079	7.809	7.498	7.437	8.000	8.075
16	6.817	7.954	7.626	7.454	8.493	8.136	7.523
17	7.514	7.467	7.472	7.477	7.407	7.186	6.878
18	7.498	7.745	7.523	7.520	7.490	7.102	6.949
19	7.250	6.878	6.388	7.505	7.498	7.679	7.000
20	8.505	8.508	7.909	8.198	7.461	7.186	7.915
21	8.471	8.487	8.000	7.659	7.005	8.071	7.878

Colony Forming Units of samples in MSB agar as logCFU

Sample	Baseline	Before Essential Oil	After Essential Oil	Before Mangosteen Extract	After Mangosteen Extract	Before CHX	After CHX
1	5.303	4.294	3.762	5.075	2.347	2.824	2.347
2	4.865	5.417	3.949	5.340	4.817	3.192	2.523
3	5.331	5.067	4.508	5.219	5.150	-	-
4	4.301	3.444	4.087	3.753	3.276	-	-
5	3.087	4.207	3.727	3.444	3.125	5.156	4.250
6	3.903	4.213	4.046	4.274	4.699	4.831	4.932
7	4.745	4.347	4.426	5.114	4.000	4.985	5.050
8	2.648	3.301	4.000	4.087	4.125	4.146	3.718
9	3.614	4.160	2.824	3.932	4.325	3.160	3.125
10	4.121	4.786	4.000	4.207	4.845	4.050	4.301
11	3.778	5.091	4.659	4.063	4.347	4.426	4.301
12	4.368	4.301	4.368	5.456	5.230	5.456	5.351
13	3.648	5.192	4.985	2.949	4.325	5.071	4.125
14	3.753	3.250	4.878	5.095	2.648	3.909	2.949
15	5.358	5.143	4.885	3.250	4.000	4.550	5.398
16	5.210	4.943	4.477	3.087	4.160	4.689	3.950
17	3.927	3.087	4.443	4.786	5.023	5.271	2.046
18	3.125	3.938	3.325	3.160	4.368	4.460	3.824
19	3.537	3.087	3.046	2.950	4.689	3.560	3.000
20	4.054	4.284	3.325	3.577	2.824	3.770	4.460
21	4.228	4.745	4.648	4.625	4.477	4.709	3.885

Questionnaire Form before intervention

แบบสอบถามงานวิจัยผลการต้านเชื้อแบคทีเรียและเชื้อราในน้ำลายเมื่อใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง

Age อายุ.....ปี ชื่อ.....HN.....

Gender, เพศ

☐ ชาย ☐ หญิง

occupation, อาชีพ

- O ปัจจุบันยังประกอบอาชีพอยู่ โปรดระบุ.....
- O เกษียณแล้ว
- O แม่บ้าน

medical conditions,โรคประจำตัว (ระบุได้มากกว่า 1 โรค

- O โรคความดันโลหิตสูง
- O โรคเบาหวาน
- O โรคไขมันโลหิตสูง
- O โรคอื่นๆ โปรดระบุ.....

marital family situation สถานภาพ

- O สมรส มีบุตร.....คน
- O โสด
- O หย่าร้าง

ปัจจุบันอาศัยอยู่กับ.....

oral hygiene care การดูแลสุขภาพช่องปาก

ความถี่ในการแปรงฟัน

☐ น้อยกว่า 1 ครั้งต่อวัน

☐ 1 ครั้งต่อวัน

☐ มากกว่า 1 ครั้งต่อวัน

ท่านได้ใช้อุปกรณ์เสริมในการช่วยทำความช่องปากหรือไม่ เช่น ไม้จิ้มฟัน ไหมขัดฟัน

- ○ ใช้ โปรดระบุ.....
- ○ ไม่ใช้

dietary habits พฤติกรรมการรับประทานอาหาร

ความถี่ในการทานขนม

- ○ มากกว่า 3ครั้งต่อวัน
- ○ 1-2 ครั้งต่อวัน
- ○ ทานเฉพาะมื้ออาหาร

smoking habits ประวัติการสูบบุหรี่

- ○ ปัจจุบันสูบ ความถี่.....
- ○ เคยสูบ
- ○ ไม่เคยสูบ

ประวัติการดื่มแอลกอฮอล์

- ○ ปัจจุบันดื่ม ความถี่.....
- ○ เคยดื่ม
- ○ ไม่เคยดื่ม

presence of prosthesis ปัจจุบันท่านได้ใส่ฟันปลอมหรือไม่

- ○ ไม่มีฟันปลอม
- ○ มีฟันปลอม
  - ○ ถอดได้
  - ○ ติดแน่น

Consent Form

เอกสารยินยอมเข้าร่วมการวิจัย

(Consent Form)

การวิจัยเรื่อง ผลการต้านเชื้อแบคทีเรียและเชื้อราในน้ำลายเมื่อใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง

ข้าพเจ้า (นาย/ นาง/ นางสาว).....

