The Study of Potential in Inhibiting the Oral Bacterial by
Chemical Substances extracted from Different Breeds of
Guava Leaves

By

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ID. 4417522

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A special project submitted to the School of Biotechnology, Assumption University in part fulfillment of the requirements of the degree of Bachelor of Science in Biotechnology

2006

Special Project

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ABSTRACT

This study, the study of chemical substances from different breeds of guava leaves which contains potential in inhibiting the oral bacterial, was inspired to be study by many factors. The selected breeds of Guava in this study were Local Thai Guava, Bangkok Apple Guava, and Sali Glom Guava. The observable features were the differences of leaves from each breed of Guava. It was found that the leaves of Bangkok Apple Guava had lowest observable area of insect damages, whereas Sali Glom Guava has many spots and holes on its leaves.

The experiments to identify the difference among them were the technique of clear zone measurement by its diameters and the second turbidity measurement by using spectrophotometer measure the different wave length of the liquid nutrient at 540nm in Trypticase Soy Broth. *Staphylococcus aureus* was used as the selected microorganisms to demonstrate the growth development of oral bacterial in this study. The comparison of antimicrobial substances level in Guava leaves demonstrated that Bangkok Apple Guava contained greater concentration

than the others, which demonstrated the highest ability to inhibit the growth of oral microbial. In contrary, Glom Sali Guava contained least amount of antimicrobial substance with lowest ability to inhibit the growth of oral microbial. The reason came from the different sources of their starter breeds that was took for cross breeding. According to this study, Guava leaves definitely contained antimicrobial substances with sufficient level to inhibit the growth of *Staphylococcus aureus*, an oral microbial. However the growth of *Staphylococcus aureus* could be inhibited at different levels as different breeds of Guava leaves contained different concentration of antimicrobial substances and Bangkok apple guava matured leaves at dilution 0.4 g/ml gave the best diameter of clear zone at 1.87 cm. and the lowest was Glom Sali guava young leaves at dilution 0.004 g/ml had diameter of clear zone at 0.10 cm.

For further study, it is quite important to proceed with the idea of inoculating the extracted antimicrobial substance with other oral microbial. If possible *Streptococcus mutans* is further experiment that would do to identify the inhibiting ability. Moreover, test with other bacteria that the cause of mouth bad odor should be performed also.

Acknowledgement

I would like to show my deep gratitude towards many people that has continuously support me throughout the years of studies and experiments.

Firstly, my advisor A. Suchawadee Wiratthikowit for her guidance, advises supports, and patience which lead me through this project. Special thanks to A. Nootruedee Siriboon, for her supportive suggestions, as well as her motivation for me to finish the project in time.

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APPENDIX D

LIST OF ABBREVIATIONS

1. ha	abbreviates from	hectare
2. %	abbreviates from	percentage
3. CAS	abbreviates from	Chemical Abstracts Service (registry number)
4. MW	abbreviates from	molecular weight
5. mm	abbreviates from	millimeter (s)
6. mg	abbreviates from	milligram (s)
7. g	abbreviates from	gram (s)
8. min	abbreviates from	minute (s)
9. nm	abbreviates from	nanometer (s)
10. μg	abbreviates from	microgram (s)
11. cm	abbreviates from	centimeter (s)
11. ^{0}C	abbreviates from	Celsius degree
12. kg	abbreviates from	kilogram (s)
13. ml	abbreviates from	millimeter (s)
14. SD	abbreviates from	standard deviation
15. ANOVA	abbreviates from	analysis of variance

The Study of Potential in Inhibiting the Oral Bacterial by Chemical

Substances extracted from Different Breeds of Guava Leaves

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Thai health services and Thai herbs are well-known around the world. They were indicated to be products for health, following the governor policy of Thailand medical health hub of Asia. Nowadays, many Thai herbs have been used as medium and supplementary foods that help to increase income of our country. Thai herbs are accepted not only in Thailand but in other countries also. Therefore, it is important that innovated products from Thai herbs must be considered.

Guava leaves is one of the herb that had been used by ancient Thai people as protecting a bad breath. It is common that Thai elders chewed the leaves after meal. By it own medical property of inhibiting the growth of oral bacterial, Thai elders believe that the leaves contain antimicrobial substances. The study of Thepanom Meungman et al (9) found that guava leaves can inhibit mouth odor and bad breath which were created by oral microbial. Also Therdpong Treerut et al (10) found that the chemical substances in guava leaves may resist the growth of bacteria.

First of all, it is well known that Guava leaves contain many substances, in which are mostly beneficial to human. There are many breeds of Guava in Thailand, so the chemical substances in each breed may be different. The analysis for bacterial resistance is beneficial for selecting the breed of Guava leaves in business of herbal production. Whereas, the most interesting benefit found in Guava leaves is the ability to inhibit the growth of bacterium. Secondly, Thailand is well known as the Kitchen of the World, since we have numerous varieties of agricultural products, in which Guava is one of the most common fruit. In addition, with varieties of breed grown and developed in Thailand, it was a challenge to find out the differences of each breed. Therefore, the study was mainly focused on Guava leaves, in order to eliminate the waste, and proved its well known herbal capacity of inhibiting the microbial growth.

As an alternative way to achieve value-added products from disposing Guava leaves, this study was focus on the potential of applying a certain breed of Guava leaves as a herbal product for eliminating bad breath by inhibiting the growth of oral microbial in human.

1.2 Objectives

The objectives of this study were to study the antimicrobial substances extracted from different breeds of Guava leaves, to differentiate the concentration of antimicrobial substances from different breeds of Guava leaves at young and mature stage, and to study the potential to inhibit the growth of oral bacterial in human.

