CHAPTER 2

BACKGROUNDS AND LITERATURE REVIEW

All information summarized in this chapter provides the whole picture of theoretical backgrounds. This chapter reviewed overview knowledge including *Boesenbergia Pandurata,* Dental carries, *Streptococcus mutans,* extraction techniques, and tooth care products.

2.1 Boesenbergia Pandurata

Boesenbergia Pandurata (B. Pandurata) was the herbal plant which general mixed in food, and medicine (Figure 2.1) (https://sites.google.com/site/prodifactproducts2/boesenbergia-pandurata). *B. pandurata* is a member of the Zingberaceae family. *B. pandurata,* commonly known as finger root, lesser galangal or Chinese ginger, is a medicinal and culinary herb from China and Southeast Asia. In English, the root has traditionally been called finger root, because the shape of the rhizome resembles that of fingers growing out of a center piece. *B. pandurata* contains 1 to 3% of an essential oil. Several aroma components have been identified, 1-8 cineol, camphor, d-borneol and methyl cinnamate being the most important. Trace components are d-pinene, zingiberene, zingiberone, curcumin, zedoarin and others (Patoomratana el al., 2002). Rhizome also contains many vitamins such as vitamin A, B12 and calcium. Its origin was found in Southern China, and Southeast Asia. Its therapeutic applications were in Anthelmintic, Anti-allergic, Carminative, Digestive, and Stomachic. It is traditionally included in the diet to aid sluggish digestion, flatulence, and indigestion. The rhizome is also used for tooth and gum disease, diarrhea, dysentery, and as a general diuretic. *B. pandurata* was called in Thai including Ka-aen, Wan-phraathit, Khingsai khingkaeng, Ka chai, Kra chai.

Figure 2.1 Fresh rhizomes of *B. pandurata*

The preliminary studied on hydrodistilate of *B. pandurata* indicated that it can inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*. The ability of *B. pandurata* proved to inhibit biofilm formation by various bacteria. Yanti et al. (2008) did a series of study on the effects concentrating on its ability to inhibit the growth of oral pathogens in particular *Porphyromonas gingivalis*. Moreover, Panduratin A has also been found to be a very potent inhibitor of Staphylococcus strains (Figure 2.2) (Yanti et al., 2010). Finally, the other studies indicated that *B. pandurata* can use for reducing the symptoms such as inhibition of melanin biosynthesis, anti-aging activity, cytotoxic activity, anti-obesity activity (Tewtrakul et al., 2009).

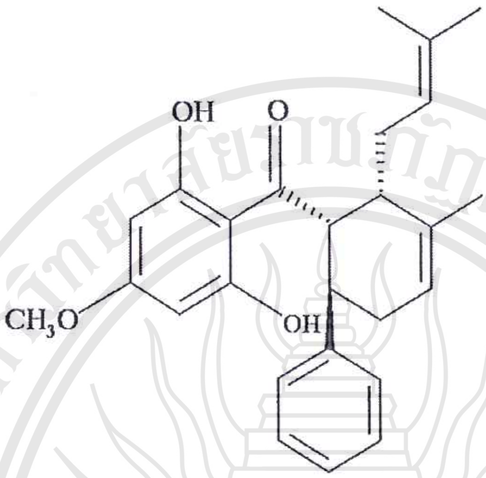

Figure 2.2 Structure of Panduratin A

Kaempferia pandurata Roxb. (Zingiberaceae), locally known as temu kunci in Indonesia or krachai in Thailand, has been traditionally used in Southeast Asia as a food ingredient and a folk medicine for treatment of dental caries, colic disorder, fungal infection, dry cough, rheumatism and muscular pains (Trakoontivakornet al., 2001). Its rhizome contains pinostrobin, cardamonin, boesenbergin, 5, 7-dimethoxyflavone, 1, 8-cineole, panduratin A, isopanduratin A and other compounds (Mahidol et al., 1984). Recently, the rhizome extracts of Kaempferia pandurata have been tested for various pharmacological effects including antibacterial, anti inflammatory, antitumor, antidiarrhea, antidysentry, antiflatulence and antiepidermophytid activities (Calliste et al.,2001; Tuchinda et al., 2002). Furthermore, our previous studies reported that panduratin A and isopanduratin A, bioactive constituents isolated from the ethanolic extract of Kaempferia pandurata Roxb., exhibited strong anticariogenic activities against periodontopathogens especially Streptococcus mutans and Porphy romonas gingivalis (Hwang et al., 2004; Park et al., 2005). Therefore, Kaempferia pandurata Roxb is thought to be useful for treatment of periodontal disease. However, little is known about its efficacy underlyingMMP-9 inhibition for preventing periodontal inflammation.

2.2 Dental carries

Dental carries, also known as tooth decayora cavity, is an infection, bacterial in origin, that causes demineralization and destruction of the hard tissues (enamel, dentin and cementum), usually by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface. If demineralization exceeds saliva and other demineralization factors such as from calcium and fluoridated toothpastes, these hard tissues progressively break down, producing dental carries (cavities, holes in the teeth). The bacteria most responsible for dental cavities are the mutans streptococci, most prominently *Streptococcus mutans* and *Streptococcus sobrinus*, and lactobacilli. If left untreated, the disease can lead to pain, tooth loss and infection. Today, carries remain one of the most common diseases throughout the world. The presentation of carries is highly variable. However, the risk factors and stages of development are similar. Initially, it may appear as a small chalky area (smooth surface carries), which may eventually develop into a large cavitation. Sometimes, carries may be directly visible. However, other methods of detection such as X-rays are used for less visible areas of teeth and to judge the extent of destruction. Lasers for detecting carries allow detection without ionizing radiation and are now used for detection of interproximal decay (between the teeth). Disclosing solutions are also used during tooth restoration to minimize the chance of recurrence.