อยู่บ้านเลขที่.....ถนน.....ตำบล/แขวง.....

อำเภอ/เขต.....จังหวัด.....รหัสไปรษณีย์.....

หมายเลขโทรศัพท์.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้

1. ข้าพเจ้าได้รับทราบรายละเอียดข้อมูลคำอธิบายสำหรับอาสาสมัครที่เข้าร่วมในการวิจัย รวมทั้งได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการทำวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการทำวิจัย

หรือจากยาที่ใช้รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดและมีความเข้าใจดีแล้ว

2. ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

3. ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น และผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับ

การรักษาพยาบาลโดยไม่คิดมูลค่า

4. ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้และการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้าพเจ้าจึงสมัครใจเข้าร่วมโครงการวิจัยนี้ตามที่ระบุในเอกสารข้อมูลคำอธิบายสำหรับอาสาสมัครและได้ลง นามในใบยินยอมนี้ด้วยความเต็มใจ และได้รับสำเนาเอกสารใบยินยอมที่ข้าพเจ้าลงนามและลงวันที่ และเอกสารยกเลิกการเข้าร่วมวิจัย อย่างละ 1 ฉบับ เป็นที่เรียบร้อยแล้ว

ลงนาม..... (อาสาสมัคร) (.....) วันที่...../...../.....	ลงนาม.....-.....(ผู้ปกครอง) (.....) วันที่...../...../.....
ลงนาม.....(ผู้วิจัยหลัก) (นายชานน สุวรรณประพิศ) วันที่...../...../.....	ลงนาม..... (พยาน) (.....) วันที่...../...../.....

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้วข้าพเจ้าจึงลงนาม หรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม..... (อาสาสมัคร) (.....) วันที่...../...../.....	ลงนาม.....-.....(ผู้ปกครอง) (.....) วันที่...../...../.....
ลงนาม.....(ผู้วิจัยหลัก) (นาย ชานน สุวรรณประพิศ) วันที่...../...../.....	ลงนาม.....(พยาน) (.....) วันที่...../...../.....

Information Form for participants

1

Essential oil

MYHERBAL MYBACIN TROSPRAY

Each 1 ml. contains :

Chamomile	35.014	mg.	Anise oil	1.26	mg.
(provides 3.68 mg% chamazulene)			Menthol	1.2	mg.
Peppermint oil	13.808	mg.	Methyl salicylate	1	mg.
Aloe vera	10	mg.	Thymol	0.3	mg.
Witch hazel	10	mg.	Spearmint Oil	0.25	mg.
Xylitol	6.5	mg.	Basil oil	0.0532	mg.
Sage	6.084	mg.	Pine oil	0.014	mg.
Eucalyptol	5	mg.	Bergamot oil	0.0028	mg.

มายเฮอบัล มายบาซิน โทรสเปรย์

น้ำใสสีน้ำตาล

(บรรจุขวดละ 15 มล. และชนิดรีฟิล 30,60 มล.)

- เป็นสเปรย์พ่นช่องปากและลำคอ ที่ให้ความรู้สึกเย็น โล่ง ชุ่มคอ ด้วยส่วนผสมของสารสกัดจากธรรมชาติ 14 ชนิด มีส่วนผสมของ Xylitol ปราศจากน้ำตาล
- ช่วยลดการสะสมของเชื้อแบคทีเรีย ทำให้ลมหายใจหอมสดชื่น ลดกลิ่นปาก

วิธีการใช้ : ใช้พ่น 1-2 ครั้ง เข้าในปาก หลังรับประทานอาหาร หรือทุกครั้งที่ต้องการความเย็น โล่ง ชุ่มคอ