1.3 Conceptual Framework

```
                    ┌─────────────────┐
                    │  Farmer Grow a  │
                    │  Lot of Guava   │
                    └─────────────────┘
                             ⇩
┌──────────────┐    ┌─────────────────┐    ┌──────────────┐
│ Insecticides │ ⇦  │ Benefit Get Only│ ⇨  │ Selling Price│
│   Attract    │    │  From Its Fruit │    │   Decrease   │
└──────────────┘    └─────────────────┘    └──────────────┘
                             ⇩
                    ┌─────────────────┐
                    │  Getting More   │
                    │   Benefit from  │
                    │   Others Parts  │
                    └─────────────────┘
                             ⇩
                    ┌─────────────────┐
                    │ Use the Leaves to│
                    │ Produce Medicine │
                    │    or Herbs      │
                    └─────────────────┘
                             ⇩
                    ┌─────────────────────┐
                    │ Identify the Different│
                    │ Between Various Kinds │
                    │      of Guava         │
                    └─────────────────────┘
              ⇩                             ⇩
  ┌──────────────────┐          ┌──────────────────┐
  │  Oral bacterial  │          │Other applications│
  │    inhibition    │          │                  │
  └──────────────────┘          └──────────────────┘
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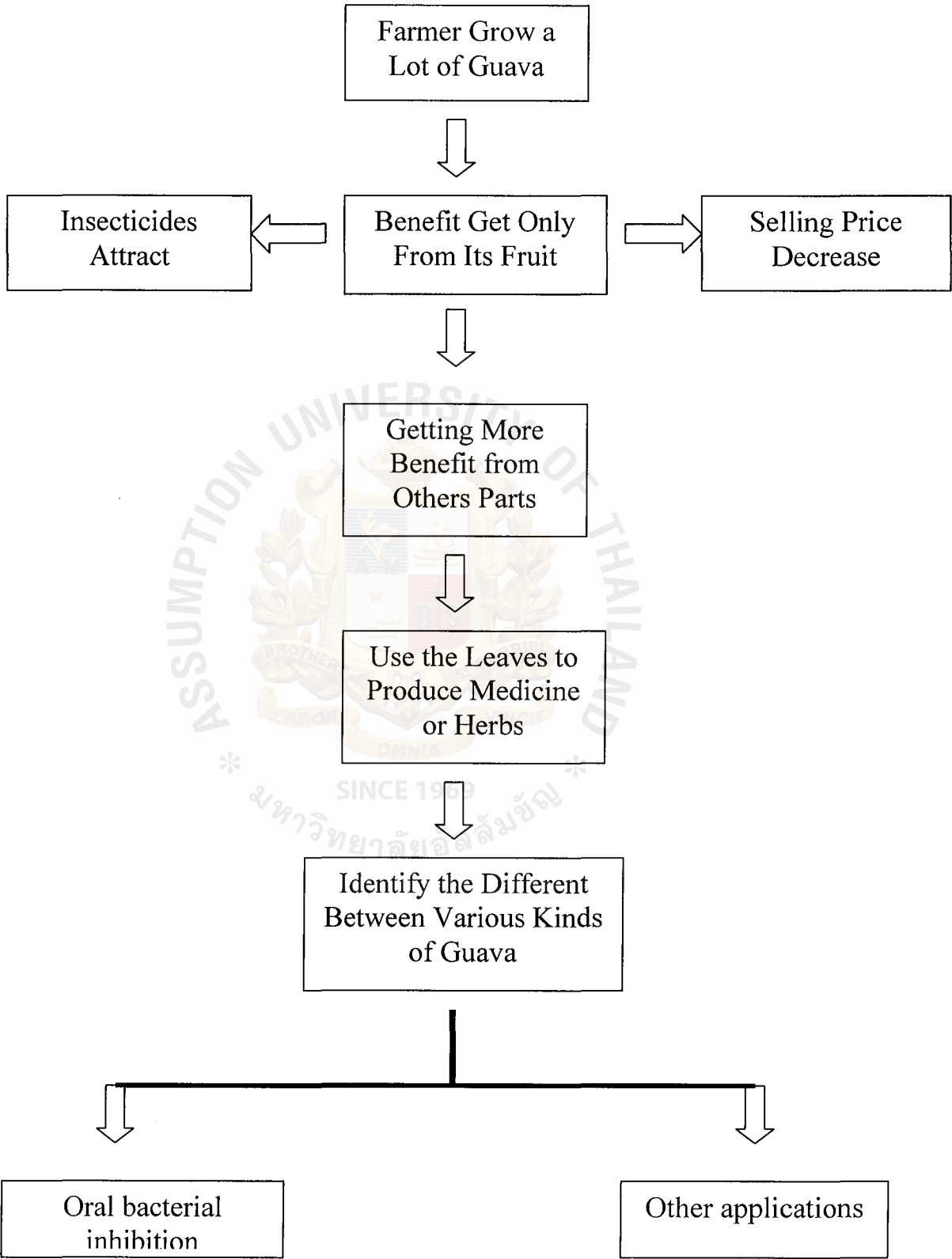
1.4 Scope

This study was firstly scoped for innovation of Thai herbs in order to prevent bad breath. However, due to the lack of resources and information, the researcher started the study with the intention to prove the potential in oral microbial inhibition using the extracted antimicrobial from different breeds and stage of Guava leaves, either at young and matured stage of Bangkok Apple Guava, Local Thai Guava, and Sali Glom Guava. *Staphylococcus aureus*, taken from stock culture of Microbial laboratory of School of Biotechnology Assumption University, was used as the selected oral microbial in this study. The ability of microbial inhibition was measured by observing the clear zone of the plate after incubated at $37^{\circ}C$, stimulating the human's mouth condition.

1.5 Hypothesis

1.5.1 Guava extracted solution from Guava Leaves might inhibit oral microbial growth.

1.5.2 Guava leaves took from the different breeds contains different concentration of antimicrobial substances and might inhibit the growth of oral microbial at different level.

1.5.3 Extracted solution from Guava leaves at different growth stage might result with different concentration of antimicrobial substances and might inhibit the growth of oral microbial at different level.

1.6 Anticipated Benefits

1.6.1 To increase the value-added of waste residue from fresh Guava leaves.

1.6.2 To innovate OTOP idea and with the wide-spread medicinal application of well-known Thai herbs for bad breath prevention.

1.7 Definitions

1.7.1 *Staphylococcus aureus*; gram-positive spherical cells, usually arranged in irregular clusters. They grow readily on a variety of media and are active metabolically, fermenting many carbohydrates and producing many pigments that vary from white to deep yellow (12).

1.7.2 Oral microbial; is dedicated to the dissemination of knowledge pertaining to fundamental and applied aspects of oral infections, including their etiologic agents, diagnosis and epidemiology, oral microbial ecology, virulence factors of oral microbes, non-specific host resistance factors in oral infections, and the immunology of oral infections. Advances in microbiology are making vital and exciting contributions in many areas and not least in the diagnosis, treatment and prevention of oral diseases. *Oral Microbiology and Immunology* will keep its readers fully in touch with all the latest theoretical and applied developments across a broad, interdisciplinary spectrum(21).

1.7.3 Cross Breeding; or *crossbred* refers to a hybrid animal of two purebred parents. *Crossbreed* may also refer to a domestic animal where the breed status of only one parent or grandparent is known. *Crossbreeding*, also known as *mixing* refers to the process of breeding such an animal, often with the intention of creating offspring that share the traits of both parent lineages. The term is sometimes used for plants but is more commonly applied to domesticated animals (22).

1.7.4 Spread plate; Trypticase Soy Agar was used for spread plate technique to growth *Staphylococcus aureus* bacteria. Then take a sterile hockey stick and spread the specimen with the flat, short area of the stick in one direction while turn the plate in a circle. Be sure that you move the hockey stick around for couples of times. The specimen will be soaking into the agar.

1.7.5 Trypticase Soy Agar (TSA)/TSA medium contain enzymatic digests of casein and soybean meal which provides amino acids and other nitrogenous substances making it a nutritious medium for a variety of organisms. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium, while dipotassium phosphate acts as buffer to maintain pH. The medium may be supplemented with blood to facilitate the growth of more fastidious bacteria or antimicrobial agents to permit the selection of various microbial groups from mixed flora (20).

1.7.6 Trypticase Soy Broth (TSB)/TSB medium contains casein and soybean peptones which provide amino acids and other nitrogenous substances making it a nutritious medium for a variety of organisms. Dextrose is the energy source and sodium chloride maintains the osmotic equilibrium. The dipotassium phosphate is added as a buffer to maintain the pH (20).

CHAPTER II

LITERATURE REVIEW

2.1 Classification of Guava

Guava is one of the most gregarious of fruit trees. *Psidium guajava L.* of the myrtale, Myrtaceae family, is almost universally known by its common English name, Guava (5). The fruit of Guava is commonly eaten fresh in Thai, whereas its juice is suitable for refreshment and thirst-quencher for lots of Thai consumers. Since Guava is one of Thailand's tropical fruit, it is a popular OTOP item well known for fruit pickled, either salted or sweetened type.

<u>Guava</u>

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Myrtales
Family:	Myrtaceae
Genus:	Psidium
species:	*Psidium cattleianum* - Strawberry Guava
	Psidium friedrichsthalium - Costa Rica Guava
	Psidium guajava - Apple Guava
	Psidium guineense - Guinea Guava
	Psidium littorale - Cattley Guava, etc..

Guava tree is a low evergreen tree with shrub of six to twenty-five feet high, and wide spreading branches and downy twigs. The branches are very strong and highly tolerant to strong winds. The leaves are oblong or oval and blunt, three to six inches long, and feather-veined. The flowers are about an inch or more across, the calyx bell-shaped and splitting irregularly, the four to six petals are white, and the stamens are white with yellow anthers. The guava is easy to recognize because of its smooth, thin, copper-colored bark that flakes off, showing the greenish layer beneath, and also because of the attractive, "bony" aspect of its trunk which may in time attain a diameter of twenty-five centimeters. Young twigs are quadrangular and downy. The leaves gives aroma when crushed, are evergreen, opposite, short-petiole, oval or oblong-elliptic, somewhat irregular in outline; seven to fifteen centimeters long, with three to five centimeters wide, leathery, with conspicuous parallel veins, and more or less downy on the underside. Faintly fragrant, the white flowers, borne singly or in small clusters in the leaf axils, are two point five centimeters wide, with four or five white petals which are quickly shed, and a prominent tuft of perhaps two hundred fifty white stamens tipped with pale-yellow anthers(5).

The fruit, exuding a strong, sweet, musky odor when ripe, may be round, ovoid, or pear-shaped, five to ten centimeters long, with four or five protruding floral remnants (sepals) at the apex; and thin, light-yellow skin, frequently blushed with pink. Next to the skin is a layer of somewhat granular flesh, three to twelve point five millimeters thick, white, yellowish, light- or dark-pink, or near-red, juicy, acid, sub acid, or

sweet and flavorful. The central pulp, concolorous or slightly darker in tone, is juicy and normally filled with very hard, yellowish seeds, three millimeters long. Though, some rare types may have soft and chewable seeds. Actual seed counts have ranged from one hundred twelve to five hundred thirty five but some guavas are seedless or nearly so. When immature and until a very short time before ripening, the fruit is green, hard, gummy within and very astringent (5).

The guava has believed to be an area extending from southern Mexico into or through Central America. It is common throughout all warm areas of tropical America and in the West Indies (since 1526), the Bahamas, Bermuda and southern Florida where it was reportedly introduced in 1847 and was common over more than half the State by 1886. Early Spanish and Portuguese colonizers were quick to carry it from the New World to the East Indies and Guam. It was soon adopted as a crop in Asia and in warm parts of Africa. Egyptians have grown it for a long time and it may have traveled from Egypt to Palestine. It is occasionally seen in Algeria and on the Mediterranean coast of France. In India, guava cultivation has been estimated at 125,327 acres (50,720 ha) yielding 27,319 tons annually.

Leaves and barks: The leaves and barks are rich in tannin (10 % in the leaves on a dry weight basis, 11-30 % in the bark). The bark is used in Central America for tanning hides. Malayans use the leaves with other plant materials to make a black dye for silk. In Southeast Asia, the leaves are employed to give a black color to cotton; and in Indonesia, they serve to dye matting.

2.2 Application of Guava

Medicinal Uses: The roots, bark, leaves and immature fruits, because of their astringency, are commonly employed to halt gastroenteritis, diarrhea and dysentery, throughout the tropics. Crushed leaves are applied on wounds, ulcers and rheumatic places, and leaves are chewed to relieve toothache. The leaf decoction is taken as a remedy for coughs, throat and chest ailments, gargled to relieve oral ulcers and inflamed gums; and also taken as an emmenagogue and vermifuge, and treatment for leucorrhea. It has been effective in halting vomiting and diarrhea in cholera patients. It is also applied on skin diseases. A decoction of the new shoots is taken as a febrifuge. The leaf infusion is prescribed in India in cerebral ailments, nephritis and cachexia. An extract is given in epilepsy and chorea and a tincture is rubbed on the spine of children in convulsions. A combined decoction of leaves and bark is given to expel the placenta after childbirth.

The leaves, in addition to tannin, possess ca 1.3% essential oil with 14–65% cineole (or eucalyptol), sesquiterpene hydrocarbons caryophyllene, β-bisabolene, aromadendrene, β-selinene, nerolidiol, caryophyllene oxide and sel-11-en-4x -ol, also some triterpenoids and β -sitosterol. The bark contains tannin, crystals of calcium oxalate, ellagic acid and starch. The young fruits are rich in tannin(5).

2.3 Chemical Structure of the Extracted Substances

2.3.1 Sesquiterpene hydrocarbons caryophyllene
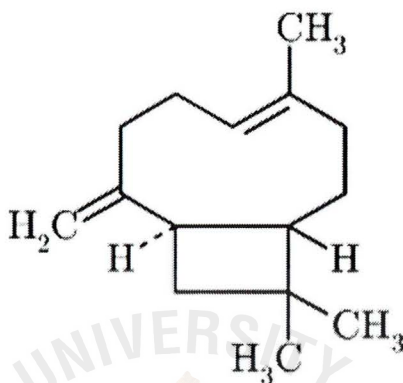
Figure 2-1 The Structure of 4,11,11-trimethyl-8-methylene bicycle
[7.2.0]undec-4-ene(21)

4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene   consist
of three isoprene units and have the molecular formula $C_{15}H_{24}$. Like
monoterpenes, sesquiterpenes may be acyclic or contain rings, including
many unique combinations. is a natural bicyclic sesquiterpene that is a
constituent of some essential oils, especially clove oil and the oil from the
stems and flowers of *Syzygium aromaticum*. It is usually found as a
mixture with isocaryophyllene (the *cis* double bond isomer) and α-
humulene (obsolete name: α-caryophyllene), a ring-opened isomer.
Caryophyllene is notable for having a cyclobutane ring, a rarity in nature.
Caryophyllene is one of the chemical compounds that contributes to the
spiciness of black pepper(4).

2.3.2   β-bisabolene,   6-methyl-2-(4-methyl-3-cyclohexen-1-yl)-1,5-heptadiene
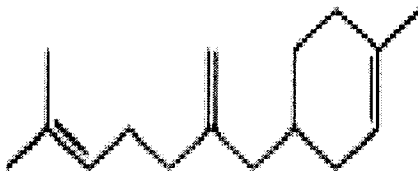
CAS 495-61-4 - MW 204.2

Figure 2-2 The Structure of 6-methyl-2-(4-methyl-3-cyclohexen-1-yl)-1,5-heptadiene(21)

2.3.3 Aromadendrene, [1ar (1aalpha, 4aalpha, 7aalpha, 7abeta, 7 balpha)] -decahydro-1,1,7-trimethyl-4-methylene-1Hcycloprop[e]azulene
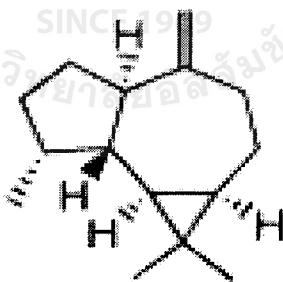
CAS 489-39-4 - MW 204.35

Figure 2-3 The Structure of [1ar (1aalpha, 4aalpha, 7aalpha, 7abeta, 7 balpha)] -decahydro-1,1,7-trimethyl-4-methylene-1Hcycloprop[e]azulene(21)

Aromodendrene is a member of a group of a naturally occurring sesquiterpines structurally characterized by fusion of a cyflopropane ring to a hydroazulene skeleton(21).

2.3.4 β-selinene
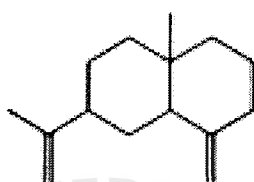
CAS 17066-67-0 - MW 204.2

Figure 2-4 The Structure of β-selinene(21)

2.3.5 Caryophyllene oxide

(1R, 4R, 6R, 10S)-4, 12, 12-trimethyl-9-methylene-5 oxatricyclo [8.2.0.0] 4,6)] dodecane
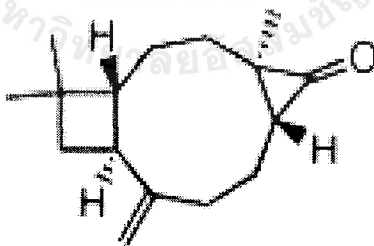
CAS 1139-30-6 - MW 220.36

