Effect of Anti-Acne Gel from *Aloe vera* and Samrong on *Staphylococcus aureus*.

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Ms. Kanyapak Sapsanyakorn

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A special project is submitted to the Faculty of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Bachelor of Science

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By:	**Ms. Kanyapak Sapsanyakorn**
Advisor	**1. A. Pornpen Panjapiyakul**
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Level of study:	**Bachelor of Science**
Department :	**Agro-Industry**
Faculty :	**Biotechnology**
Academic Year :	**2005**

Pornpen Panj. Advisor,

(A. Pornpen Panjapiyakul)

Advisor,

(Dr. Viyada Kunathigan)

Abstract

The aim of this project was to formulate *Aloe vera* and Samrong anti-acne gel for inhibiting the growth of *S. aureus* which is a major cause of skin and soft tissue infection. The experiment was divided into two parts which were raw material preparation and gel formulation.

Three treatments of *A. vera* preparation was studied including manual chopped, blended, and blended and pasteurized. All treatments showed similar result which could inhibit 7.51×10^8 CFU/ml (46%) of *S. aureus*. Then blended treatment was chosen to prepare *A. vera* for gel formulation.

Samrong concentration was varied into four levels consist of 25%, 50%, 75%, and 100% of Samrong gel. It was found that 75% Samrong has the highest antimicrobial activity. However 25% Samrong was chosen to formulate *Aloe vera* and Samrong anti-acne gel because the color is the most suitable to use in gel.

The plain gel formulation was done in order to determine suitable amount of water content in *Aloe vera* and Samrong anti-acne gel so 40ml, 60ml, 80ml, and 100ml water were studied. There was no significant difference among treatments at $p>0.05$. Then water at 100ml was chosen.

Aloe vera and Samrong anti-acne gel was formulated by varying three levels of samrong concentration which were 5%, 15%, and 25% of Samrong. *A. vera* gel was added in the formula as 20% *A. vera*. Moreover, the effect of parabens to *Aloe vera* and Samrong anti-acne gel was studied and showed no differences to the control. *Aloe vera* and Samrong anti-acne gel containing 5% Samrong was the most preferred by panelists.

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Lastly, I would like to thank my family and all friends who I hold dear. I could not have done without them.

Ms. Kanyapak Sapsanyakorn

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Abbreviation

AFI	=	Retentate fraction of *A. vera*
AFII	=	Filtrate fraction of *A. vera*
ASAG	=	*A. vera* and Samrong Anti-acne Gel
MAG	=	Mucilaginous *A. vera* Gel
MSG	=	Mucilaginous Samrong Gel
NA	=	Agar Diffusion Method
NB	=	Broth Culture Method
SFI	=	Retentate fraction of Samrong
SFII	=	Filtrate fraction of Samrong from the first filtration
SFIII	=	Filtrate fraction of Samrong from the second filtration (like MSG)

Introduction

The rapid growing of natural skincare market has increased in the last decade (House, 2003). The utilization of chemical or synthetic components is reducing. The plants are considered to substitute those chemical agents. Nowadays, most people want to go back to the original which means they prefer natural product based on the idea that natural product has less side effect. There are many plants that are commonly added in the cosmetic products such as *Aloe vera*, tumeric, cucumber, etc. Each plant has the unique properties and characteristics so it can be used in cosmetic products in the different forms and different ways. The tumeric is currently used in the formulation of some sunscreens and skincare products. (Anonymous, 2006[b]) Substances in cucumbers help to reduce the swelling around eyes or the bags under the eyes. Cucumbers, then are used to improve the hydration of skin. (Anonymous, 2006[b]) *A. vera* is also popular as moisturizer, healer, antiinflammation and antimicroorganism in cosmetic products. Reynolds and Dweck (1999) reviewed *A. vera* can inhibit growth of *Bacillus subtilis, Mycobacterium tuberculosis,* and *Streptococcus faecalis* while *Staphylococcus aureus* and *Escherichia coli* were still had controversy. However, Orafidiya *et al.* (2004) reported that *A. vera* gel can enhance the anti-acne properties of *Ocimum* oil. Recently, Samrong or Malva nut antimicrobial activity on *S. aureus* has been reported. (Hoonsaard, 2005) Traditionally, Samrong has been used as beverage or dessert products. Alternatively, Samrong should be used as another natural antimicrobial agent in cosmetic product. This is a new application in the market and would be able to increase the value of Samrong. In last decade, the novel natural cosmetic products use the combination of many natural ingredients to gain multiple cosmetic or synergetic effects. Therefore, the application of plants' combination is attractive and may provide the new and better products. In this study, *A. vera* and Samrong were tested for their antimicrobial activity to produce novel acne gel.