Tooth decay disease is caused by specific types of bacteria that produce acid in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. To be specific, a tooth (which is primarily mineral in content) is in a constant state of back-and-forth demineralization between the tooth and surrounding saliva. For people with little saliva, especially due to radiation therapies that may destroy the salivary glands, there also exists remineralization gel. These patients are particularly susceptible to dental carries. When the pH at the surface of the tooth drops below 5.5, demineralization proceeds faster than remineralization (meaning that there is a net loss of mineral structure on the tooth's surface). Most foods are in this acidic range and without remineralization, these results in the ensuing decay. Depending on the extent of tooth destruction, various treatments can be used to restore teeth to proper form, function, and aesthetics, but there is no known method to regenerate large amounts of tooth structure. Instead, dental health organizations advocate preventive and prophylactic measures, such as regular oral hygiene and dietary modifications, to avoid dental carries.

There are four main criteria required for carries formation: a tooth surface (enamel or dentin); carries-causing bacteria; fermentable carbohydrates (such as sucrose); and time. The carries process does not have an inevitable outcome, and different individuals will be susceptible to different degrees depending on the shape of their teeth, oral hygiene habits, and the buffering capacity of their saliva. Dental carries can occur on any surface of a tooth that is exposed to the oral cavity, but not the structures that are retained within the bone. All carries occur from acid demineralization that exceeds saliva and fluoride remineralization, and almost all acid demineralization occurs where food (containing carbohydrate like sugar) is left on teeth. Because most trapped food is left between teeth, over 80% of cavities occur inside pits and fissures on chewing surfaces where brushing, fluoride, and saliva cannot reach to remineralize the tooth as they do on easy-to-reach surfaces that develop few cavities.

There are certain diseases and disorders affecting teeth that may leave an individual at a greater risk for carries. Amelogenes is imperfect, which occurs between 1 in 718 and 1 in 14,000 individuals, is a disease in which the enamel does not fully form or forms in insufficient amounts and can fall off a tooth. In both cases, teeth may be left more vulnerable to decay because the enamel is not able to protect the tooth.

In most people, disorders or diseases affecting teeth are not the primary cause of dental carries. Ninety-six percent of tooth enamel is composed of minerals. These minerals, especially hydroxyapatite, will become soluble when exposed to acidic environments. Enamel begins to demineralize at a pH of 5.5. Dentin and cementum are more susceptible to carries than enamel because they have lower mineral content. Thus, when root surfaces of teeth are exposed from gingival recession or periodontal disease, carries can develop more readily. Even in a healthy oral environment, however, the tooth is susceptible to dental carries.

The evidence for linking malocclusion and/or crowding to the dental carries is weak; however, the anatomy of teeth may affect the likelihood of carries formation. Where the deep grooves of teeth are more numerous and exaggerated, pit and fissure carries are more likely to develop. Also, carries are more likely to develop when food is trapped between teeth.

The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental carries: *S. mutans* and *Lactobacilli* among them. These organisms can produce high levels of lactic acid following fermentation of dietary sugars, and are resistant to the adverse effects of low pH, properties essential for cariogenic bacteria. As the cementum of root surfaces is more easily demineralized than enamel surfaces, a wider variety of bacteria can cause root carries including *Lactobacillus acidophilus*, *Actinomyces spp.*, *Nocardia spp.*, and *S. mutans*. Bacteria collect around the teeth and gums in a sticky, creamy-colored mass called plaque, which serves as a biofilm. Some sites collect plaque more commonly than others, for example, sites with a low rate of salivary flow (molar fissures). Grooves on the surfaces of molar and pre molar teeth provide microscopic retention sites for plaque bacteria, as do the approximate sites. Plaque may also collect above or below the gingival where it is referred to as supra- or sub-gingival plaque, respectively.

These bacterial strains, most notably *S. mutans* can be inherited by a child from a care takers kiss or through feeding food Bacteria in a person's mouth convert glucose, fructose, and most commonly sucrose (table sugar) into acids such as lactic acid through a glycolytic process called fermentation. If left in contact with the tooth, these acids may cause demineralization, which is the dissolution of its mineral content. The process is dynamic, however, as remineralization can also occur if the acid is neutralized by saliva or mouthwash. Fluoride toothpaste or dental varnish may aid remineralization. If demineralization continues over time, enough mineral content may be lost so that the soft organic material left behind disintegrates, forming a cavity or hole. The impact such sugars have on the progress of dental carries is called carcinogenicity. Sucrose, although a bound glucose and fructose unit, is in fact more cariogenic than a mixture of equal parts of glucose and fructose. This is due to the bacteria utilizing the energy in the saccharide bond between the glucose and fructose subunits. *S. mutans* adheres to the biofilm on the tooth by converting sucrose into an extremely adhesive substance called dextran polysaccharide by the enzyme dextran sucranase.

Worldwide, approximately 2.43 billion people (36% of the population) have dental carries in their permanent teeth. In baby teeth, it affects about 620 million people or 9% of the population. The disease is most prevalent in Latin American countries, countries in the Middle East, and South Asia, and least prevalent in China. In the United States, dental carries is the most common chronic childhood disease, being at least five times more common than asthma. It is the primary pathological cause of tooth loss in children. The number of cases has decreased in some developed countries, and this decline is usually attributed to increasingly better oral hygiene practices and preventive measures such as fluoride treatment. Nonetheless, countries that have experienced an overall decrease in cases of tooth decay continue to have a disparity in the distribution of the disease. Among children in the United States and Europe, twenty percent of the population endures sixty to eighty percent of cases of dental carries. A similarly skewed distribution of the disease is found throughout the world with some children having none or very few carries and others having a high number Australia, Nepal, and Sweden have a low incidence of cases of dental carries among children, whereas cases are more numerous in Costa Rica and Slovakia. The classic "DMF" (decay/missing/filled) index is one of the most common methods for assessing carries prevalence as well as dental treatment needs among populations. This index is based on in-field clinical examination of individuals by using a probe, mirror and cotton rolls. Because the DMF index is done without X-ray imaging, it underestimates real carries prevalence and treatment needs.