Figure 2-5 The Structure of (1R, 4R, 6R, 10S)-4, 12, 12-trimethyl-9-methylene-5 oxatricyclo [8.2.0.0] 4,6)] dodecane(21)

2.3.6 Triterpenoids

Figure 2-6 The rubber that can get from the plants(21)

Plant terpenoids are extensively used for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for **antibacterial**, antineoplastic and other pharmaceutical effects.

2.3.7 β -sitosterol or **Phytosterols**
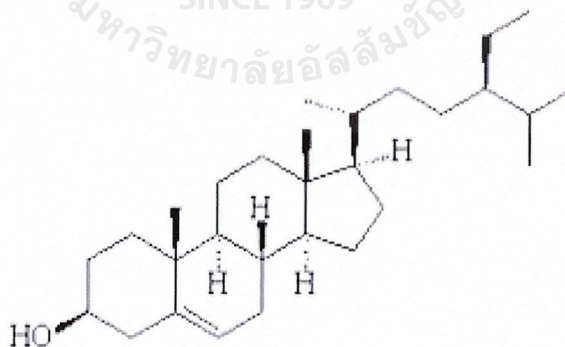
Figure 2-7 The Structure of β -sitosterol or Phytosterols(21)

(Also called **plant sterols**) are a group of steroid alcohol, phytochemicals naturally occurring in plants. They are white powders with mild, characteristic odor, insoluble in water and soluble in alcohols.

They have many applications as food additives, and in medicine and cosmetics. β-sitosterol reduces blood levels of cholesterol. There is some belief that it plays some role in the possible benefits of herbal therapy of benign prostatic hypertrophy (BPH). β-sitosterol itself is used as a medicine in Europe for BPH (21).

2.4 Breeds of Guava

All three selected breeds of Guava leaves taken for samples grew from the same place, so the climate condition and environment are practically same. None of them were given any pesticides, so it's certain that they were growth naturally and with none fertilizer. The time that the leaves were collected was early in the morning about 6:00 to 8:00 AM during October and November of 2006, which are the rainy season. The breeds selected for samples are as followed:

2.4.1 Local Thai Guava

This guava was growth in normal climate of Thailand without controlling of anything or selecting breed, in other words, it is a naturally growth type. The leaves are in medium size, at the rim of leaves has quite a round shape, and the green color of leaves is not as dark as other breeds. This breed of Guava has smallest branch and the leaves lines are thin in both upper side and lower side.
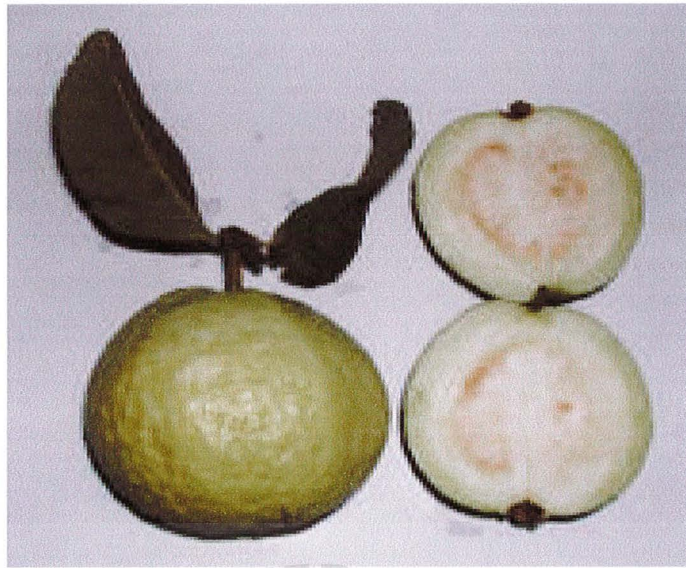
Figure 2-8 Photo of guava fruit: local Thai breeding
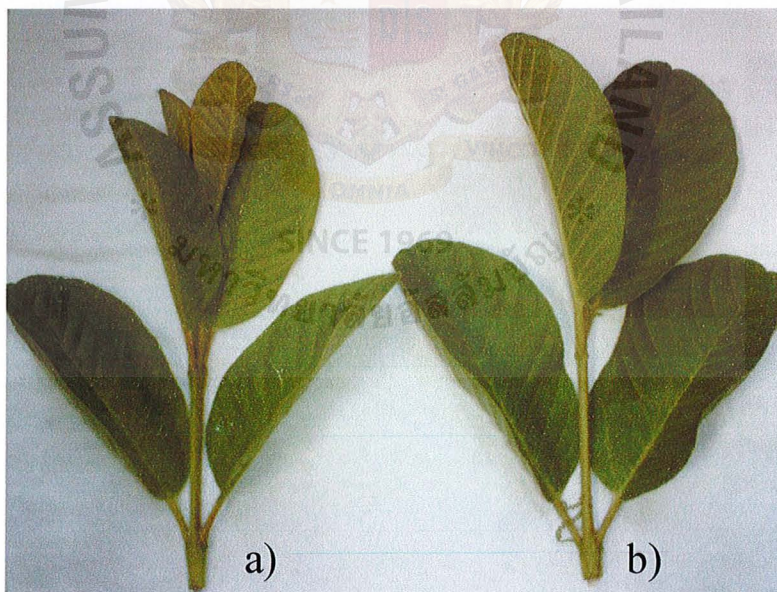
Figure 2-9 Photo of guava leave: Local Thai breeding a) Young leaves,

b) Matured leaves

2.4.2 Bangkok Apple Guava

Bangkok apple came from the cross breeding between Indian Stain (E. Haew species) and Vietnam stain (from Glom Sali Guava). The fruit has medium size, slightly oblate; deep-pink skin, creamy-white flesh, moderate amount of seeds, very sweet flavor (0.34 - 2.12 % acid, 9 to 11.36 % sugar); heavy bearer; good keeping quality; and good for canning quality. The young branch has shape similar to square, leaves look circling big, and a little waving at its rim. The upper side of leaves has deep lines and spread out clearly, which is different from the others. In addition, the branch of this breed has larger size than the others.
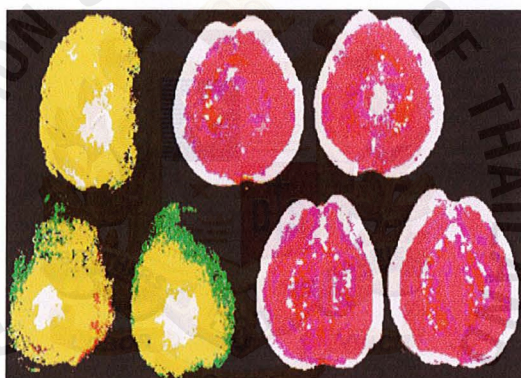
Figure 2-10 Photo of guava fruit: Bangkok apple breeding (3)
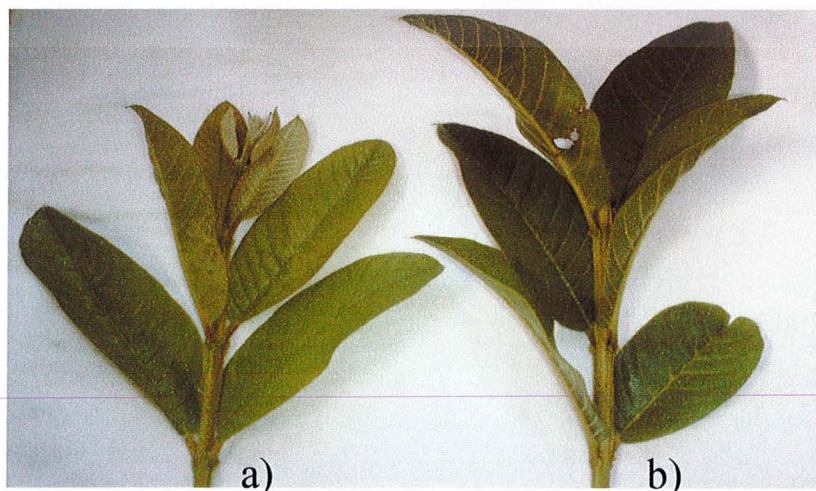
Figurure 2-11 Photo of guava leave: Bangkok apple breeding a) Young leaves, b) Matured leaves.

2.4.3 Glom Sali Guava

Glom Sali Guava was derived from the Vietnam stain with the shape quite round and very big when compare to other breeds. This breed of Guava has light green skin color with very thick and crispy meat and seedless. The fruit has sweet and slightly sour taste. The size of leaves is smaller than the others breeds and the leaves lines are quite clearly observable. Both upper and lower sides of leaves are mostly covered with white powder.
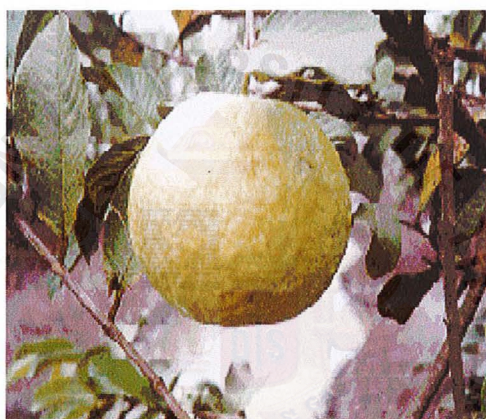
Figure 2-12 The picture of guava fruit : Glom Sali Guava breeding
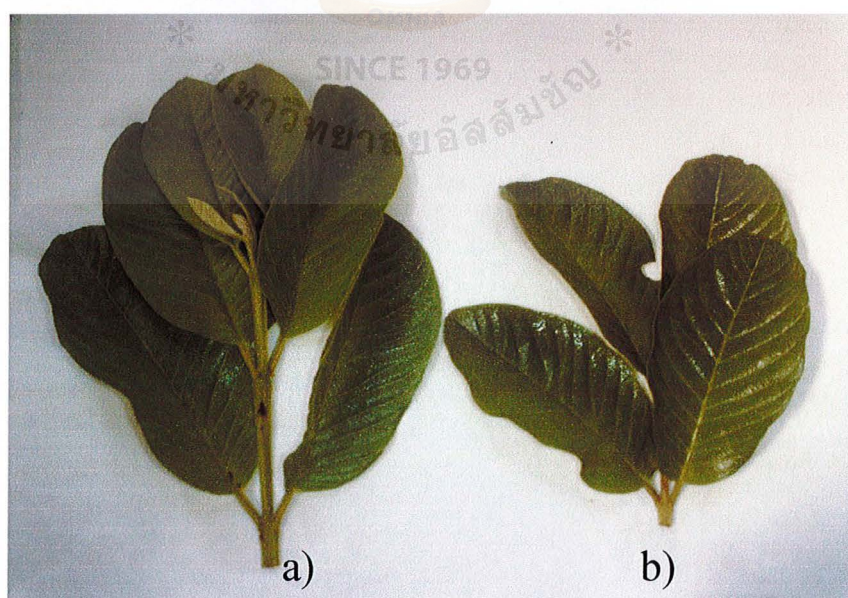
Figure 2-13 Photo of guava leave: Glom Sali Guava a) Young leaves, b) Matured leaves

2.5 Microorganism (Oral Bacterial)

There is a lot of food that gets stuck in one's mouth that doesn't make it to the stomach right away in which those bacteria helps to break it down. However, there is a trade off, because parts of their waste are acidic and may contribute to tooth decay. Human have some harmful bacteria in their mouths. The bacteria that cause tooth decay could be considered harmful. This is why one should brush his or her teeth after eating and not eat too many sweets which they love. Every time one opens his or her mouth, the person gets exposed to bacterium. Human's mouth contains enzymes that can kill bacteria. Normally, most of the oral bacteria does not cause any harm, and may even help to support your immune because they take up space and crowd out harmful bacteria (1).

The only harmful species of oral bacteria is *Streptococcus mutans*, the cavity causing bacteria. Traditionally, microbiologists believe that this bacterial played a useful role, just as the other species of bacteria do. As man progressed down the evolutionary timeline and began refining raw sugar, *Streptococcus mutans* became the enemy. This species thrives upon refined sugar and, as a part of its digestive process, converts sugar into acid (1).

Before man began refining sugar, the bicarbonate ions in the saliva possessed the ability to counteract the acid it produced. With the arrival of refined sugar on the scene, and *Streptococcus mutans* insatiable appetite for it, acid production increased to the point where the saliva

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could no longer counteract it. The excess acid produced erodes the teeth and causes cavities (1).

The example of bacteria, fungi, protozoa, and viruses that normally found in our mouth are as followed;

1. *Streptococcus mutans*
2. *Staphylococcus aureus*
3. *Streptococcus salivarius*
4. *Lactobacillus sp.*
5. *Actinomyces sp.*
6. *Fusobacterium sp.*
7. *Anaerobic micrococci*
8. *Anaerobic streptococci*
9. *Vibrios*

The microorganism which was selected to use in this experiment is *Streptococcus aureus*, as it is an oral microbial with gram positive coccus, that can normally be found in mouth during aspiration.
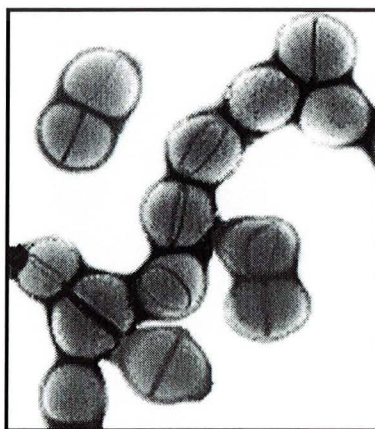
Figure 2-14   *Staphylococcus aureus* (23)
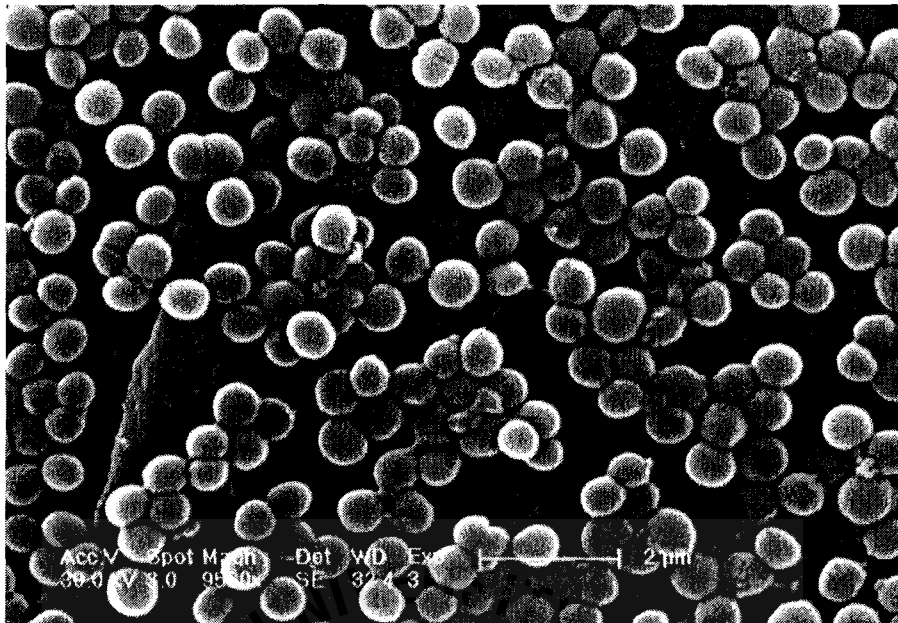
Figure 2-15 *Staphylococcus aureus* An electron micrograph of the

microbe (18)

2.6 Microorganism Growth Inhibition Test

The most popular method for identifying or testing the anti microbial growth is the clear zone identification and it's perform on the agar nutrient. For this lab test, the microorganism used is *Staphylococcus aureus* growth on the Trypticase Soy Agar medium, because it contain enzymatic digests of casein and soybean meal which provides amino acids and other nitrogenous substances. When added the antimicrobial the diffuse out into the agar at each antimicrobial. The diffusion rate is directly related to the chemical structure. If the antimicrobial can inhibit the microorganism growth, a zone of no growth appears around the disc. Zones of inhibition have been correlated with the Minimum Inhibitory Concentration (MIC) of each solution extracted from guava; therefore,

the diameter of the zone determines whether the organism is sensitive (susceptible) or resistant to the particular antibiotic or not (7).

2.7 Relevant Research

Mr. Chanin Jirapongwattana (2) had studied on the production of chitosan patch and the inhibition of *Staphylococcus aureus* growth test. The cutllefish's cuttlebone (*Sepia pharaonis* Ehrenberg) wastes obtained from fresh and frozen cuttlefish industry were used as the raw materials. Some compositions and the patch at different concentration forming were performed. The Inhibition of *Staphylococcus aureus* growth test was performed in order to investigate the anti-microbial patch which was friendly to the environment, reduce the waste, convenience use, and value-added product and other applications of chitosan product could be produced. The results revealed that the extracted chitosan in this study had comparable quality to the commercial one. The moisture content was 9.2 % with 83.64 % deacetylation. For the patch formation different mixture were observed, 1.0 % chitosan solution in 1.0 % of lactic acid with 1.0% of gelatin(1:1); 1.5 % chitosan solution in 1.0 % of lactic acid with 1.0% of gelatin(1.5:1); 2.0 % chitosan solution in 1.0 % of lactic acid with 1.0% of gelatin(2:1); 2.5 % chitosan solution in 1.0 % of lactic acid with 1.0% of gelatin(2.5:1); 1.0 % chitosan solution in 1.0% of lactic acid purely (1:0); 1.5 % chitosan solution in 1.0 % of lactic acid purely (1.5:0); 2.0 % chitosan solution in 1.0% of lactic acid purely (2:0) and 2.5 % chitosan solution in 1.0 % of lactic acid purely (2.5:0). It was found that 1.0 % chitosan solution in 1.0 % of lactic acid purely (1:0) could not form the patch. The inhibition of *Staphylococcus aureus* test was performed, disc diffusion was selected to determine the inhibitory ability. Sterilized paper disc at 0.5 cm in diameter and chitosan patches at

every treatment with 1.0 cm in diameter were observed and compared. At 1.0 % chitosan solution in 1.0 % of lactic acid with 1.0 % of gelatin(1:1) was the suitable concentration in the inhibition of *Staphylococcus aureus*. it was the lowest amount of chitosan solution that could inhibit the growth of *Staphylococcus aureus* or MIC (Minimum Inhibitory Concentration). According to the results of this study, extracted chitosan obtained from the cutllefish's cuttlebone (*Sepia pharaonis* Ehrenberg) wastes could be used to produce the chitosan patches and they can inhibit the growth of *Staphylococcus aureus* which concerned as the causative of some skin diseases such as acne and folliculitis.

Ms. Tatsaporn Todhanakasem (8) had studied on the inhibition of *Staphylococcus aureus* by Khaa, Fa thalaai and Wattle, Thailand has high variety of medicinal science as potential source of new useful chemical to treat the diseases or inhibit microbial growth. Medicinal plants that commonly available in Thailand are used to inhibit *Staphylococcus aureus* growth of this project. The plants used are namely *Andrographis paniculata (Burm.f.) Nees (Fa Thalaai joan), Alpinia galangal* or *Languas galangal* (Khaa) and *Acacia auriculaeformis* (wattle of Kratjinarong). Disc diffusion method was used to study the effective of extracted medicinal plants on *S. aureus* growth and observe the clear zone radius out of the disc. The larger the clear zone radius out of the disc, the higher the effective in inhibition. The solvents that used in this method are varied in each plant to find the highest potential solvent in extraction the microbial inhibition compound in the medicinal plants. The concentration of each extracted medicinal plants are also control to be in the range 13 – 16 mg/disc. Ampicillin, solvents (water, ethanol 95 %, hexane, chloroform) are provided an inhibition control condition. The

result shown that wattle extracted with ethanol 95 % gives the larger clear zone out of the disc (4.49 mm) than Fa Thalaai and Khaa in any kinds of solvent in concentration range 13 – 16 mg/disc. It also gave the higher effective inhibit *S. aureus* growth than Ampicillin with its larger clear zone radius out of disc than Ampicillin (concentration 30 µg/disc) which gave the clear zone radius out of disc 2.43 mm. The factors that effect on the efficiency of medicinal plant extraction can be the extraction method and medicinal plant itself such as the age, environment of medicinal plant growth and cultivation season. Therefore, these factors must carefully consider when there is a research on medicinal plant extract.

Pranee Jaiarj ed al (6), The Anticough and antimimrobial activities of *Psidium guajava* Linn. (guava) leaf extract was evaluated in rats and guinea pigs. The results showed that water extract of the plant at doses of 2 and 5 g : kg, p.o. decreased the frequency of cough induced by capsaicin aerosol by 35 and 54 %, respectively, as compared to the control, within 10 min after injection of the extract, (*P*<0.01). However, the anticough activity is less potent than that of 3 mg:kg dextromethorphan which decreased frequency of cough by 78% (*P*B0.01). An experiment on isolated rat tracheal muscle showed that the extract directly stimulated muscle contraction and also synergized with the stimulatory effect of pilocarpine. This effect was antagonized by an atropine. Moreover, growth of *Staphylococcus aureus* and b-streptococcus group A, as determined by the disc diffusion method, was inhibited by water, methanol and chloroform extract of dry guava leaves (*P*<0.001). The LD_{50} of guava leaf extract was more than 5 g : kg, p.o. These results suggest that guava leaf extract is recommended as a cough remedy.

CHAPTER III

RESEARCH METHODOLOGY

This study were to study with the intention to prove the potential in oral microbial inhibition using the extracted antimicrobial from different breeds and stage of Guava leaves, either at young and matured stage of Bangkok Apple Guava, Local Thai Guava, and Glom Sali Guava. *Staphylococcus aureus*, taken from stock culture of Microbial laboratory of Faculty of Biotechnology Assumption University, was used as the selected oral microbial in this study. Then, differentiate the concentration of antimicrobial substances from different breeds of Guava leaves at young and mature stage, and to study the potential to inhibit the growth of oral bacterial in human. The ability of microbial inhibition was measured by observing the clear zone of the plate after incubated at $37^{\circ}C$, stimulating the human's mouth condition.