Literature review

Skin and soft tissue infection is not very severe but it has the psychological effect which is a huge concern, especially in developing personalities. *S. aureus* is a microorganism which involves in the skin and soft infection. In this study, *A. vera* and Samrong are used as raw material inwhich both of them may exert contribution to the effectiveness of the *A. vera* and Samrong anti-acne gel (ASAG).

Acne (Anonymous, 2006[g])

Acne is the medical word used to describe anything from the occasional blemish through the entire spectrum of pimples, blackheads, whiteheads, nodules and large cysts. Acne is a skin condition that occurs when excess oil (sebum) production combined with dead skin cells clog your pores. Then, bacteria forms in the clogged pores resulting in red inflamed pimples, pus filled whiteheads, or blackheads. Acne is extremely common. So common that it's considered a normal part of growing up

There are three basic elements that can cause acne which are hormones, plugged pores, and bacteria. An increasing in testosterone hormones causes sebaceous glands to over-produce sebum (oil) which makes skin oily. When the duct of the oil gland (sebaceous gland) or the pore becomes blocked by skin cells, this leads to the production of comedones (whiteheads or blackheads). *Propionibacterium acnes* is the presented bacterium in skin pores. Once pore gets blocked, the *P. acnes* bacteria will infect the sebaceous gland as well as the pore. *P. acnes* thrives on the sebum and creates the large infections within the skin.

Skin and soft tissue infections (Danziger *et al.*, 1992)

The conditions that may predispose a patient to the development of skin infections including; a high concentration of bacteria ($>10^5$ microorganisms), excessive moisture of the skin, inadequate blood supply, availability of bacterial nutrients, and damage to corneal layer allowing for bacterial penetration.

The majority of skin and soft tissue infections are resulted from the disruption of normal host defenses by processes such as skin puncture, abrasion, or underlying diseases. The nature and severity of the infection depends on both the type of microorganisms present and the site of inoculation. A large percentage of these infections are caused by normal

skin flora and the exposed area of the body, such as face, neck, which generally have the highest bacterial density.

The types of skin and soft tissues infections by *S. aureus* include folliculitis, impetigo, and pimples. Follicutis is a superficial infection surrounding the hair follicles. This condition is commonly referred to as a stye when it occurs at the base of the eyelid. Most cases of folliculitis are caused by *S. aureus*. Impetigo is another distinct type of superficial cellulites that is caused by *S. aureus* and/or group A streptococci (*S. pyogenes*). This superficial skin infection is most common during hot, humid weather, which facilitates the microbial colonization of the skin. Minor trauma, such as scratches or insect bites, then allows entry of organisms into the superficial layers of skin, and infection ensues. Impetigo occurs most commonly in children. Pimples are a small solid rounded bump rising from the skin that is usually less than 1 cm in diameter. They may open when scratched and become crusty and infected. Dermatologists call any small solid circumscribed bump in the skin a papule, as opposed to a vesicle which contains fluid or a macule which is flat and even with the surrounding skin. (Anonymous, 2006[c])

Pimples are known as acne in the state of numerous pimples. The preliminary stage of a pimple is the comedone. In addition to the large comedons, it is small rascals as well, which is called micro comedons. (Anonymous, 2006[i])

Staphylococcus aureus (Madigan *et al.*, 2003)

S. aureus are facultative aerobic gram-positive cocci which are relatively resistant to reduce water potential and tolerate drying and high salt fairly well. Their ability to grow in media with high salt provides a selective means for isolation. This organism is pigmented which aids in selecting *S. aureus*. It is a yellow pigmented species that is most commonly associated with pathological conditions, including boils, pimples, pneumonia, osteomyelitis, meningitis, and arthritis.

S. aureus can be represented as the normal flora of humans on the skin, in the respiratory tract, gastrointestinal tract, and urogenital tract. When bacteria contact host tissues at mucous membranes, they may associate either loosely or firmly. If *S. aureus* associate loosely with the mucosal surface, they are usually swept away by physical processes, but they may also adhere to the epithelial surface as a result of specific cell-cell recognition between pathogen and host. From there, actual tissue infection may follow. When the infection occurs, the mucosal barrier is breached, allowing the pathogen to invade deeper tissues.

Nowadays, there are many anti acne product. The active ingredient is that generally used are chemical such as sodium salicylate (Anonymous, 2006[f]), salicylic acid (Anonymous, 2006[k]), and benzoyl peroxide (Anonymous, 2006[h]). There are many forms of cosmetic products such as gel, cream, lotion, and foam but the gel form was chosen for the formulation because its process is quite easy and requires less chemical agents.