2.3 Streptococcus mutans

The important dental carries bacteria were *Streptococcus mutans* and *Streptococcus sobrinus*. The *Streptococcus mutans* were about 90% and *Streptococcus sobrinus* were approximately 8% to 40%. Figure 2.3 showed the shape of both bacteria that cause the dental carries. Both bacteria were used as sucrose as carbon source and change the sucrose to lactic acid. The lactic acid caused the acid inside mouth and destroyed the teeth enamel.

Streptococcus mutans is a Gram-negative bacteria. (The Gram stain techniques is a technique used to classify bacteria from the difference of the chemical composition of the cell wall. A facultatively anaerobic bacteria can live without O_2 but will grow better with O_2 normally found inside of the humans mouth.

Inside human mouth has specifical good receptor for both bateria binding to teeth and produce dextransucrase for changing of sucrose to Polysaccharide dextran. Polysaccharidedextran was a cohesive teeth coating and cause a plaque.

Sucrose was the only sugar that *S. mutans* use for producing of dextran. The other sugar such as glucose, fructose, lactose can directly digested by *S. mutans* and change to lactic acid as the final product. This acid bacteria eventually cause tooth decay. This bacteria were discovered by Clarke in 1924.

(a) *Streptococcus mutans*

(Casey and Jesse, 2010)

(b) *Streptococcus sobrinus*

(Kenyon and et al., 2011)

Figure 2.3 Dental carries bacteria

2.4 Mouthwash

Plaque control can be achieved by use of mechanical and chemical methods. Mechanical methods include: tooth brushing, interproximal cleaning using dental floss, tape or interproximal brushes. Chemical methods involve the use of a mouthwash. In the UK, there has been an increase in the variety of mouthwashes available and in sales of these products. Patients can buy mouthwashes from supermarkets and chemist shops; typically start using them in addition to the mechanical plaque removal. There are reports of some patients using mouthwashes as often as up to six times per day. Many over-the-counter and prescription mouthwashes contain a high volume percentage of alcohol and have a low pH. Studies have shown that the use of mouthwashes with a low pH can cause erosion of enamel and dentine and adversely affect the physical properties of some restorative materials. The surface finish of a restoration is important for plaque retention, staining and patient comfort. In a recent study, Jones et al. found that patients were able to detect and distinguish between surface roughness's levels within the range of 0.25–0.50 mm. They concluded that restorations should be finished with a maximum roughness of 0.50 mm in order to make the restoration undetectable by a patient, tongue (Sadaghiani, L., M. A. Wilson, et al., 2007).

Mouthwash is a liquid oral product designed to freshen breath. Certain varieties may also kill bacteria and/or whiten teeth. Mouthwashes are made by combining the appropriate raw materials in large, stainless steel tanks and then filling the product into individual packages. First used by ancient societies, technological advances in chemistry have resulted in steadily improving formulas (Antonio, A. G., N. L. P. Iorio, et al. 2011).

The need for mouthwash is a result of a condition called halitosis, or bad breath. It is estimated that over half the population occasionally has foul-smelling breath. This typically occurs upon first awakening or after a meal with garlic or onions. It has been found that bad breath is mostly due to bacterial activity in an unclean mouth. Specifically, anaerobic bacteria that grow on the protein-rich food debris stuck between the teeth or on the tongue. As the bacteria breaks down the proteins, those containing sulphur give off foul odor molecules such as methylmercaptan and hydrogen sulphide which result in bad breath. Mouthwashes are designed to eliminate bad breath in two ways. First, they relieve it by killing the bacteria responsible for producing the foul odor. The best of these products prevent bad breath for as long as eight hours. The second way that mouthwashes help reduce bad breath is by masking the odor. This is a much less effective method which lasts no more than 30 minutes (Hwang, J.-K., and J.-S. Shim, et al., 2004).

Products used for freshening breath or cleaning teeth have been in existence for centuries. Many of the ancient societies—including the Egyptians, Chinese, Greeks, and Romans—had recipes for such preparations. They used a variety of ingredients; from edible materials like fruit, honey, or dried flowers to less appealing compounds such as ground lizard, minced mice, or urine. These products were generally ineffective and in some cases were harmful to the sensitive enamel which coats each tooth (Yenjai, C., K. Prasanphen, et al., 2004).

While tooth cleaning preparations steadily improved over the years, it was not until the early 1800s—when the modern toothpaste was developed—that truly effective oral products became available. The first mouthwashes were basically solutions of grain alcohol and were likely developed accidentally during this era. One of the most famous brands, Listerine, was developed during the 1880s and is still sold today. The antibacterial effect of fluoride was an important discovery for the development of modern mouthwashes. In the early 1900s, a dentist named Frederick McKay found that some of his patients had a condition called mottled enamel. He found that this condition was linked to a reduction in tooth decay. In 1931, he tested the drinking water that these patients consumed and found a high level of natural fluoride. By the early 1940s, other workers had determined that fluoride in drinking water at one part per million would reduce tooth decay without causing mottling. Various testing went on during the rest of this decade and by the 1950s, it was recommended by the United States federal government that all public water sources be fortified with fluoride. This discovery led to the development of toothpastes and mouthwashes that contained fluoride compounds. During the years that followed, various raw materials have been developed that have an antibacterial effect but do not contain alcohol. Additionally, materials that prevent tartar, whiten teeth, and reduce cavities have also been discovered and added to mouthwash formulas (Tewtrakul, S., S. Subhadhirasakul, et al., 2008).