```
                    ┌─────────────────────────────────┐
                    │  Preparation of the Guava leaves │
                    └─────────────────────────────────┘
                                     │
                                     ▼
        ┌──────────────────────────────────────────────────────┐
        │ Blend leaves, filtered and collected the extracted    │
        │ solution and kept it in the refrigerator for next     │
        │ process.                                              │
        └──────────────────────────────────────────────────────┘
                                     │
                                     ▼
        ┌──────────────────────────────────────────────────────┐
        │ Added the guava extracted solution by using the       │
        │ capillary tube                                        │
        └──────────────────────────────────────────────────────┘
                                     │
                                     ▼
```

┌──┐
│ Treatment 1: Control tube(pure broth)   │
├──┤
│ Treatment 2: extracted solution from young leave of Local │
│ Thai Guava.   │
├──┤
│ Treatment 3: extracted solution from mature leave of   │
│ Local Thai Guava.   │
├──┤
│ Treatment 4: extracted solution from young leave of   │
│ Bangkok apple Guava.   │
├──┤
│ Treatment 5: extracted solution from mature leave of   │
│ Bangkok apple Guava.   │
├──┤
│ Treatment 6: extracted solution from young leave of   │
│ Glom Sali Guava.   │
├──┤
│ Treatment 7: extracted solution from mature leave of   │
│ Glom Sali Guava.   │
└──┘