Gel (Allen *et al.*, 2005)

Gels are defined as semisolid systems consisting of dispersions made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by a liquid. Gels are also defined as semirigid systems in which the movement of dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. A high degree of physical or chemical cross-linking may be involved. The increased viscosity caused by interlacing and consequential internal friction is responsible for the semisolid state. A gel may consist of twisted matted strands often wound together by stronger types of Van Der Waals forces to form crystalline and amorphous regions throughout the system.

1. Classification and types of gels

There are two classification schemes (table 1). The first scheme divides gels into inorganic and organic. Most inorganic hydrogels are two-phase system, such as aluminum hydroxide gel and bentonite magma while most organic gels are single- phase systems and may include such gelling agents as carbomer and tragacanth and those that contain an organic liquid, such as plastibase.

The second classification scheme divides gels into hydrogels and organogels with some additional subcategories. Hydrogels include ingredients that are dispersible as colloidals or soluble in water; they include organic hydrogels (e.g. pectin paste, tragacanth jelly), natural and synthetic gums (e.g. methylcellulose, sodium carboxymethylcellulose), and inorganic hydrogels (e.g. silica, bentonite gel) while organogels include hydrocarbons (e.g. mineral oil, polyethylene gel), animal and vegetable fats, soap base greases, and the hydrophilic organogels (e.g. carbowax bases).

Table 1: General classification and description of gels

Class	Description	Examples
Inorganic	Usually two-phase systems	Aluminum hydroxide gel
		Bentonite magma
Organic	Usually single-phase systems	Carbopol
		Tragacanth
Hydrogels jelly	Organic hydrogels	Pectin paste, Tragacanth
	Natural and synthetic gums	Methylcellulose, sodium carboxymethylcellulose, Pluronic
	Inorganic hydrogels	Bentonite gel (10-25%), Veegum, silica
Organogels	Hydrocarbon type	Petrolatum, mineral oil/ polyethylene gel (Plastibase)
	Animal, vegetable fats	Lard, cocoa butter
	Soap base greases	Aluminum stearate with heavy mineral oil gel
	Hydrophilic organogels	Carbowax bases (PEG ointment)
	Polar	
	Nonionic	

From Allen *et al.*, 2005

2. Characteristic of gels

The characteristics of gels include imbibition, swelling, syneresis, thixotropy, and xerogel. Imbibition is the taking up of a certain amount of liquid without a measurable increase in volume. Swelling is the taking up of a liquid by a gel with an increase in volume. Only liquids that solvate a gel can cause swelling. The swelling of protein gels is influenced by pH and presence of electrolytes. Syneresis occurs when the interaction between particles of the dispersed phase becomes so great that on standing, the dispersing medium is squeezed out in droplets and the gel shrinks. Syneresis is a form of instability in aqueous and nonaqueous gels. Thixotropy is a reversible gel-sol formation with no change in volume or temperature, a type of non-Newtonian flow. Xerogel is formed when the liquid is removed from a gel and only the framework remains.

Moreover, the commercial gel products, especially skin gel, should have the followings properties. Gel is water-soluble. After applying gel on the skin, gel is easily absorbed and then water evaporates so the applicants feel cool but not oily. After that, there is a thin film coating on the skin in order to prevent the skin from the environment. It acts as moisturizer for a skin. pH of gel is usually 5.5 to 6.5 which is similar to the pH of the skin. The gel is thixotropic which is flow by agitating or shaking and then it becomes semi-solid or solid state after standing undisturbed.

3. Standard formula of gel

3.1	Carbopol 940	2 g
3.2	Triethanolamine	1.65 ml
3.3	Parabens	0.2 g
3.4	Purified water	100 ml

- Glycerine is optional to help dispersing carbopol 940 or gelling agent in solution.

4. Ingredients in gel

4.1 Gelling agent

There are many types of gelling agent such as acacia, alginic acid, bentonite, carbomer, sodium carboxymethylcellulose, gelatin, guar gum, hydroxypropyl cellulose, methylcellulose, propylene glycol alginate, sodium alginate, starch, tragacanth, and xanthan gum. However, carbomer group is commonly used in commercial cosmetic product.

Carbomer (carbopol) resins are ingredients in variety of dosage systems, including controlled-release tablets, oral suspensions, and topical gels. Carbomer resins are high-molecular-weight allyl pentaerythritol-cross-linked acrylic acid-based polymers modified with C-10 and C-30 alkyl acrylates. They are fluffy white dry powders with large bulk density. The 0.5% and 1.0% aqueous dispersions are pH 2.7 to 3.5 and 2.5 to 3.0, respectively. Carbomer 940 is the one type of carbomer which forms sparkling clear water or hydroalcoholic gels. It is the most efficient of all the carbopol resins and has very good nondrip properties.