2.4.1 Mouthwash manufacturing process

After a mouthwash formula is designed, it is tested to ensure that minimal changes will occur over time regardless of the storage conditions. This testing, called stability testing, helps detect physical changes in such things as color, odor, and flavor. It can also provide information about product performance over time. In the United States, the Food and Drug Administration (FDA) requires that specific stability testing be done to ensure product performance during long term storage (Solórzano-Santos, F. and M. G. Miranda-Novales, 2012). In general, the process for creating a mouthwash occurs in 4 distinct steps which are compounding, batch analysis, filling and quality control.

Compounding: Mouthwash is made via a batch process in an area of the manufacturing plant called compounding. Mouthwash was made batches of 2,000-3,000 gal (7,571-11,356 L) of mouthwash following specific formula instructions. The raw materials are delivered to the compounding area by fork lift trucks. Compounders add them to the main batch tank where they are thoroughly mixed. Depending on the formula instructions, the batch is heated and cooled to get the raw materials to rapidly combine. Materials which are used in large quantities—such as alcohol or water—are then pumped directly into the tank. This is done by simply setting computer controls to the appropriate amount and pushing a button. Computers also control the mixing speed and temperature of the batch. Depending on the size of the batch and the number of raw materials, a mouthwash can take anywhere from one to three hours to make.

Batch analysis: When the batch is completed, a sample is taken to the Quality Control (QC) lab. The appearance and flavor of the batch is examined to ensure that it meets the specifications laid out in the formula. QC chemists may also run pH determinations and viscosity checks. If some characteristic of the batch is found to be out of a specified range, adjustments may be made at this point. For example, colors can be modified by adding more dye. After the batch is approved, it is pumped from the main tank to a holding tank. This holding tank may be directly hooked up to the filling lines where the product is put into individual packaging.

Filling: At the beginning of the filling line there is a large bin called a hopper which contains the empty bottles that will be filled. In this bin, the bottles are physically manipulated so that they come out standing upright on a conveyor belt. They are then moved to the filling carousel which contains the bulk mouthwash product. The filling carousel has a series of piston filling heads that are designed to deliver an exact amount of mouthwash. As the bottles move around the carousel, the piston moves down and the mouthwash product is dispensed into the bottle. After the bottles are filled, they are sent on a conveyor belt to a capping machine. The caps are also held in a large bin and correctly aligned. As the bottles pass the capping hopper, the caps are put on and either twisted or pushed in place. From the capping station, the bottles are moved to a labeling machine. The labels are held on large spools and threaded through the machine. As the bottles pass by, the label is either stuck on using an adhesive or heat pressed. Beyond labeling, the bottles are next moved to a boxing station. They are typically gathered in a group of 12 or 24 and dropped into a box. The boxes then move to a palliating machine and stacked. The pallets are moved via fork lifts to large trucks and shipped to distributors. High speed production lines like these can produce over 20,000 bottles per hour.

Quality Control: While quality control is a critical step in the batching process, it is also done at other points during manufacture. Workers are stationed at various points on the filling lines to inspect the production process. They examine things such as bottle quality, fill levels, and label placement. They also make sure that all the caps are put on correctly. Microbial contamination is also routinely checked during the filling process. Additionally, the packaging is checked for things such as bottle thickness, appearance, and weight to make sure the final product has the desired characteristics. With advances in chemical technology, mouthwashes of the future will be designed with a larger array of and more improved functions. In the past, mouthwashes were primarily powerful breath fresheners. They eventually evolved into tooth protectors. Today, products are available to not only fight bad breath but whiten teeth and help battle cavity formation and gum recession. Some new technologies that will undoubtedly be adapted to mouthwash products have recently been discovered. For example, researchers have found a peptide known as p1025, which can bond to the teeth and prevent the growth of naturally occurring bacteria. This prevents the cavity-causing bacteria to adhere to the tooth and thus inhibits cavity formation. Using this technology, they have created a mouthwash that may prevent tooth decay for up to three months. Another new mouthwash may actually contain good bacteria to kill the odor-and cavity-causing germ Streptococcus mutans. Using genetic engineering, scientists at the University of Florida, College of Dentistry developed this bacterium and are now testing it in humans to determine whether it can be used. Ultimately, this new bacteria may be added to mouthwash products and thereby revolutionizing oral care (Sae-wong, C., P. Tansakul, et al., 2009).

2.4.2 Composition of mouth wash

Mouthwashes are generally composed of diluents, antibacterial agents, soaps, flavorings, and colorants. The primary ingredient in most mouthwashes is water, a diluent, making up over 50% of the entire formula. The water is specially treated to remove various particles and ions which might impact flavor. Water treated as such is called deionized water. The sources of water vary, coming from reserves such as underground wells, lakes, and rivers. Alcohol is another diluent typically used in up to 20% of the formula. While early mouthwashes used alcohol extensively; today its use is limited because of governmental regulations and consumer desires (Ahrens, W., H. Pohlabeln, et al., 2014).

Numerous antibacterial agents have been employed in mouthwash formulations. These include ingredients like phenols, thymol, salol, tannic acid, hexachloraphene, chlorinated thymols, and quaternary ammonium compounds. Chlorinated phenols like parachlormetacresol have both an antibacterial effect and a desired flavor. Thymol, which is obtained from volatile oils, is used at low concentrations and in conjunction with other ingredients. Hexachloraphene is substantive to the mucous membrane which makes it ideal for longer lasting formulas. Quatemary compounds are often used because of their non-toxic and non-irritant nature. They are effective against plaque (Rujjanawate, C., D. Kanjanapothi, et al., 2005; Cheenpracha, S., C. Karalai, et al., 2006).