```
                                     │
                                     ▼
```

┌──┐
│ The inhibition growth of *Staphylococcus aureus* by   │
│ measuring the diameters of the clear zone and measure the │
│ level of growth by spectrophotometer with the wavelength   │
│ at 540 nm.   │
└──┘

Figure 3-1 Experimental Procedure

3.1 Experiment Location

3.1.1 Antimicrobial substances extracted were conducted at Microbiology Laboratory, School of Biotechnology, Assumption University.

3.1.2 *Staphylococcus aureus* stock culture and media formation were taken and performed at Microbiology Laboratory, School of Biotechnology, Assumption University.

3.1.2 Inhibition of *Staphylococcus aureus* growth was performed at Microbiology Laboratory, School of Biotechnology, Assumption University.

3.2 Material

3.2.1 Agar, Broth and Reagents

1. Trypticase Soy Agar (TSA)
2. Trypticase Soy Broth (TSB)
3. Distilled water
4. 95 % Ethyl alcohol

3.2.2 Equipments

1. Blender (Moulinex M2111 92 D 70)

2. Glassware

3. Glass hockey sticks

4. Autoclave (model Hirayama HA 300MII)

5. Incubator (model Jouan, EB 280)

6. UV light cabinet (Dwyer Instrument Inc, USA)

7. Spectrophotometer (Spectronic Genesys5, Milton Roy)

8. Plastic Cuvettes

9. Capillary Tube

3.2.3 Three Breeds of Guava Leaves

The samples of guava leaves were collected from a garden at Petchaburi Province, which composed of three different breeds as followed.

- Local Thai Guava
- Bangkok apple Guava
- Glom Sali Guava

3.3 Preparation Methodology

Initially, cleaned the guava leaves by soaking in the water then, separated the leaves from the branches. In order to separate between young leaves and old leaves from each branch, young leaves were taken from the first four leaves on the top of each branch, and the six leaves below were taken as matured leaves. Cut each type of leaves into the smallest piece using blender (100g/ 250 ml of water). Furthermore, filtered the blended leaves by white filter cotton cloth. Collected the extracted solution in the bottle and let it stand in the refrigerator for next process. And kept at most not more than 1 week.

3.4 Experimental Methodology

3.4.1. To confirm the chemical substance in the Guava leaves has potential to inhibit the growth of oral microbial (*S. aureus*)

The growth of *Staphylococcus aureus* was used to stimulate the oral condition in human. Therefore, its inhibition which results the plate with clear zone would be a sample of the ability to inhibit the growth of oral bacterial in human by such substances contained in sampled Guava leaves.

A. Preparation

1. Sterilizing the equipment:

Dipped the short part of the hockey stick into 70 % alcohol and swiftly pass it through the flame so that the alcohol catches on fire. Do not hold the stick in the flame too long, just runs it through in order to catch it on fire. The glass is now sterile and can be used as a spreader (repeat this with every spread plate).

2. Distribution of the diluted specimens:

Dropped 1 ml of specimens (*S. aureus*) into TSA agar plate. Took a sterile hockey stick and spread the specimen with the flat, short area of the stick in one direction while turn the plate in a circle. Be sure that you move the hockey stick around for couples of times. The specimen would be soaking into the agar.

3. Added of guava extract solution:

Directly added the guava extracted solution by using the capillary tube by placing the solution on the surface of agar.

4. Incubation:

Stack the plates, and hold them together with masking tape. Incubate at $37^{\circ}C$ incubator for twenty four hours.

B. Growth inhibition of *Staphylococcus aureus*

The test for microorganism growth inhibition was observed by measuring diameter size of a colony at the center that was seeded of pure culture (*Staphylococcus aureus*) with different dilutions. The inhibition was determined by measuring the diameters of the clear zone.

3.4.2. To find a suitable concentration of antibacterial solution for comparing the difference breeds of guava.

Three dilutions of guava extracted solution at 10^{0}, 10^{-1}, and 10^{-2} were compared. (The concentration at 10^{0} is contain solution from guava extracted 0.4 g/ml, the concentration at 10^{-1} is contain solution from guava extracted 0.04 g/ml, and the concentration at 10^{-2} is contain solution from guava extracted 0.004 g/ml)

A. Method for Identification of microbial inhibited zone

1. Liquefied trypticase soy agar, which had been cooled down to 45 °C was poured into 54 sterile petridishes. Then they were allowed to become harden solid as temperature decreased. Each plate was separated into four zones with four different bacteria inhibitors.
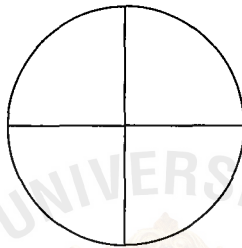
2. Then the *Streptococcus aureus*, which was grown in the broth, was inoculated using spread plate technique.

3. Guava extracted solution was added into each of four zones in the plate by using capillary tube.
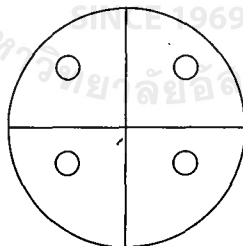
4. Procedures were repeated with the other breed of guava extracted solutions. All six different extracted solutions would be tested with *S. aureus*.

5. Plates were incubated in the incubator at 37°C for twenty four hours

6. After incubation, the dimension of clear zones were measured from each plate using protractor ruler to measure

3.4.3. To test in TSB broth and measure the level of growth by spectrophotometer.

Three treatments for each stage of leaves collected were used for variations with the wavelength at 540 nm for the measurement of microbial growth. It was interpreted that the higher the growth resulted the solution with higher concentration, and the higher turbidity of the solution analyzed by Spectrophotometer.

Treatment 1: Control tube (pure broth)

Treatment 2: *S. aureus* growth in TSB broth + extracted solution from young leave of Local Thai Guava.

Treatment 3: *S. aureus* growth in TSB broth + extracted solution from mature leave of Local Thai Guava.

Treatment 4: *S. aureus* growth in TSB broth + extracted solution from young leave of Bangkok apple Guava.

Treatment 5: *S. aureus* growth in TSB broth + extracted solution from mature leave of Bangkok apple Guava.

Treatment 6: *S. aureus* growth in TSB broth + extracted solution from young leave of Glom Sali Guava.

Treatment 7: *S. aureus* growth in TSB broth + extracted solution from mature leave of Glom Sali Guava.

3.5 Data Analysis

For the inhibition of *Staphylococcus aureus* test by using capillary tube (one capillary tube can contain the solution 0.1 ml, and gave the diameter of the antimicrobial solution around 1.3 to 1.5 cm) three replications were done in each treatment. The mean and standard deviation were calculated. The differences among them were compared using analysis of variance (ANOVA).

When F-ratios of data were statistically different at level 0.05, the average inhibition zone's differences in each treatment were calculated using Duncans' new multiple range test (DMRT) to rank physical properties values in form of different letters such as a, ab, b, bc or c. The comparison of the *Staphylococcus aureus* inhibition test between each treatment was compared by using Paired Sample T-test. All of the statistical analysis was calculated with a computer package program SPSS 11.5 for Window.

3.6 The period in this study.

Research start : September 25, 2006

Research end : December 1, 2006

Activities	Sep.	Oct.	Nov.	Dec.
Planning	←——→			
Implement (Proposal preparation)	←———→			
Chemical analysis		←———————→		
Preparation stock nutrient solution		←————→		
Microorganism Lab Testing			←———→	
Collection Data			←———→	
Data Analysis			←———	———→

CHAPTER IV

RESULTS AND DISCUSSIONS

In this study, the antimicrobial solution was extracted from the young and matured guava leaves by water extraction method. It was taken care that the extraction performance must be done immediately after the leaves were collected.

4.1 To confirm that the chemical substance in the guava leaves can inhibit the growth of *Staphylococcus aureus*.

As the knowledge that guava leaves have potential to kill bacteria, Table 4-1 below illustrated the result of the tests in microbial growth inhibiting ability amongst different breeds of Guava. This result will be taken to the further experiment in order to confirm that every breeds of guava contain microbial inhibiting substances.

Table 4-1 Result of Inhibiting Ability Test.

Breeds of Guava		Result
Local Thai Guava	Young leaves	+
	Mature leaves	++
Bangkok apple	Young leaves	++
	Mature leaves	+++
Glom Sali	Young leaves	+
	Mature leaves	+

Remarks: +   =   small clear zone diameter

++   =   medium clear zone diameter

+++   =   large clear zone diameter

According to Table 4-1, the substances extracted from guava leaves of Local Thai Guava, Bangkok Apple, and Glom Sali demonstrate the possibility in inhibiting the growth of *Staphylococcus aureus* in TSA agar. The positive result could be observed with the clear zone, showing absolutely no growth of bacterial. This revealed that the extracted substances from guava leaves, both young and mature leaves, were capable of preventing the growth of a certain oral bacterial, *S. aureus*. Though, this experiment would be more effective if other oral microbial could be testify together with *S. aureus*, it is impossible for the researcher to find another target. Therefore, this research would concentrate more on the comparison on effectiveness of antimicrobial capability between each breed of Guava, as well as the condition of Guava leaves as collected for sample.

4.2 To find a suitable concentration of antibacterial solution for comparing the differences of Guava breeds.

This experiment mainly concentrated on the identification of the most suitable concentration of antimicrobial substances extracted from the Guava leaves of different breeds at each stage. Three dilutions were analyzed in comparison using the observation of the radius of each colony grew at each plate with spread plate technique. In addition, the results of each dilution were found from the average results of triplicate trials.

Table 4-2 Identify the inhibiting ability in different concentration of three breeds of Guava Leaves

Breeds of Guava			Result (cm)
Local Thai Guava	Young	10^0	0.97d
		10^{-1}	0.63fghi
		10^{-2}	0.53ghi
	Mature	10^0	1.50b
		10^{-1}	1.00d
		10^{-2}	0.70fgh
Bangkok apple Guava	Young	10^0	1.53b
		10^{-1}	0.77ef
		10^{-2}	0.63fghi
	Mature	10^0	1.87a
		10^{-1}	1.33c
		10^{-2}	1.00d
Glom Sali Guava	Young	10^0	0.733efg
		10^{-1}	0.50i
		10^{-2}	0.10j
	Mature	10^0	1.233c
		10^{-1}	0.90de
		10^{-2}	0.567ghi

(The same letter means no significance difference at 95% level confidence)

(See Appendix C. Table 1-3)

Table 4-2 demonstrates the width of clear zone in centimeters from different breeds of guava, different leaves' condition, and different dilution of extracted solution. Among the three breeds of Local Thai Guava, Bangkok Apple Guava, and Glom Sali Guava, it was observed from the result that Bangkok Apple Guava had the most capability to inhibit the microbial growth with the clear zone width of 0.6 to 1.9 cm. In contrary, the smallest size of clear zones, came from the extract of Glom Sali Guava, which it also showed the incapability in inhibiting the oral bacterial. It could also be observed from this table that the different condition of leaves contains different concentration of antimicrobial substances, in which the mature leaves extracted contained higher concentration than the young leaves extracted. This could be observing from the comparison of three breeds.