2

3

0.2% chlorhexidine mouthwash - control

ข้อมูลงานวิจัย เรื่อง ผลการต้านเชื้อแบคทีเรียและเชื้อราในน้ำลายเมื่อใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง โดยสังเขป

งานวิจัยนี้ เป็นการวัดผลในการต้านเชื้อราและเชื้อแบคทีเรียในช่องปากจากการใช้สเปรย์ที่ผลิตจากสารธรรมชาติโดยวัดจากน้ำลาย โดยการเก็บน้ำลายทั้งหมดรวม 7 ครั้งนับตั้งแต่วันแรกที่เข้าร่วม ดังนี้

ครั้งที่1

- สอบถามข้อมูลทั่วไป
- ได้รับการตรวจในช่องปาก และ ประเมินความเสี่ยงของโรคฟันผุและโรคปริทันต์
- อาสาสมัครจะถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลาย
- การขูดหินปูนและทำความสะอาดช่องปาก

หลังจากนั้น 2 สัปดาห์

ครั้งที่2

- อาสาสมัครจะถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง
- อาสาสมัครจะได้รับสเปรย์ขวดที่ 1 เพื่อนำกลับไปใช้ที่บ้านเป็นเวลา 2 สัปดาห์

ครั้งที่3

- อาสาสมัครจะต้องคืนสเปรย์ขวดที่ 1 และถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง

หลังจากนั้น 2 สัปดาห์

ครั้งที่4

- อาสาสมัครจะถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง
- อาสาสมัครจะได้รับสเปรย์ขวดที่ 2 เพื่อนำกลับไปใช้ที่บ้านเป็นเวลา 2 สัปดาห์

ครั้งที่5

- อาสาสมัครจะต้องคืนสเปรย์ขวดที่ 2 และถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง

หลังจากนั้น 2 สัปดาห์

ครั้งที่6

- อาสาสมัครจะถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง
- อาสาสมัครจะได้รับสเปรย์ขวดที่ 3 เพื่อนำกลับไปใช้ที่บ้านเป็นเวลา 2 สัปดาห์

ครั้งที่7

- อาสาสมัครจะต้องคืนสเปรย์ขวดที่ 2 และถูกขอให้บ้วนเพื่อเก็บตัวอย่างน้ำลายอีกครั้ง

ตัวอย่างน้ำลายที่เก็บได้จะถูกนำไปหาปริมาณเชื้อราแคนดิดาและเชื้อแบคทีเรีย ซึ่งเป็นเชื้อโรคประจำถิ่นที่อาศัยอยู่บริเวณเยื่อเมือกในช่องปากและลำคอ โดยวิธีการเพาะเชื้อและการทำ Quantitative PCR โดยจะมีระยะเวลาประมาณ 3 เดือน (86 วัน)โดยตลอดโครงการ โดยขอให้อาสาสมัครปฏิบัติตามที่ผู้วิจัยแนะนำได้แก่

- งดรับประทานอาหาร แปรงฟันมาก่อนอย่างน้อย 120 นาที
- งดใช้น้ำยาบ้วนปากอย่างน้อย 12 ชั่วโมง ก่อนเก็บตัวอย่างน้ำลาย
- ไม่เคยได้รับยาปฏิชีวนะ, ยาต้านเชื้อรา และยาต้านเชื้อไวรัส หรือยาประเภทสเตียรอยด์ ภายใน 1 เดือนก่อนเข้าร่วมโครงการ หากได้รับภายใน 1 เดือน โปรดแจ้งทางผู้วิจัยทราบ
- ไม่เคยได้รับการวินิจฉัยว่าเป็นมะเร็งบริเวณใบหน้าและลำคอ หรือมีโรคภูมิคุ้มกันบกพร่องใดๆ

วิธีใช้ผลิตภัณฑ์

- ใช้วันละ 2 ครั้ง เช้า-เย็น
- โดยแต่ละครั้งที่ใช้ ฉีดที่บริเวณกระพุ้งแก้มทั้งซ้ายและขวา ด้านละ 2 ครั้ง