Color and flavor are added to the formulas to improve the consumer acceptability of the mouthwash ingredients. Flavor is an essential feature of a mouthwash because it has the most consumer perceptible impact. In the United States, flavorants such as peppermint, menthol, methyl salicylate, and eugenol are commonly used. The most common colors, blue and green, are the result of adding governmentally approved and certified FD&C dyes. Some mouthwash formulas also include a synthetic detergent to give extra foaming and cleansing action. (Wong, R. W. K., U. Hägg, et al., 2010).

In general there are three types of mouthwashes. There are antibacterial products that reduce the bacterial population of the mouth. These products have a fresh taste and improve breath odor. The second type is fluoride mouthwashes, which help to improve the fluoride layer on tooth enamel. Finally, there are demineralizing mouthwashes that help repair various lesions in the mouth. Mouthwashes are sold in a variety of flavors and colors. The most popular is the golden colored, medicinal-tasting Listerine. Blue or green mint varieties are also common. There are mouthwash products that are geared specifically toward smokers. These products are designed to remove tobacco tar stains as well as fresh breath and whiten teeth. There are also tartar control mouthwashes and those that kill the germs responsible for gingivitis and plaque. The packaging for mouthwashes is typically a clear, plastic bottle. Since the products generally contain alcohol, child resistance and tamper evidence closures are typically used. Additionally, some mouthwashes have a dosing feature which lets the consumer squeeze out an exact amount each time it is used.

Mouthwash helps to clean the mouth and to improve teeth health care. The teeth brushing and flossing required for teeth cleaning and food residue eliminating. The mouthwash used only to reduce oral bacteria, to give temporary fresh breath, and to increase the mouth moist. Mouthwash is divided into three types such as ready use, concentrated, and mixed before use. The mouthwash ingredients had to use as same as cosmetic criteria following Ministry of Public Health regulation. The common properties for mouthwash products were included as followings (Ahrens, W., H. Pohlabeln, et al., 2014).

- The liquid is clear solution, no sediment, and no layer separation.
- The pH of product was 3.0 to 5.0.
- The total concentration of all heavy metals combination were less than 20 mg/kg and tested by atomic absorption spectroscopy photometer or other equivalent tool. The concern heavy metals were zinc, copper, lead, arsenic and mercury.
- The preservation must not show any deterioration as a separate sediment layers opaque color.
- The total number of bacteria, yeast and mold (total colony count) shall not exceed 100 colonies per gram or cubic centimeters.
- *Clostridium, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* cannot found.

The main compositions of mouthwash consist of flavors, antiseptic or disinfectant, astringent, drug extract, surfactant, colors, and humectants.

1. Flavors: The main function used for help mouth fresh, cool and clean after use. The used flavors can reduce the bad mouth odor, no toxic, non-irritating to mucous membranes in the mouth. The general ingredients were essential oils, high aromatic aldehyde, peppermint oil, menthol, aniseed oil, and cinnamon oil.

2. Antiseptic or disinfectant reduces the number of bacteria in the mouth which causes bad breath. Disinfectants must be safe to eat such as chlorinated phenol, beta-naphthnol, hexyl resorcinol, thymol, and hydrogen peroxide.

3. Astringent used for preventing of inflammation in oral mucosa. It helps to reduce the accumulation of protein in saliva, mucous secretion by the precipitation

and it can easily wash off including zinc chloride, zinc acetate, alum, zinc phenol sulfonate, and tannic acid.

4. Drug Extract may be filled with overly astringent.
5. Surfactant fills to increase the absorption of disinfectants on the oral mucosa.
6. The colors used from plant colors such as saffron, carmine, and phoxine.
7. Humectants used to help prevent crystallization of the fluid around the bottle cap, adjust the viscosity, and improve the taste of alcohol. It also has the power to heal a mild astringent and reduces tension.

The traditional medicinal use of Salvadora persica as an antimicrobial stick toothbrush for oral hygiene and to treat gum inflammation is a centuries old practice and a part of the Greeko Arab system of medicine. The miswak chewing stick is used by the majority of Muslim people in developing countries where it is growing. This traditional toothbrush stick can be used by the vast majority of people who cannot afford to buy commercial western toothbrushes and toothpastes, especially those in developing countries. The extent of carious frequency and the total lack of oral hygiene can be explained by socioeconomic conditions, seldom curative care (expensive and painful) associated with the socio-cultural context, especially in children. For these reasons, the World Health Organization recommends the use of chewing sticks as an effective tool for oral hygiene in areas where such use is customary. Thus, during recent years, many researchers throughout the world have studied miswak as a helpful plant in mouth and tooth hygiene and have demonstrated the antimicrobial, anti-caries and antiperiopathic properties of aqueous extracts of various chewing sticks (Chelli-Chentouf, N., A. Tir Touil Meddah, et al., 2012).