When compared all dilution results together, mature part of Bangkok apple Guava of pure extracted substance or 10^0 was the best result with the clear zone width 1.87 cm. inhibition test. The dilution 10^0 of mature part of Local Thai Guava and young part of Bangkok apple Guava shown that the medium inhibition zone with no significant difference between them which were 1.53 and 1.50 cm., respectively. While the remaining dilution also gave the smaller size inhibition zone even if there were significant difference between them.

According to the significant difference between breed of guava results in each dilution were determined separately as shown in table 4-3, 4-4 and 4-5 orderly

Table 4-3 The Comparison of Clear Zone from Different Breeds at the dilution of 10^0

Breeds of Guava			Result (cm)
Local Thai Guava	Young	10^0	0.97d
	Mature	10^0	1.50b
Bangkok Apple Guava	Young	10^0	1.53b
	Mature	10^0	1.87a
Glom Sali Guava	Young	10^0	0.73e
	Mature	10^0	1.23c

(The same letter means no significance difference at 95% level confidence)

(See Appendix C. Table 1-3)

The result of table 4-3 demonstrated the comparison of the efficiency between each breed at the dilution of 10^0. It was found that Matured leaves of Bangkok Apple Guava could best inhibit the growth of bacterial at this dilution. Young leaves of Bangkok Apple Guava and Matured leaved of Local Thai Guava showed second efficacy in inhibiting the growth of bacterial. Then, Matured leaves of Glom Sali Guava, young leaves of Local Thai Guava, Young leaves of Glom Sali Guava, respectively showed the efficacy in order.

Table 4-4 The Comparison of Clear Zone from Different Breeds at the dilution of 10^{-1}

Breeds of Guava			Result (cm)
Local Thai Guava	Young	10^{-1}	0.63de
	Mature	10^{-1}	1.00b
Bangkok Apple Guava	Young	10^{-1}	0.77cd
	Mature	10^{-1}	1.33a
Glom Sali Guava	Young	10^{-1}	0.50e
	Mature	10^{-1}	0.90bc

(The same letter means no significance difference at 95% level confidence)

(See Appendix C. Table 1-3)

The result of table 4-4 demonstrated the comparison of the efficiency between each breed at the dilution of 10^{-1}. It was found that Matured leaves of Bangkok Apple Guava could best inhibit the growth of bacterial at this dilution. Matured leaves of Local Thai Guava and Glom Sali showed second efficacy in inhibiting the growth of bacterial. Matured leaves of Glom Sali and Young leaves of Bangkok Apple demonstrated the similar result in inhibiting the growth at the third efficacy. Young leaves of Bangkok Apple Guava and Young leaves of Local Thai Guava demonstrated the forth efficacy. Lastly, young leaves of Local Thai Guava and Glom Sali Guava demonstrated the least efficiency in inhibiting the growth of bacterial.

Table 4-5 The Comparison of Clear Zone from Different Breeds at the dilution of 10^{-2}

Breeds of Guava			Result (cm)
Local Thai Guava	Young	10^{-2}	0.53b
	Mature	10^{-2}	0.70b
Bangkok Apple Guava	Young	10^{-2}	0.63b
	Mature	10^{-2}	1.00a
Glom Sali Guava	Young	10^{-2}	0.10c
	Mature	10^{-2}	0.57b

(The same letter means no significance difference at 95% level confidence)

(See Appendix C. Table 1-3)

The result of table 4-5 demonstrated the comparison of the efficiency between each breed at the dilution of 10^{-1}. It was found that Matured leaves of Bangkok Apple Guava could best inhibit the growth of bacterial at this dilution. Matured leaves of Local Thai Guava, young leaves of Bangkok Apple Guava, matured leaves of Glom Sali, and young leaves of Local Thai Guava, together, demonstrated the similar efficiency in inhibiting the growth of bacterial. While, young leaves of Glom Sali demonstrated the least efficiency.

Table 4-6 The Comparison Between Different Dilutions of Local Thai Guava Leaves Extracted

Breeds of Guava			Result (cm)
Local Thai Guava	Young	10^0	0.97a
		10^{-1}	0.63b
		10^{-2}	0.53b
	Mature	10^0	1.50a
		10^{-1}	1.00b
		10^{-2}	0.70c

(The same letter means no significance difference at 95% level confidence)

(Note that the Young and Matured data are being analyzed separately)

(See Appendix C. Table 5-3)

As different dilutions were compared, it was demonstrated that the antimicrobial substances extracted from Local Thai Guava could best inhibit the growth of microbial at the dilution of 10^0. However, there was no significant difference between the dilution of 10^{-1} and 10^{-2} of young leaves extracted, as well as the dilution of 10^{-1} from the extracted solution of matured leaves. Therefore, it was important to note that there was no difference in diluting the solution to 10^{-1} or 10^{-2} to affect the efficiency of microbial growth inhibition. This also shows that there is a possibility to safe cost of production. Lastly, the matured leaves extraction demonstrated the poorest efficiency in inhibiting the growth of microbial.

Table 4-7 The Comparison Between Different Dilutions of Bangkok
Apple Guava Leaves Extracted

Breeds of Guava			Result (cm)
Bangkok apple Guava	Young	10^0	1.53a
		10^{-1}	0.77b
		10^{-2}	0.63b
	Mature	10^0	1.87a
		10^{-1}	1.33b
		10^{-2}	1.00c

(The same letter means no significance difference at 95% level
confidence)

(Note that the Young and Matured data are being analyzed separately)

(See Appendix C. Table 6-3)

The comparison within this breed demonstrated similar result to the
previous table, which stated that the dilution of 10^0 had the best efficiency
in inhibiting the microbial growth, while there was no significance
different between the dilution of 10^{-1} and 10^{-2}. As for the extract of
Matured leaves, all three dilutions demonstrated significant difference
from each other.

Table 4-8 The Comparison Between Different Dilutions of Glom Sali Guava Leaves Extracted

Breeds of Guava			Result (cm)
Glom Sali Guava	Young	10^0	0.73a
		10^{-1}	0.50a
		10^{-2}	0.10b
	Mature	10^0	1.23a
		10^{-1}	0.90b
		10^{-2}	0.57c

(The same letter means no significance difference at 95% level confidence)

(Note that the Young and Matured data are being analyzed separately)

(See Appendix C. Table 7-3)

The comparison within this breed demonstrated that the extract of matured leaves had significant difference most of all. The dilution of 10^0 and 10^{-1}, of the young leaves extract showed no significance difference from each other.

4.3 To measure the level of growth by spectrophotometer using TSB as the growing condition.

Trypticase soy broth was used as the second source of microbial nutrition in order to stimulate the oral condition. The extracted solution from Guava leaves were added to the broth in order to observe the growth of microorganisms. As the bacterium growth in the agar broth, some

kinds produce wastes that make nutrient broth becomes turbid and some kind produce gases as well. Therefore, the percent growths were observed in this experiment by Spectrophotometer with the wavelength at 540 nm in order to observe the best differences length result.

Table 4-9 OD Value of Bacterial growth in TSB mixed with Guava Leaves Extracted Solution

Breeds of Guava		OD Value 540nm
Control		0.0000
Local Thai Guava	Young	0.5796c
	Mature	0.3433b
Bangkok apple Guava	Young	0.4584b
	Mature	0.0973a
Glom Sali Guava	Young	0.8736d
	Mature	0.6675c

(The same letter means no significance difference at 95% level confidence)

(See Appendix C. Table 14-3)

From table 4-9, the amount of growth bacterial could be observed by the higher OD value. The turbidity of solution indicated the great quantity of microbial growth, whereas the clear solution demonstrated that microbial growth were inhibited by the Guava leaves extracted solution. The result of this experiment had conquered to part 2, as the guava leaves extracted solution with dilution of 10^{-1} could inhibit the microbial growth more effectively. It could also be concluded from this experiment that the more mature the leaves of Guava were, the higher concentration of antimicrobial substance that they contained. The

capability to inhibit *S. aureus* could be rank accordingly as followed ; Extracted solution of matured Bangkok Apple Guava leaves, matured Local Thai Guava Leaves, matured Glom Sali Guava leaves, young Bangkok Apple Guava leaves, young Local Thai Guava leaves, and young Glom Sali Guava leaves.

Table 4-10 OD Value of Bacterial growth in TSB mixed with young Guava Leaves Extracted Solution comparing between three breeds of guava.

Breeds of Guava		OD Value 540 nm
Local Thai Guava	Young	0.5796b
Bangkok Apple Guava	Young	0.4584a
Glom Sali Guava	Young	0.8736c

(The same letter means no significance difference at 95% level confidence)
(See Appendix C. Table 9-3)

This table illustrated that the young leaves extracted solution of Bangkok Apple Guava had the most efficiency to inhibit the microbial growth in TSB. In contrary, the highest amount left by Glom Sali Guava demonstrated that this solution did not prevent or inhibit the microbial growth and production of wastes and gases.

Table 4-11 OD Value of Bacterial growth in TSB mixed with matured
        Guava Leaves Extracted Solution comparing between three
        breeds of guava

Breeds of Guava		OD Value 540 nm
		Average of 3 Trials
Local Thai Guava	Matured	0.3433b
Bangkok Apple Guava	Matured	0.0973a
Glom Sali Guava	Matured	0.6675c

(The same letter means no significance difference at 95% level
confidence)

(See Appendix C. Table 10-3)

This table illustrated that the matured leaves extracted solution of
Bangkok Apple Guava had the most efficiency to inhibit the microbial
growth in TSB. In contrary, the highest amount left by Glom Sali Guava
demonstrated that this solution did not prevent or inhibit the microbial
growth and production of wastes and gases. This table could also
compare with Table 4-10 in the means of illustrating the most effective
stage of leaves to be extracted. It was very obvious that the matured
leaves could inhibit microbial growth better than the young leaves
extracted solution. This might be happened due to the higher
concentration of the antimicrobial substances that the matured leaves
contain.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

5.1.1 Guava leaves of Local Thai Guava, Bangkok Apple Guava, and Glom Sali Guava, were capable in controlling and inhibiting *Staphylococcus aureus*, which was considered as one of the oral microbial.

5.1.2 The matured leaves from each breed contain higher concentration of antimicrobial substances than younger leaves.

5.1.3 Bangkok Apple Guava demonstrated the most effective result of antimicrobial amongst the three breeds, where as, Glom Sali Guava demonstrated the least effective result.

5.2 Recommendation

This study had done in the laboratory scale which the further studies are very important to improve the qualities of the products or to scale up as follows:

5.2.1   Other   oral   microbial   should   be   used   instead   of *Staphylococcus aureus* in   order   to   test   the   capability   of antimicrobial   substances   extracted   from   Guava   leaves. Especially *Streptococcus mutans*.

5.2.2 In order to obtain the best extracted solution, Alcohol extraction should be performed.

5.2.3 This study was only the laboratory scale which is needed to improve to use in the larger scale. The scale up results may differ from this.

5.2.4 Research and development in the economical production may be needed more concerns.
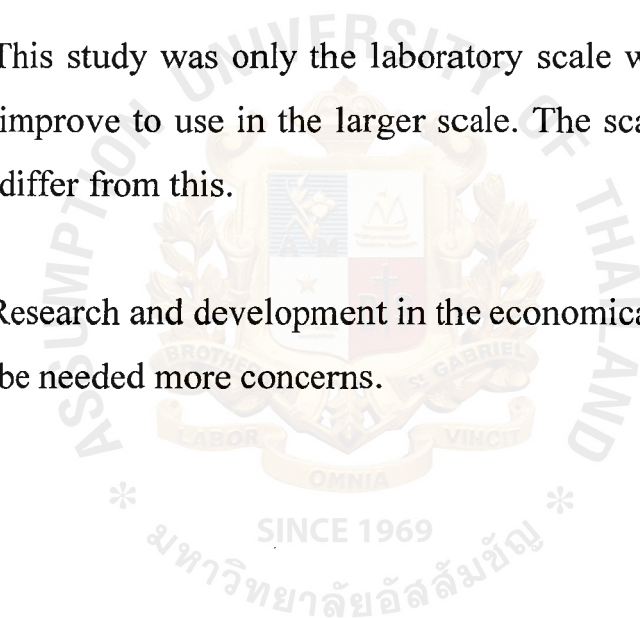
1. Trypticase soy broth is a general purpose enrichment broth for the isolation and cultivation of a wide variety of microorganisms. This medium is widely used for the isolation of bacteria from clinical specimens and will support the growth of the majority of pathogenic bacteria. (20)

Trypticase Soy Broth

Trypticase Soy Broth	30 g
H_2O	1,000 ml
Clave Temperature/Time	$121^{\circ}C$ / 15 minutes

The agar was heated with gentle agitation to dissolve. Dispense 225 ml into 500 ml Erlenmeyer flasks. Then, taken to autoclave at the temperature of 121 Degree Celsius for fifteen minutes. Final pH is at 7.3 ± 0.2.(11)