ค่าตอบแทนในการเดินทาง 1,000 บาท ตลอดงานวิจัย

ต้องการข้อมูลเพิ่มเติม ติดต่อได้ที่ ทพ.ชานน สุวรรณประพิศ

เบอร์โทรศัพท์ 099-2968542

Patients Satisfaction Form

แบบทดสอบความพึงพอใจต่อการใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง

1. อาสาสมัครวิจัยได้ใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่องตามคำแนะนำของผู้วิจัยหรือไม่
   - ○ ได้ใช้ตามคำแนะนำตลอดระยะเวลา 2 สัปดาห์ (ข้ามไปตอบข้อที่ 3)
   - ○ ได้ใช้แต่ไม่อย่างต่อเนื่อง
   - ○ ไม่ได้ใช้ตามคำแนะนำ

   } กรุณาตอบข้อที่ 2
2. เหตุผลที่อาสาสมัครไม่ได้ใช้ผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติอย่างต่อเนื่อง
   - ○ ลืมใช้ตามระยะเวลาที่กำหนด
   - ○ ไม่ชอบในผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติที่ใช้
   - ○ อื่นๆ โปรดระบุเหตุผล .....
3. ท่านมีความพึงพอใจอย่างไรต่อผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติที่ได้ใช้

ในแง่ของรสชาติ

พึงพอใจน้อย พึงพอใจมาก

ในแง่ของกลิ่น

พึงพอใจน้อย พึงพอใจมาก

ใช้แล้วรู้สึกสดชื่น ไม่แสบปาก

พึงพอใจน้อย พึงพอใจมาก

ในแง่ของการใช้งาน

พึงพอใจน้อย พึงพอใจมาก

โดยรวมที่มีผลต่อผลิตภัณฑ์ดูแลสุขภาพช่องปากที่ผลิตจากสารธรรมชาติชนิดนี้

พึงพอใจน้อย พึงพอใจมาก

REFERENCES

1. Bodineau A, Folliguet M, Séguier S. Tissular senescence and modifications of oral ecosystem in the elderly: risk factors for mucosal pathologies. Current aging science. 2009;2(2):109-20.

2. Barnett ML. The rationale for the daily use of an antimicrobial mouthrinse. The Journal of the American Dental Association. 2006;137:S16-S21.

3. Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of Streptococcus mutans and the ability to form biofilms. European Journal of Clinical Microbiology & Infectious Diseases. 2014;33(4):499-515.

4. Rezaei S, Rezaei K, Mahboubi M, Jarahzadeh MH, Momeni E, Bagherinasab M, et al. Comparison the efficacy of herbal mouthwash with chlorhexidine on gingival index of intubated patients in Intensive Care Unit. J Indian Soc Periodontol. 2016;20(4):404-8.

5. Janjić-Pavlović O, Stančić I, Cicmil S, Stojanović Z, Lečić J, Elenčevski S. The use of essential oils based antiseptic solution in the treatment of denture stomatitis. Serbian Dental Journal. 2017;64(1):7-13.

6. Kaomongkolgit R, Jamdee K, Chaisomboon N. Antifungal activity of alpha-mangostin against Candida albicans. Journal of oral science. 2009;51(3):401-6.

7. Tadtong S, Viriyaroj A, Vorarat S, Nimkulrat S, Suksamrarn S. Antityrosinase and antibacterial activities of mangosteen pericarp extract. J Health Res. 2009;23(2):99-102.

8. Tiwari M. Science behind human saliva. Journal of natural science, biology, and medicine. 2011;2(1):53.

9. Vissink A, Spijkervet FKL, Amerongen AVN. Aging and saliva: a review of the literature. Special Care in Dentistry. 1996;16(3):95-103.

10. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. Journal of oral biology and craniofacial research. 2016;6(1):67-76.

11. Denepitiya L, Kleinberg I. A comparison of the microbial compositions of pooled human dental plaque and salivary sediment. Archives of oral biology. 1982;27(9):739-45.

12. Melo NId, Carvalho CEd, Fracarolli L, Cunha WR, Veneziani RCS, Martins CHG, et al. Antimicrobial activity of the essential oil of Tetradenia riparia (Hochst.) Codd.(Lamiaceae) against cariogenic bacteria. Brazilian Journal of Microbiology. 2015;46(2):519-25.

13. Prada-López I, Quintas V, CASARES-DE-CAL MDL, SUAREZ-QUINTANILLA JA, Suárez-Quintanilla D, TOMÁS CARMONA I. Ex vivo vs. in vivo antibacterial activity of two antiseptics on oral biofilm. Frontiers in microbiology. 2015;6:655.