The results of the antimicrobial activity of methanolic extracts of Hoggar Salvadora persica L. are showed a varying degree of antimicrobial activity against the microbial species isolated from the oral cavity. The miswak had an inhibitory effect on the growth of Staphylococcus species, Streptococcus mutans. The highest inhibition was seen against Escherichia coli. Overall, the methanolic extract exhibited a stronger antibacterial activity against Gram negative (6.5–12 mm) than Gram positive (1–8 mm) bacteria. This difference is probably due to a difference in the chemical composition of the bacterial cell-wall (Leclerc et al., 1986), especially in the lipopolysaccharids (LPS) layer composition (Nostro et al., 2000). This layer of LPS can be considered as a barrier to diffusion, making Gram negative bacteria less likely to be susceptible to antimicrobial agents. Al-Bayati and Sulaiman (2008) specify that the antibacterial effect can also be due to various chemical substances contained in the extract such as saponins, tannins, alkaloids and terpenoids. A study by Sofrata et al. (2008) on the antibacterial effect of suspended and embedded miswak species on oral bacteria indicated that this may be due to different assays used to test the antibacterial activity. Well-standardized studies are needed to identify which components of the extract exert antibacterial effect against Gram positive and Gram negative bacteria. In addition, the pH of Salvadora persica L. extract was 5.5 which is acid and can disturb the development of a nitrophile flora. According to Guiraud (1998), enterobacteria, Staphylococcus and enterococci grow easily in neutral medium, while an acid pH make their growth more difficult. Nevertheless, Staphylococcus and Streptococcus can develop at this pH. The virulence of Streptococcus mutans resides in three core attributes, its abilities to form biofilms on the tooth surface, to produce large quantities of organic acids (acidogenicity) from a wide range of carbohydrates, and to tolerate environmental stresses, particularly low pH (Lemos and Burne, 2008). The methanolic extract of Salvadora persica was active against all fungal strains, but its effectiveness varied between them. At the concentration of 400 mg/ml, Candida albicans was the most sensitive one, following by Candida sp. The weakest activity of the same plant extract was demonstrated against Penicillium sp. Noumi et al. (2010) showed that methanolic extracts of dry Salvadora persica stems were active on only one oral Candida albicans isolate. Another study showed that 20% miswak extract was effective against the Candida albicans with different contact times, namely 1, 6 and 24 h (Al-Obaida Mohammad et al., 2010). The methanolic extract either inhibited or enhanced the growth depending on which test strain was used. For Staphylococci, a significant reduction in growth was revealed after 2 h of incubation. Streptococcus mutans and Lactobacillus sp. were also susceptible to this extract. The antibacterial effect was more pronounced on Gram negative (Escherichia coli) than Gram positive (Staphylococcus, Streptococcus and Lactobacillus). For fungal strains, a significant reduction in the counts of Candida albicans and Candida sp. was witnessed while a lesser effect was seen on Penicillium sp. Our findings are in accordance with other studies. According to Guiraud (1998), the activity of the antimicrobial agent depends on the physiological state of bacteria, bacteria in the exponential phase of growth being more sensitive.

2.5 Toothpaste

In 1850, Dr. Washington Wentworth Sheffield, a dental surgeon and chemist, invented the first toothpaste. Dr. Sheffield had been using his invention, which he called "Cream Dentifrice", in his private practice. The positive response of his patients encouraged him to market the paste. He constructed a laboratory to improve his invention and a small factory to manufacture it. Modern toothpaste was invented to aid in the removal foreign particles and food substances, as well as cleans the teeth. When originally marketed to consumers, toothpaste was packaged in jars. Chalk was commonly used as the abrasive in the early part of the twentieth century.

The next big milestone in toothpaste development happened in the mid-twentieth century (1940-60, depending on source). After studies proving fluoride aided in protection from tooth decay, many toothpastes were reformulated to include sodium fluoride. Fluoride's effectiveness was not universally accepted. Some consumers wanted fluoride-free toothpaste, as well as artificial sweetener-free toothpaste. The most commonly used artificial sweetener is saccharin. The amount of saccharin used in toothpaste is minuscule. Companies like Toms of Maine responded to this demand by manufacturing both fluoridated and non-fluoridated toothpastes, and toothpastes without artificial sweetening (Kanaffa-Kilijańska, U., U. Kaczmarek, et al., 2013).

Many of the innovations in toothpaste after the fluoride breakthrough involved the addition of ingredients with "special" abilities to toothpastes and toothpaste packaging. In the 1980s, tartar control became the word in the dentifrice industry. Tartar control toothpastes claimed they could control tartar build-up around teeth. In the 1990s, toothpaste for sensitive teeth was introduced. Bicarbonate of soda and other ingredients were also added in the 1990s with claims of aiding in tartar removal and promoting healthy gums. Some of these benefits have been largely debated and have not been officially corroborated. Packaging toothpaste in pumps and stand-up tubes was introduced during the 1980s and marketed as a neater alternative to the collapsible tube. In 1984, the Colgate pump was introduced nationally, and in the 1990s, stand-up tubes spread throughout the industry, though the collapsible tubes are still available (Chu, C. H., S. S. S. Wong, et al., 2013).

Every toothpaste contains the following ingredients: binders, abrasives, sudsers, humectants, flavors (unique additives), sweeteners, fluorides, tooth whiteners, a preservative, and water. Binders thicken toothpastes. They prevent separation of the solid and liquid components, especially during storage. They also affect the speed and volume of foam production, the rate of flavor release and product dispersal, the appearance of the toothpaste ribbon on the toothbrush, and the rinsibility from the toothbrush. Some binders are karaya gum, bentonite, sodium alginate, methylcellulose, carrageenan, and magnesium aluminum silicate. Abrasives scrub the outside of the teeth to get rid of plaque and loosen particles on teeth. Abrasives also contribute to the degree of opacity of the paste or gel. Abrasives may affect the paste's consistency, cost, and taste (Yim, N.-H., Y. P. Jung, et al., 2013).

The most commonly used abrasives are hydrated silica (softened silica), calcium carbonate (also known as chalk), and sodium bicarbonate (baking soda). Other abrasives include dibasic calcium phosphate, calcium sulfate, tricalcium phosphate, and sodium metaphosphate hydrated alumina. Each abrasive also has slightly different cleaning properties, and a combination of them might be used in the final product.