2. Trypticase soy agar is a general purpose medium for the isolation and cultivation of a wide variety of organisms. TSA slants may be used to cultivate, store, and ship pure cultures of bacteria.

Trypticase Soy Agar

Trypticase Soy Broth	40 g
NaCl	30 g
H^2O	1000 ml
Clave	121°C / 15 minutes

The agar was heated with agitation to dissolve agar. Boil 1 min. Dispense into suitable tubes or flasks. Then, taken to autoclave at 121 degree Celsius for fifteen minutes. Final pH is at 7.3 ± 0.2. (19)

APPENDIX B

RAW DATA
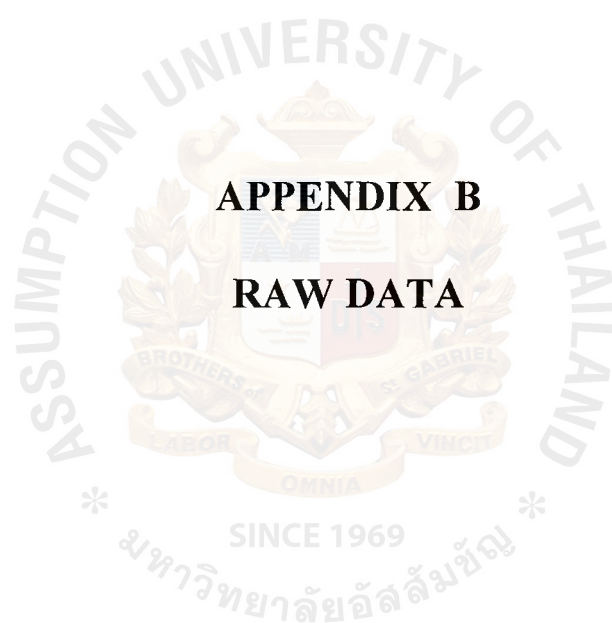
Appendix B Table 1 Identify the inhibiting ability in different
concentration of three breeds of Guava Leaves

Breeds of Guava			Result (cm)		
			Trial 1	Trial 2	Trial 3
Local Thai Guava	Young	10^0	1.0	1.0	0.9
		10^{-1}	0.6	0.6	0.7
		10^{-2}	0.5	0.6	0.5
	Mature	10^0	1.6	1.4	1.5
		10^{-1}	0.9	1.1	1.0
		10^{-2}	0.8	0.6	0.7
Bangkok Apple Guava	Young	10^0	1.5	1.5	1.6
		10^{-1}	0.9	0.7	0.7
		10^{-2}	0.6	0.7	0.6
	Mature	10^0	1.8	1.9	1.9
		10^{-1}	1.3	1.4	1.3
		10^{-2}	1.0	0.9	1.1
Glom Sali Guava	Young	10^0	0.9	0.7	0.6
		10^{-1}	0.5	0.5	0.5
		10^{-2}	0.3	-	-
	Mature	10^0	1.2	1.1	1.4
		10^{-1}	0.9	1.0	0.8
		10^{-2}	0.5	0.6	0.6

Appendix B Table 2 Bacterial growth in TSB mixed with Guava Leaves

Extract at dilution 10^{-1}

Breeds of Guava		OD Value 540nm		
		Trial 1	Trial 2	Trial 3
Control		0.0000	0.0000	0.0000
Local Thai Guava	Young	0.5210	0.6384	0.5793
	Mature	0.3096	0.4182	0.3022
Bangkok apple Guava	Young	0.3214	0.2112	0.3101
	Mature	0.1034	0.0879	0.1006
Glom Sali Guava	Young	0.8214	0.9650	0.8345
	Mature	0.6159	0.6847	0.7018
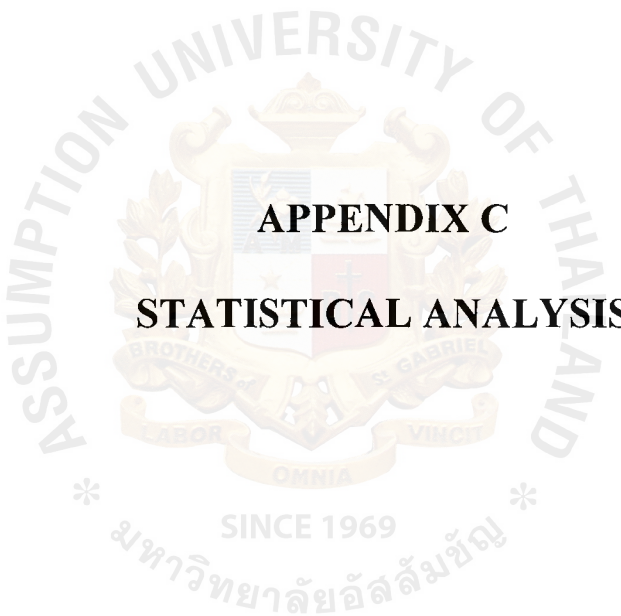
APPENDIX C

STATISTICAL ANALYSIS

Description

In this study, some codes were used to abbreviate the treatment as the following;

Dilution 1 = extraction of guava at 10^{-0}
Dilution 2 = extraction of guava at 10^{-1}
Dilution 3 = extraction of guava at 10^{-2}

Local Y   = Local Thai guava young
Local M   = Local Thai guava mature
Bangkok Y = Bangkok Apple guava young
Bangkok M = Bangkok Apple guava mature
Glom Y   = Glom Sali guava young
Glom M   = Glom Sali guava mature

Appendix C Table 1-1 Statistical analysis of the inhibiting ability to *S. aureus* between each dilution.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
DILUTE1	Local Y	3	.96667	.057735	.033333	.82324	1.11009	.900	1.00
	Local M	3	1.50000	.100000	.057735	1.25159	1.74841	1.40	1.60
	Bangkok Y	3	1.53333	.057735	.033333	1.38991	1.67676	1.50	1.60
	Bangkok M	3	1.86667	.057735	.033333	1.72324	2.01009	1.80	1.90
	Glom Y	3	.73333	.152753	.088192	.35388	1.11279	.600	.900
	Glom M	3	1.23333	.152753	.088192	.85388	1.61279	1.10	1.40
	Total	18	1.30556	.397747	.093750	1.10776	1.50335	.600	1.90
DILUTE2	Local Y	3	.63333	.057735	.033333	.48991	.77676	.600	.700
	Local M	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
	Bangkok Y	3	.76667	.115470	.066667	.47982	1.05351	.700	.900
	Bangkok M	3	1.33333	.057735	.033333	1.18991	1.47676	1.30	1.40
	Glom Y	3	.50000	.000000	.000000	.50000	.50000	.500	.500
	Glom M	3	.90000	.100000	.057735	.65159	1.14841	.800	1.00
	Total	18	.85556	.285373	.067263	.71364	.99747	.500	1.40
DILUTE3	Local Y	3	.53333	.057735	.033333	.38991	.67676	.500	.600
	Local M	3	.70000	.100000	.057735	.45159	.94841	.600	.800
	Bangkok Y	3	.63333	.057735	.033333	.48991	.77676	.600	.700
	Bangkok M	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
	Glom Y	3	.10000	.173205	.100000	-.33027	.53027	.000	.300
	Glom M	3	.56667	.057735	.033333	.42324	.71009	.500	.600
	Total	18	.58889	.286744	.067586	.44629	.73148	.000	1.10

Appendix C Table 1-2 ANOVA of the inhibiting ability to *S. aureus* between each dilution.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DILUTE1	Between Groups	2.556	5	.511	46.010	.000
	Within Groups	.133	12	.011		
	Total	2.689	17			
DILUTE2	Between Groups	1.304	5	.261	39.133	.000
	Within Groups	.080	12	.007		
	Total	1.384	17			
DILUTE3	Between Groups	1.278	5	.256	25.556	.000
	Within Groups	.120	12	.010		
	Total	1.398	17			

Appendix C Table 1-3 Multiple comparison of the inhibiting ability to *S. aureus* between each dilution.

DILUTE1

Duncan[a]

		Subset for alpha = .05				
CONPAREB	N	1	2	3	4	5
Glom Y	3	.73333				
Local Y	3		.96667			
Glom M	3			1.23333		
Local M	3				1.50000	
Bangkok Y	3				1.53333	
Bangkok M	3					1.86667
Sig.		1.000	1.000	1.000	.705	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DILUTE2

Duncan[a]

CONPAREB	N	Subset for alpha = .05
		1	2	3	4	5
Glom Y	3	.50000				
Local Y	3	.63333	.63333			
Bangkok Y	3		.76667	.76667		
Glom M	3			.90000	.90000	
Local M	3				1.00000	
Bangkok M	3					1.33333
Sig.		.069	.069	.069	.159	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DILUTE3

Duncan[a]

CONPAREB	N	Subset for alpha = .05
		1	2	3
Glom Y	3	.10000		
Local Y	3		.53333	
Glom M	3		.56667	
Bangkok Y	3		.63333	
Local M	3		.70000	
Bangkok M	3			1.00000
Sig.		1.000	.082	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 2-1 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava young leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	.96667	.057735	.033333	.82324	1.11009	.900	1.00
dilution2	3	.63333	.057735	.033333	.48991	.77676	.600	.700
dilution3	3	.53333	.057735	.033333	.38991	.67676	.500	.600
Total	9	.71111	.202759	.067586	.55526	.86697	.500	1.00

Appendix C Table 2-2 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava young leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.309	2	.154	46.333	.000
Within Groups	.020	6	.003		
Total	.329	8			

Appendix C Table 2-3 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava young leaves.

TRIAL

Duncan[a]

		Subset for alpha = .05	
CONPAREB	N	1	2
dilution3	3	.53333	
dilution2	3	.63333	
dilution1	3		.96667
Sig.		.078	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 2-4 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava matured leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	1.50000	.100000	.057735	1.25159	1.74841	1.40	1.60
dilution2	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
dilution3	3	.70000	.100000	.057735	.45159	.94841	.600	.800
Total	9	1.06667	.360555	.120185	.78952	1.34381	.600	1.60

Appendix C Table 2-5 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava matured leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.980	2	.490	49.000	.000
Within Groups	.060	6	.010		
Total	1.040	8			

Appendix C Table 2-6 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Local Thai guava matured leaves.

TRIAL

Duncan[a]

CONPAREB	N	Subset for alpha = .05		
		1	2	3
dilution3	3	.70000		
dilution2	3		1.00000	
dilution1	3			1.50000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 3-1 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava young leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	1.53333	.057735	.033333	1.38991	1.67676	1.50	1.60
dilution2	3	.83333	.115470	.066667	.54649	1.12018	.700	.900
dilution3	3	.63333	.057735	.033333	.48991	.77676	.600	.700
Total	9	1.00000	.415331	.138444	.68075	1.31925	.600	1.60

Appendix C Table 3-2 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava young leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.340	2	.670	100.500	.000
Within Groups	.040	6	.007		
Total	1.380	8			

Appendix C Table 3-3 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava young leaves.

TRIAL

Duncan[a]

CONPAREB	N	Subset for alpha = .05		
		1	2	3
dilution3	3	.63333		
dilution2	3		.83333	
dilution1	3			1.53333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 3-4 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava matured leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	1.83333	.057735	.033333	1.68991	1.97676	1.80	1.90
dilution2	3	1.33333	.057735	.033333	1.18991	1.47676	1.30	1.40
dilution3	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
Total	9	1.38889	.368932	.122977	1.10530	1.67248	.900	1.90

Appendix C Table 3-5 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava matured leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.056	2	.528	95.000	.000
Within Groups	.033	6	.006		
Total	1.089	8			

Appendix C Table 3-6 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Bangkok Apple guava matured leaves.

TRIAL

Duncan[a]

CONPAREB	N	Subset for alpha = .05		
		1	2	3
dilution3	3	1.00000		
dilution2	3		1.33333	
dilution1	3			1.83333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 4-1 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava young leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	.73333	.152753	.088192	.35388	1.11279	.600	.900
dilution2	3	.50000	.000000	.000000	.50000	.50000	.500	.500
dilution3	3	.10000	.173205	.100000	-.33027	.53027	.000	.300
Total	9	.44444	.300463	.100154	.21349	.67540	.000	.900

Appendix C Table 4-2 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava young leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.616	2	.308	17.313	.003
Within Groups	.107	6	.018		
Total	.722	8			

Appendix C Table 4-3 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava young leaves.

TRIAL

Duncan[a]

CONPAREB	N	Subset for alpha = .05	
		1	2
dilution3	3	.10000	
dilution2	3		.50000
dilution1	3		.73333
Sig.		1.000	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 4-4 Statistical analysis of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava matured leaves.

Descriptives

TRIAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
dilution1	3	1.23333	.152753	.088192	.85388	1.61279	1.10	1.40
dilution2	3	.90000	.100000	.057735	.65159	1.14841	.800	1.00
dilution3	3	.56667	.057735	.033333	.42324	.71009	.500	.600
Total	9	.90000	.304138	.101379	.66622	1.13378	.500	1.40

Appendix C Table 4-5 ANOVA of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava matured leaves.

ANOVA

TRIAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.667	2	.333	27.273	.001
Within Groups	.073	6	.012		
Total	.740	8			

Appendix C Table 4-6 Multiple comparison of the inhibiting ability to *S. aureus* in each dilution of Glom Sali guava matured leaves.

TRIAL

Duncan[a]

		Subset for alpha = .05		
CONPAREB	N	1	2	3
dilution3	3	.56667		
dilution2	3		.90000	
dilution1	3			1.23333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 5-1 Statistical analysis of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves of Local Thai Guava.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
YOUNG	dilution1	3	.96667	.057735	.033333	.82324	1.11009	.900	1.00
	dilution2	3	.63333	.057735	.033333	.48991	.77676	.600	.700
	dilution3	3	.53333	.057735	.033333	.38991	.67676	.500	.600
	Total	9	.71111	.202759	.067586	.55526	.86697	.500	1.00
MATURE	dilution1	3	1.50000	.100000	.057735	1.25159	1.74841	1.40	1.60
	dilution2	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
	dilution3	3	.70000	.100000	.057735	.45159	.94841	.600	.800
	Total	9	1.06667	.360555	.120185	.78952	1.34381	.600	1.60

Appendix C Table 5-2 ANOVA of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Local Thai Guava.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
YOUNG	Between Groups	.309	2	.154	46.333	.000
	Within Groups	.020	6	.003		
	Total	.329	8			
MATURE	Between Groups	.980	2	.490	49.000	.000
	Within Groups	.060	6	.010		
	Total	1.040	8			

Appendix C Table 5-3 Multiple comparison of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Local Thai Guava.

YOUNG

Duncan[a]

CONPAREB	N	Subset for alpha = .05	
		1	2
dilution3	3	.53333	
dilution2	3	.63333	
dilution1	3		.96667
Sig.		.078	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

MATURE

Duncan[a]

CONPAREB	N	Subset for alpha = .05		
		1	2	3
dilution3	3	.70000		
dilution2	3		1.00000	
dilution1	3			1.50000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 6-1 Statistical analysis of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves of Bangkok Apple Guava.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
YOUNG	dilution1	3	1.53333	.057735	.033333	1.38991	1.67676	1.50	1.60
	dilution2	3	.76667	.115470	.066667	.47982	1.05351	.700	.900
	dilution3	3	.63333	.057735	.033333	.48991	.77676	.600	.700
	Total	9	.97778	.426549	.142183	.64990	1.30565	.600	1.60