14. Araujo MW, Charles CA, Weinstein RB, McGuire JA, Parikh-Das AM, Du Q, et al. Meta-analysis of the effect of an essential oil–containing mouthrinse on gingivitis and plaque. The journal of the American dental association. 2015;146(8):610-22.

15. Piraporn Vichienroj D, Pharm SCB. Antibacterial activity of mangosteen pericarp extract against cariogenic Streptococcus mutans.

16. Balagopal S, Arjunkumar R. Chlorhexidine: the gold standard antiplaque agent. J Pharm Sci Res. 2013;5(12):270-4.

17. Van Rijkom H, Truin G, Van't Hof M. A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. Journal of dental research. 1996;75(2):790-5.

18. Samaranayake L. Essential Microbiology for Dentistry E-Book: Elsevier Health Sciences; 2011.

19. Chaudhary D, Patthi B, Singla A, Gupta R, Muchhal M, Kumar JK, et al. The Anticariogenic Efficacy of 5000 ppm Fluoridated Toothpaste: A Systematic Review. Journal of Clinical & Diagnostic Research. 2018;12(1).

20. Xiao J, Huang X, Alkhers N, Alzamil H, Alzoubi S, Wu TT, et al. Candida albicans and Early Childhood Caries: A Systematic Review and Meta-Analysis. Caries research. 2018;52(1-2):102-12.

21. Badet C, Thebaud N. Ecology of lactobacilli in the oral cavity: a review of literature. The open microbiology journal. 2008;2:38.

22. Teanpaisan R, Piwat S, Dahlen G. Inhibitory effect of oral Lactobacillus against oral pathogens. Letters in applied microbiology. 2011;53(4):452-9.

23. Cho T, Nagao J, Imayoshi R, Tanaka Y. Importance of Diversity in the Oral Microbiota including Candida Species Revealed by High-Throughput Technologies. Int J Dent. 2014;2014:454391.

24. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Critical reviews in microbiology. 2010;36(1):1-53.

25. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. J Oral Microbiol. 2011;3.

26. Bondaryk M, Kurzątkowski W, Staniszewska M. Antifungal agents commonly used in the superficial and mucosal candidiasis treatment: mode of action and resistance development. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii. 2013;30(5):293.

27. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Sciepuk M, Zaremba M, et al. Occurrence rate of oral Candida albicans in denture wearer patients. Advances in medical sciences. 2006;51:77-80.

28. Zawadzki PJ, Perkowski K, Padzik M, Mierzwinska-Nastalska E, Szaflik JP, Conn DB, et al. Examination of Oral Microbiota Diversity in Adults and Older Adults as an Approach to Prevent Spread of Risk Factors for Human Infections. Biomed Res Int. 2017;2017:8106491.

29. PERCIVAL RS, CHALLACOMBE SJ, MARSH PD. Age-related microbiological changes in the salivary and plaque microflora of healthy adults. Journal of Medical Microbiology. 1991;35(1):5-11.

30. Leibovitz A, Plotnikov G, Habot B, Rosenberg M, Segal R. Pathogenic colonization of oral flora in frail elderly patients fed by nasogastric tube or percutaneous enterogastric tube. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2003;58(1):M52-M5.

31. ten Cate JM, Klis FM, Pereira-Cenci T, Crielaard W, de Groot PW. Molecular and cellular mechanisms that lead to Candida biofilm formation. J Dent Res. 2009;88(2):105-15.

32. de Annan Gutiérrez S, de Cárdenas Benito L. Effect of metabolic substances of oral Actinomyces on Candida albicans. Revista iberoamericana de micologia. 2004;21(1):29-34.

33. Collins E, Hardt P. Inhibition of Candida albicans by Lactobacillus acidophilus. Journal of dairy science. 1980;63(5):830-2.

34. Fitzsimmons N, Berry D. Inhibition of Candida albicans by Lactobacillus acidophilus: evidence for the involvement of a peroxidase system. Microbios. 1994;80(323):125-33.

35. Nair RG, Anil S, Samaranayake LP. The effect of oral bacteria on Candida albicans germ-tube formation. Apmis. 2001;109(2):147-54.

36. Teanpaisan R, Douglas C, Walsh T. Characterisation of black-pigmented anaerobes isolated from diseased and healthy periodontal sites. Journal of periodontal research. 1995;30(4):245-51.