Sudsers, also known as foaming agents, are surfactants. They lower the surface tension of water so that bubbles are formed. Multiple bubbles together make foam. Sudsers help in removing particles from teeth. Sudsers are usually a combination of an organic alcohol or a fatty acid with an alkali metal. Common sudsers are sodium lauryl sulfate, sodium lauryl sulfoacetate, dioctyl sodium sulfosuccinate, sulfolaurate, sodium lauryl sarcosinate, sodium stearyl fumarate, and sodium steady lactate.

Humectants retain water to maintain the paste in toothpaste. Humectants keep the solid and liquid phases of toothpaste together. They also can add a coolness and/or sweetness to the toothpaste; this makes toothpaste feel pleasant in the mouth when used. Most toothpastes use sorbitol or glycerin as humectants. Propylene glycol can also be used as a humectant (Laine, M. A., M. Tolvanen, et al., 2014).

Toothpastes have flavors to make them more palatable. Mint is the most common flavor used because it imparts a feeling of freshness. This feeling of freshness is the result of long term conditioning by the toothpaste industry. The American public associates mint with freshness. There may be a basis for this in fact; mint flavors contain oils that volatize in the mouth's warm environment. This volatizing action imparts a cooling sensation in the mouth. The most common toothpaste flavors are spearmint, peppermint, wintergreen, and cinnamon. Some of the more exotic toothpaste flavors include bourbon, rye, anise, clove, caraway, coriander, eucalyptus, nutmeg, and thyme (Chung, J. Y., J. H. Choo, et al., 2006).

In addition to flavors, toothpastes contain sweeteners to make it pleasant to the palate because of humectants. The most commonly used humectants (sorbitol and glycerin) have a sweetness level about 60% of table sugar. They require an artificial flavor to make the toothpaste palatable (Khan, A. V., Q. U. Ahmed, et al., 2012).

Fluorides reduce decay by increasing the strength of teeth. Sodium fluoride is the most commonly used fluoride. Sodium perborate is used as a tooth whitening ingredient. Most toothpaste contains the preservative p-hydrozybenzoate. Water is also used for dilution purposes.

2.5.1 Toothpaste manufacturing process

There are main four manufacturing process. It consists of weighing and mixing, filling the tubes, packaging and shipment, and quality Control.

Weighing and mixing: After transporting the raw materials into the factory, the ingredients are both manually and mechanically weighed. This ensures accuracy in the ingredients' proportions. Then the ingredients are mixed together. Usually, the glycerin-water mixture is done first.

All the ingredients are mixed together in the mixing vat. The temperature and humidity of vat are watched closely. This is important to ensuring that the mix comes together correctly. A commonly used vat in the toothpaste industry mixes a batch that is the equivalent of 10,000 four-ounce (118 ml) tubes.

Filling the tubes: Before tubes are filled with toothpaste, the tube itself passes under a blower and a vacuum to ensure cleanliness. Dust and particles are blown out in this step. The tube is capped, and the opposite end is opened so the filling machine can load the paste. After the ingredients are mixed together, the tubes are filled by the filling machine. To make sure the tube is aligned correctly, an optical device rotates the tube. Then the tube is filled by a descending pump. After it is filled, the end is sealed (or crimped) closed. The tube also gets a code stamped on it indicating where and when it was manufactured.

Packaging and shipment: After tubes are filled, they are inserted into open paperboard boxes. Some companies do this by hand. The boxes are cased and shipped to warehouses and stores.

Quality Control: Each batch of ingredients is tested for quality as it is brought into the factory. The testing lab also checks samples of final product.

2.5.2 Herbal Toothpaste

Many kinds of Thailand herbs were used in toothpaste such as Khoi, Clove, sea salt, cuttlebone, and menthol. Cloves are often used as an oil flower. It has the effect of mild antiseptics. Also, it has salt and cuttlebone was used as polishing powder. Foreign herbs were mixed in toothpaste for other expectations. Chamomile has anti-inflammatory effect. Mercedes, Pepper mint, sage, Eck Chip Surratt, Catania euthanasia was mixed in toothpaste for treatment of gum disease (Chaveewan, 2545). The ingredients in herbal toothpaste consist of the followings. The part of clove was utilized for remedy disease such as bark, leaves, buds of clove and essential oil.

The main components of the toothpaste include cuttlebone polishing duty for clean the teeth. There are many kinds of toothpaste containing abrasive rough bark as a component of cuttlebone. Despite this rough abrasive scrub teeth clean, it will destroy the enamel is very useful for some down. The standard of toothpaste has developed quality abrasive powder to clean the teeth. Powder is one point that should be understood by consumers. Cuttlebone is a limestone with calcium Cabot. It located within the core of the body of cuttlefish.

Currently, camphor is used as a flavoring, mostly for sweets, in Asia. There are anti-itch gels and cooling gels with camphor as the active ingredient. Camphor may also be administered orally in small quantities (50 mg) for minor heart symptoms and fatigue. Camphor has been used in ancient Sumatra to treat sprains, swellings, and inflammation. Camphor is a component of paregoric, an opium/camphor tincture from the 18th century. Camphor is available as an essential oil for aromatherapy or topical.

Liquorice extract is produced by boiling liquorice root and subsequently evaporating most of the water, and is traded both in solid and syrup form. Its active principle is glycyrrhizin, a sweetener between 30 to 50 times as sweet as sucrose, and which also has pharmaceutical effects. Liquorice flavoring is also used in soft drinks and in some herbal infusions where it provides a sweet aftertaste. The flavor is common in medicines to disguise unpleasant flavors. The root of the plant is simply dug up, washed and chewed as a mouth freshener.