MATURE	dilution1	3	1.86667	.057735	.033333	1.72324	2.01009	1.80	1.90
	dilution2	3	1.33333	.057735	.033333	1.18991	1.47676	1.30	1.40
	dilution3	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
	Total	9	1.40000	.384057	.128019	1.10479	1.69521	.900	1.90

Appendix C Table 6-2 ANOVA of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Bangkok Apple Guava.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
YOUNG	Between Groups	1.416	2	.708	106.167	.000
	Within Groups	.040	6	.007		
	Total	1.456	8			
MATURE	Between Groups	1.147	2	.573	103.200	.000
	Within Groups	.033	6	.006		
	Total	1.180	8			

Appendix C Table 6-3 Multiple comparison of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Bangkok Apple Guava.

YOUNG

Duncan[a]

		Subset for alpha = .05	
CONPAREB	N	1	2
dilution3	3	.63333	
dilution2	3	.76667	
dilution1	3		1.53333
Sig.		.092	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

MATURE

Duncan[a]

		Subset for alpha = .05		
CONPAREB	N	1	2	3
dilution3	3	1.00000		
dilution2	3		1.33333	
dilution1	3			1.86667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 7-1 Statistical analysis of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves of Glom Sali Guava.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
YOUNG	dilution1	3	.73333	.152753	.088192	.35388	1.11279	.600	.900
	dilution2	3	.50000	.000000	.000000	.50000	.50000	.500	.500
	dilution3	3	.10000	.173205	.100000	-.33027	.53027	.000	.300
	Total	9	.44444	.300463	.100154	.21349	.67540	.000	.900
MATURE	dilution1	3	1.23333	.152753	.088192	.85388	1.61279	1.10	1.40
	dilution2	3	.90000	.100000	.057735	.65159	1.14841	.800	1.00
	dilution3	3	.56667	.057735	.033333	.42324	.71009	.500	.600
	Total	9	.90000	.304138	.101379	.66622	1.13378	.500	1.40

Appendix C Table 7-2 ANOVA of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Glom Sali Guava.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
YOUNG	Between Groups	.616	2	.308	17.313	.003
	Within Groups	.107	6	.018		
	Total	.722	8			
MATURE	Between Groups	.667	2	.333	27.273	.001
	Within Groups	.073	6	.012		
	Total	.740	8			

Appendix C Table 7-3 Multiple comparison of the inhibiting ability to *S. aureus* comparing between young leaves and mature leaves Glom Sali Guava.

YOUNG

Duncan[a]

CONPAREB	N	Subset for alpha = .05	
		1	2
dilution3	3	.10000	
dilution2	3		.50000
dilution1	3		.73333
Sig.		1.000	.076

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

MATURE

Duncan[a]

CONPAREB	N	Subset for alpha = .05		
		1	2	3
dilution3	3	.56667		
dilution2	3		.90000	
dilution1	3			1.23333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Description

In this study, some codes were used to abbreviate the treatment as the following;

Local Y 1   = Local Thai guava young dilution 10^0
Local Y 2   = Local Thai guava young dilution 10^{-1}
Local Y 3   = Local Thai guava young dilution 10^{-2}
Local M 1   = Local Thai guava mature dilution 10^0
Local M 2   = Local Thai guava mature dilution 10^{-1}
Local M 3   = Local Thai guava mature dilution 10^{-2}
Bkk Y 1   = Bangkok Apple guava young dilution 10^0
Bkk Y 2   = Bangkok Apple guava young dilution 10^{-1}
Bkk Y 3   = Bangkok Apple guava young dilution 10^{-2}
Bkk M 1   = Bangkok Apple guava mature dilution 10^0
Bkk M 2   = Bangkok Apple guava mature dilution 10^{-1}
Bkk M 3   = Bangkok Apple guava mature dilution 10^{-2}
Glom Y 1   = Glom Sali guava young dilution 10^0
Glom Y 2   = Glom Sali guava young dilution 10^{-1}
Glom Y 3   = Glom Sali guava young dilution 10^{-2}
Glom M 1   = Glom Sali guava mature dilution 10^0
Glom M 2   = Glom Sali guava mature dilution 10^{-1}
Glom M 3   = Glom Sali guava mature dilution 10^{-2}

Appendix C Table 8-1 Statistical analysis of the inhibiting ability to *S. aureus* between all dilutions of three breed of guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Local Y 1	3	.96667	.057735	.033333	.82324	1.11009	.900	1.00
Local Y 2	3	.63333	.057735	.033333	.48991	.77676	.600	.700
Local Y 3	3	.53333	.057735	.033333	.38991	.67676	.500	.600
Local M 1	3	1.50000	.100000	.057735	1.25159	1.74841	1.40	1.60
Local M 2	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
Local M 3	3	.70000	.100000	.057735	.45159	.94841	.600	.800
Bkk Y 1	3	1.53333	.057735	.033333	1.38991	1.67676	1.50	1.60
Bkk Y 2	3	.76667	.115470	.066667	.47982	1.05351	.700	.900
Bkk Y 3	3	.63333	.057735	.033333	.48991	.77676	.600	.700
Bkk M 1	3	1.86667	.057735	.033333	1.72324	2.01009	1.80	1.90
Bkk M 2	3	1.33333	.057735	.033333	1.18991	1.47676	1.30	1.40
Bkk M 3	3	1.00000	.100000	.057735	.75159	1.24841	.900	1.10
Glom Y 1	3	.73333	.152753	.088192	.35388	1.11279	.600	.900
Glom Y 2	3	.50000	.000000	.000000	.50000	.50000	.500	.500
Glom Y 3	3	.10000	.173205	.100000	-.33027	.53027	.000	.300
Glom M 1	3	1.23333	.152753	.088192	.85388	1.61279	1.10	1.40
Glom M 2	3	.90000	.100000	.057735	.65159	1.14841	.800	1.00
Glom M 3	3	.56667	.057735	.033333	.42324	.71009	.500	.600
Total	54	.91667	.438587	.059684	.79696	1.03638	.000	1.90

Appendix C Table 8-2 ANOVA of the inhibiting ability to *S. aureus* between all dilutions of three breed of guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.862	17	.580	62.651	.000
Within Groups	.333	36	.009		
Total	10.195	53			

Appendix C Table 8-3 Multiple comparison of the inhibiting ability to *S. aureus* between all dilutions of three breed of guava.

RESULT

Duncan[a]

CONPAREB	N	Subset for alpha = .05
		1	2	3	4	5	6	7	8	9	10
Glom Y 3	3	.10000									
Glom Y 2	3		.50000								
Local Y 3	3		.53333	.53333							
Glom M 3	3		.56667	.56667	.56667						
Local Y 2	3		.63333	.63333	.63333	.63333					
Bkk Y 3	3		.63333	.63333	.63333	.63333					
Local M 3	3			.70000	.70000	.70000					
Glom Y 1	3				.73333	.73333	.73333				
Bkk Y 2	3					.76667	.76667				
Glom M 2	3						.90000	.90000			
Local Y 1	3							.96667			
Local M 2	3							1.00000			
Bkk M 3	3							1.00000			
Glom M 1	3								1.23333		
Bkk M 2	3								1.33333		
Local M 1	3									1.50000	
Bkk Y 1	3									1.53333	
Bkk M 1	3										1.86667
Sig.		1.000	.138	.064	.064	.138	.051	.255	.211	.674	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 9-1 Statistical analysis of the inhibiting ability to *S. aureus* between all of young leaves in three breed of guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Local	3	.579567	.0587005	.0338907	.433747	.725387	.5210	.6384
Bangkok	3	.280900	.0606258	.0350023	.130297	.431503	.2112	.3214
Glom	3	.873633	.0793965	.0458396	.676402	1.070865	.8214	.9650
Total	9	.578033	.2631207	.0877069	.375781	.780286	.2112	.9650

Appendix C Table 9-2 ANOVA of the inhibiting ability to *S. aureus* between all of young leaves in three breed of guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.527	2	.264	58.884	.000
Within Groups	.027	6	.004		
Total	.554	8			

Appendix C Table 9-3 Multiple comparison of the inhibiting ability to *S. aureus* between all of young leaves in three breed of guava.

RESULT

Duncan[a]

COMPARE	N	Subset for alpha = .05		
		1	2	3
Bangkok	3	.280900		
Local	3		.579567	
Glom	3			.873633
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 10-1 Statistical analysis of the inhibiting ability to *S. aureus* between all of matured leaves in three breed of guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Local	3	.343333	.0649419	.0374942	.182009	.504658	.3022	.4182
Bangkok	3	.097300	.0082601	.0047690	.076781	.117819	.0879	.1034
Glom	3	.667467	.0454691	.0262516	.554515	.780418	.6159	.7018
Total	9	.369367	.2508463	.0836154	.176549	.562184	.0879	.7018

Appendix C Table 10-2 ANOVA of the inhibiting ability to *S. aureus* between all of matured leaves in three breed of guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.491	2	.245	115.853	.000
Within Groups	.013	6	.002		
Total	.503	8			

Appendix C Table 10-3 Multiple comparison of the inhibiting ability to *S. aureus* between all of matured leaves in three breed of guava.

RESULT

Duncan[a]

COMPARE	N	Subset for alpha = .05		
		1	2	3
Bangkok	3	.097300		
Local	3		.343333	
Glom	3			.667467
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C Table 11-1 Statistical analysis of the inhibiting ability to *S. aureus* between young and matured leaves in Local Thai guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
young	3	.579567	.0587005	.0338907	.433747	.725387	.5210	.6384
mature	3	.343333	.0649419	.0374942	.182009	.504658	.3022	.4182
Total	6	.461450	.1407378	.0574560	.313755	.609145	.3022	.6384

Appendix C Table 11-2 ANOVA of the inhibiting ability to *S. aureus* between young and matured leaves in Local Thai guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.084	1	.084	21.847	.009
Within Groups	.015	4	.004		
Total	.099	5			

Appendix C Table 12-1 Statistical analysis of the inhibiting ability to *S. aureus* between young and matured leaves in Bangkok Apple guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
young	3	.280900	.0606258	.0350023	.130297	.431503	.2112	.3214
mature	3	.097300	.0082601	.0047690	.076781	.117819	.0879	.1034
Total	6	.189100	.1077505	.0439890	.076023	.302177	.0879	.3214

Appendix C Table 12-2 ANOVA of the inhibiting ability to *S. aureus* between young and matured leaves in Bangkok Apple guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.051	1	.051	27.012	.007
Within Groups	.007	4	.002		
Total	.058	5			

Appendix C Table 13-1 Statistical analysis of the inhibiting ability to *S. aureus* between young and matured leaves in Glom Sali guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
young	3	.873633	.0793965	.0458396	.676402	1.070865	.8214	.9650
mature	3	.667467	.0454691	.0262516	.554515	.780418	.6159	.7018
Total	6	.770550	.1268854	.0518008	.637392	.903708	.6159	.9650

Appendix C Table 13-2 ANOVA of the inhibiting ability to *S. aureus* between young and matured leaves in Glom Sali guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.064	1	.064	15.232	.017
Within Groups	.017	4	.004		
Total	.080	5			

Appendix C Table 14-1 Statistical analysis of the inhibiting ability to *S. aureus* between all of young and matured leaves in three breed of guava.

Descriptives

RESULT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Local Y	3	.579567	.0587005	.0338907	.433747	.725387	.5210	.6384
Local M	3	.343333	.0649419	.0374942	.182009	.504658	.3022	.4182
Bangkok Y	3	.280900	.0606258	.0350023	.130297	.431503	.2112	.3214
Bangkok M	3	.097300	.0082601	.0047690	.076781	.117819	.0879	.1034
Glom Y	3	.873633	.0793965	.0458396	.676402	1.070865	.8214	.9650
Glom M	3	.667467	.0454691	.0262516	.554515	.780418	.6159	.7018
Total	18	.473700	.2715087	.0639952	.338682	.608718	.0879	.9650

Appendix C Table 14-2 Multiple comparison of the inhibiting ability to *S. aureus* between all of young and matured leaves in three breed of guava.

ANOVA

RESULT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.214	5	.243	73.635	.000
Within Groups	.040	12	.003		
Total	1.253	17			

Appendix C Table 14-3 ANOVA of the inhibiting ability to *S. aureus* between all of young and matured leaves in three breed of guava.

RESULT

Duncan[a]

COMPARE	N	Subset for alpha = .05			
		1	2	3	4
Bangkok M	3	.097300			
Bangkok Y	3		.280900		
Local M	3		.343333		
Local Y	3			.579567	
Glom M	3			.667467	
Glom Y	3				.873633
Sig.		1.000	.208	.085	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.
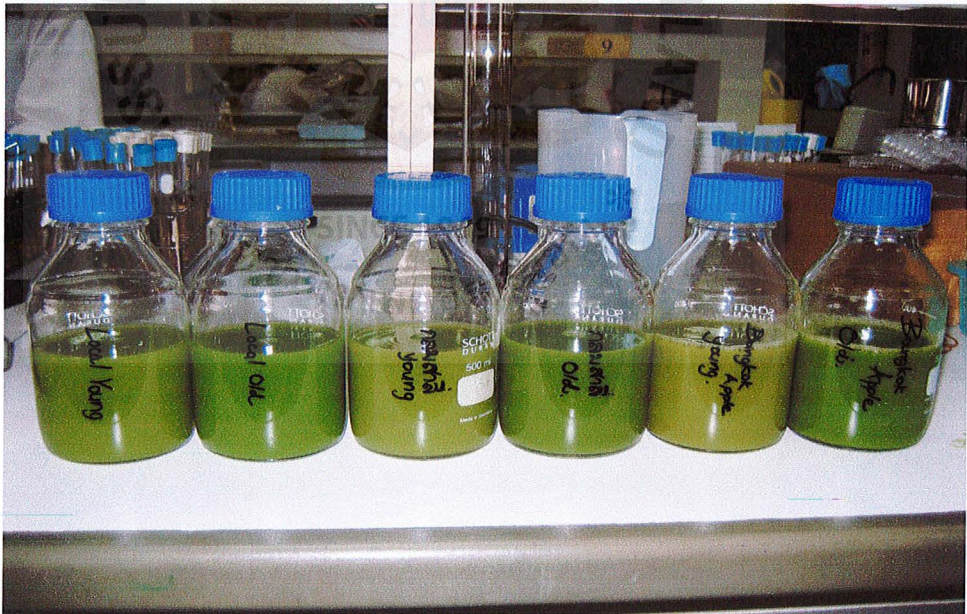
APPENDIX D

SOME FIGURES FROM THE STUDY

Appendix D figure1 The solution extracted from the Local Thai guava
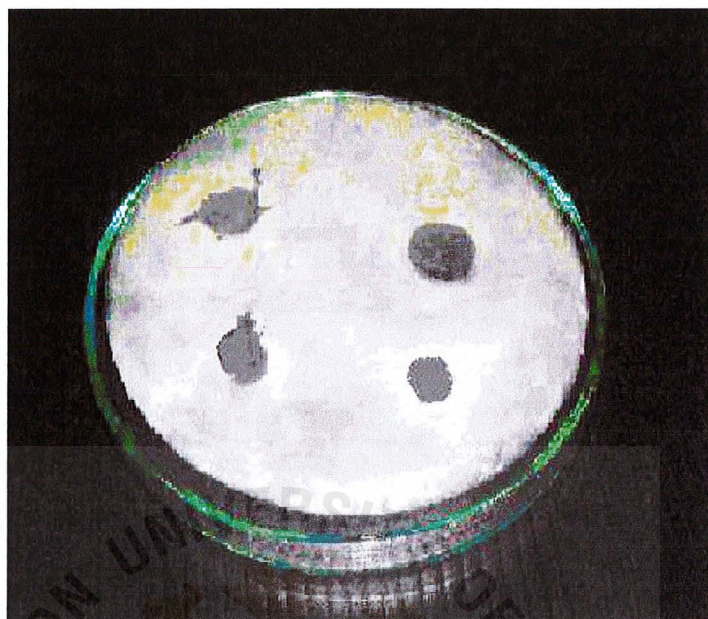leaves.

Appendix D figure 2 The solution extracted from the Bangkok Apple guava
leaves.

Appendix D figure3 The solution extracted from the Glom Sali guava
leaves.

Appendix D figure 4 The solution extracted from the Local Thai guava
leaves, Bangkok Apple guava leaves and Glom Sali
guava leaves.

Appendix D figure 5 The clear zone occurred on TSA broth growth *S. aureus* by addition of antimicrobial growth extracted from guava leaves.