37. Hogan DA, Vik Å, Kolter R. A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology. Molecular microbiology. 2004;54(5):1212-23.

38. Samaranayake L, Lamb A, Lamey PJ, MacFarlane T. Oral carriage of Candida species and coliforms in patients with burning mouth syndrome. Journal of Oral Pathology & Medicine. 1989;18(4):233-5.

39. O'Sullivan JM, Jenkinson HF, Cannon RD. Adhesion of Candida albicans to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. Microbiology. 2000;146(1):41-8.

40. El-Azizi M, Starks S, Khardori N. Interactions of Candida albicans with other Candida spp. and bacteria in the biofilms. Journal of applied microbiology. 2004;96(5):1067-73.

41. Steinberg S. A paradigm shift in the treatment of caries. General dentistry. 2002;50(4):333-8.

42. Rezaei S, Rezaei K, Mahboubi M, Jarahzadeh MH, Momeni E, Bagherinasab M, et al. Comparison the efficacy of herbal mouthwash with chlorhexidine on gingival

index of intubated patients in Intensive Care Unit. Journal of Indian Society of Periodontology. 2016;20(4):404.

43. Vargas KG, Joly S. Carriage frequency, intensity of carriage, and strains of oral yeast species vary in the progression to oral candidiasis in human immunodeficiency virus-positive individuals. Journal of clinical microbiology. 2002;40(2):341-50.

44. Cense AW. A spray based method for biofilm removal. 2005.

45. Chen Z, Saxena D, Caufield PW, Ge Y, Wang M, Li Y. Development of species-specific primers for detection of Streptococcus mutans in mixed bacterial samples. FEMS microbiology letters. 2007;272(2):154-62.

46. Zhang J, Hung G-C, Nagamine K, Li B, Tsai S, Lo S-C. Development of Candida-specific Real-Time PCR assays for the detection and identification of eight medically important Candida species. Microbiology insights. 2016;9:MBI. S38517.

47. Byun R, Nadkarni MA, Chhour K-L, Martin FE, Jacques NA, Hunter N. Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. Journal of clinical microbiology. 2004;42(7):3128-36.

48. Bascones A, Morante S, Mateos L, Mata M, Poblet J. Influence of additional active ingredients on the effectiveness of non-alcoholic chlorhexidine mouthwashes: a randomized controlled trial. Journal of periodontology. 2005;76(9):1469-75.

49. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and chemical toxicology. 2008;46(2):446-75.

50. Fine D, Furgang D, Barnett M, Drew C, Steinberg L, Charles C, et al. Effect of an essential oilcontaining antiseptic mouthrinse on plaque and salivary Streptococcus mutans levels. Journal of Clinical Periodontology. 2000;27(3):157-61.

51. McKenzie WT, Forgas L, Vernino AR, Parker D, Limestall J. Comparison of a 0.12% chlorhexidine mouthrinse and an essential oil mouthrinse on oral health in institutionalized, mentally handicapped adults: One-year results. Journal of periodontology. 1992;63(3):187-93.

52. Ibrahim MY, Hashim NM, Mariod AA, Mohan S, Abdulla MA, Abdelwahab SI, et al. α-Mangostin from Garcinia mangostana Linn: an updated review of its pharmacological properties. Arabian journal of Chemistry. 2016;9(3):317-29.

53. Jarosz LM, Deng DM, van der Mei HC, Crielaard W, Krom BP. Streptococcus mutans competence-stimulating peptide inhibits Candida albicans hypha formation. Eukaryotic cell. 2009;8(11):1658-64.

54. Thein ZM, Samaranayake YH, Samaranayake LP. Effect of oral bacteria on growth and survival of Candida albicans biofilms. Archives of oral biology. 2006;51(8):672-80.

55. Tepper BJ, Ullrich NV. Taste, smell, and the genetics of food preferences. Topics in Clinical Nutrition. 2002;17(4):1-14.

56. Malhotra R, Grover V, Kapoor A, Saxena D. Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidine gluconate at the clinical and patient level. Journal of Indian Society of Periodontology. 2011;15(4):349.

57. Axelsson P, Lindhe J, Nyström B. On the prevention of caries and periodontal disease: results of a 15-year longitudinal study in adults. Journal of clinical periodontology. 1991;18(3):182-9.

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