Marlstone is a calcium carbonate or lime-rich mud or mudstone which contains variable amounts of clays and silt. Menthol is an organic compound made synthetically or obtained from corn mint, peppermint or other mint oils. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts slightly above. The main form of menthol occurring in nature is (−)-menthol, which is assigned the (1R, 2S, 5R) configuration. Menthol has local anesthetic and counter irritant qualities, and it is widely used to relieve minor throat irritation. Menthol also acts as a weak kappa opioid receptor agonist.

2.6 Thai herbs

Traditional Thai herbs are affiliated with the Thai culture and have become integrated in the way of living of the Thai people lived through out the ages for a long time. Many Thai herbs use in Thai cuisine and have beneficial medicinal properties. Table 2.1 was shown some of Thai herbs using for food, medicals, and beauty. The medicinal properties of Thai herbs were concluded in Table 2.2.

This research interested in finger roots because the finger roots was low cost, easily found in food market, has a good smell and medical property. According to the comparison of finger roots or *B. pandurata* with garlic in mouthwash product, it has been found from a clinical trial that a mouthwash containing 2.5% fresh garlic shows good antimicrobial activity. But, the majority of the participants reported an unpleasant taste and halitosis. Among the Thai herbs, finger roots, garlic, lemon grass, spearmint, and lime leaves have medical properties to prevent bacteria. Studies have been conducted in comparing finger root and garlic extract in mouth wash (Nagata, J. Y., N. Hioka, et al., 2012). It was found that the antimicrobial agents in garlic were better than finger root extract. Finger root extract also have sufficient antimicrobial activities. However, garlic mouths wash product resulted in unpleasant smell and halitosis. Therefore, finger root was chosen for this research.

Table 2.1 List of Thai herbs

Thai Name	ภาษาไทย	**English Name**	**Description and use**
Bai makrut	ใบมะกรูด	Kaffir lime leaves	Kaffir lime leaves are widely used in spicy Thai soups and curries, either cooked whole, together with the dish, and/or finely shredded and added before serving.
Horapha	โหระพา	Thai sweet basil	A variety of the sweet basil with a taste of anise. It is used in different curries such as red and green curry and often also served separately.
Kha	ข่า	Galangal	The perfume-like scent and flavor of the galangal root is characteristic for many Thai curries and spicy soups.
Khing	ขิง	Ginger	Either served raw (shredded or diced) with dishes such as *Miang kham* and *Khanom chinsao nam*, in certain chili dips, or in stir fried dishes of Chinese origin.
Krachai	กระชาย	Fingerroot	This root has a slightly medicinal flavor and is used in certain fish dishes and curries.

Table 2.1 List of Thai herbs (Cont.)

Thai Name	ภาษาไทย	English Name	Description and use
Krathiam	กระเทียม	Garlic	Besides being used cooked or fried, garlic is used raw in many dips and salad dressings. It is also served raw on the side with several Thai dishes such as *Khao kha mu* (stewed pork served on rice) or as one of the ingredients for dishes such as Miang kham.
Saranae	สะระแหน่	Spearmint	Used in many Thai salads and sometimes as a way to suppress the 'muddy' taste of certain fish when steamed.
Takhrai	ตะไคร้	Lemon grass	Used extensively in many Thai dishes such as curries, spicy soups and salads.

Table 2.2 Medicinal properties of Thai herbs

Thai Name	ภาษาไทย	English Name	Medicinal Properties
Bai makrut	ใบมะกรูด	Kaffir lime leaves	The juice and rinds are used in traditional Indonesian medicine; for this reason the fruit is referred to in Indonesia as *jeruk obat* ("medicine citrus"). The oil from the rind has strong insecticidal properties.
Krachai	กระชาย	Finger root	In traditional medicine, rhizomes and roots are used in post-partum tonic mixtures, as a stomachic (improves appetite and digestion) and carminative (to aid digestion and reduce gas) and as a remedy for coughs and mouth ulcers. Crushed rhizomes and roots are also applied externally to treat rheumatism. Scientific research is underway to investigate their possible antioxidant, anti-inflammatory, antibacterial and anticancer properties. Finger root is also grown as an ornamental.

Table 2.2 Medicinal properties of Thai herbs (Cont.)

Thai Name	ภาษาไทย	English Name	Medicinal Properties
Krathiam	กระเทียม	Garlic	Garlic is also alleged to help regulate blood sugar levels. Regular and prolonged use of therapeutic amounts of aged garlic extracts lower blood homocysteine levels and has been shown to prevent some complications of diabetes mellitus. Garlic was used as an antiseptic to prevent gangrene during World War I and World War II. Garlic cloves are used as a remedy for infections (especially chest problems), digestive disorders, and fungal infections such as thrush. Garlic can be used as a disinfectant because of its bacteriostatic and bactericidal properties.
Saranae	สะระแหน่	Spearmint	Recent research has shown that spearmint tea may be used as a treatment for hirsutism in women. The anti- androgenic properties reduce the level of free testosterone in the blood, while leaving total testosterone and DHEA unaffected. Spearmint has been studied for antifungal activity. It can have a calming effect when used for insomnia or massages. Spearmint has also been described as having excellent antioxidant activity; its antioxidant activity was found to be comparable to the synthetic BHT.
Takhrai	ตะไคร้	Lemon grass	These species are used for the production of citronella oil, which is used in soaps, as an insect repellent especially mosquitoes. The principal chemical constituents of citronella, geraniol and citronellol, are antiseptics, hence their use in household disinfectants and soaps. It is supposed to help with relieving cough and nasal congestion