The addition of alcohol to prepare carbomer gels may reduce their viscosity and clarity. An increasing of the concentration of carbomer may be required to overcome the loss of viscosity. Also, gel viscosity depends on the presence of electrolytes and pH. Too much neutralization will result in decreased viscosity that cannot be reversed by the addition of acid. Maximum viscosity and clarity occur at pH 7, but acceptable viscosity and clarity begin at pH 4.5 to 5.0 and extend to a pH of 11.

Carbomer preparations are primarily done in aqueous systems, although other liquids can be used. In water, a single particle of carbomer will wet very rapidly, but like many other powders, carbomer polymers tend to form clumps of particles when haphazardly dispersed in polar solvents. To achieve fastest dispersion of the carbomer, it is wise to take advantage of the very small particle size of the carbomer powder by adding it very slowly into the vortex of the liquid while very rapidly stirring it. Almost any device, like a simple sieve, that can sprinkle the powder on the rapidly stirring liquid is useful. The goal is to prevent lumping by slowly sprinkling the very fine powder over the rapidly agitated water.

A neutralizer is added to thicken the gel after the carbomer is dispersed. Triethanolamine will neutralize carbomer resins containing up to 50% ethanol.

4.2 Triethanolamine (Anonymous, 2006[b])

Triethanolamine often abbreviated as TEA, is an organic chemical compound which is both a tertiary amine and a tri-alcohol. A tri-alcohol is a molecule with three hydroxyl groups. Like other amines, triethanolamine acts as a weak base due to the lone pair on the nitrogen atom. This ingredient is used as a pH balancer in cosmetic preparations in a variety of different products - ranging from skin lotion, eye gels, moisturizers, shampoos, shaving foams etc.

4.3 Preservatives

Preservatives are the substances which can inhibit microbial growth. Contamination may occur in the products via the ingredients or during the production. There are many types of preservatives such as chlorhexidine, chlorobutanol, chlorocresol, paraben, phenol, and benzoic acid. However, paraben is commonly used in commercial product and it is suitable for using with carbomer.

Parabens is very popular in the production of cosmetics and phamaceuticals. It is usually a mixture of methylparaben (MP) and propylparaben (PP). MP and PP are used mostly because they are effective, good aroma, less poison, and appropriate to many substances. The efficiency of mixture between MP and PP is better than individual because MP can suppress the growth of fungi and PP is for suppressing yeast. The limitation of parabens is its low solubility in water. If the length of alkyl group increases, the effective of preservative also increases but solubility of parabens decreases. Parabens is stable and active at pH 4 to 8.

4.4 Glycerine (Anonymous, 2006[b])

It is humectant, plasticizer, emollient, thickener, solvent, dispersing medium, lubricant, sweetener, bodying agent, antifreeze and processing aid.

5. Method for making gels

Gel can be made by two methods. The first, parabens are dissolved by 95 ml purified water under heating condition. The mixture is cooled to room temperature and then carbopol 940 is added slowly and mixed at high speed until the solution is homogenous. The mixture is stood in order to let the air bubble vapored out of the mixture. Triethanolamine is added drop by drop while stirring it in order to prevent air bubble in the gel. The remaining water is then added. The second method, carbopol 940 is mixed with glycerine thoroughly. Then water is added slowly to dissolve the mixture. The solution is stirred until it becomes homogenous. The triethanolamine is added drop by drop while stirring the solution.

6. Evaluation of gel

The gel can be evaluated by physical and microbial testing. The physical testing is to measure the viscosity, pH, and stability. The characteristics of commercial gel are 10,000 cps, pH 5.5-6.5, and no separation of gel. However, the viscosity depends on the type of gelling agent, raw materials and users' satisfaction. The clear zone is used to check the capability of gel for anti-acne together with growth in liquid media. Moreover, the sensory characteristics are also tested by human in order to check the satisfactory of formulated gel, fragrance, and viscosity.

In addition, the gel is designed to be absorbed quickly through the skin after application on the arms, shoulders or abdomen. The formulation should not cause irritation or occlusion of the skin after the application.

Due to the physicochemical properties of the excipients used in the gel formulation, and specifically due to the solubilization property, a wide range of active agents can be formulated with the gel. The solubilization properties allow higher concentrations of the active ingredient or, as in the case of the hormone replacement therapy (HRT) transdermal gel already developed, they allow an increase in the water concentration of the formulation. The increased water concentration leads to a reduction in viscosity of the formulation, facilitating the application, and spreading of the gel by the patient, while providing a better cosmetic appearance. Therefore the higher water content results in good moisturizing properties. (Anonymous, 2006[j]) *A. vera* and Samrong were chosen for formulation of anti-acne gel due to their antimicrobial activities.

Aloe vera

A. vera L. (*Aloe barbadensis Miller*) is a member of liliaceae family. There are more than 300 species of *A. vera*. *A. vera* L. is most widely accepted and used for various medical, cosmetic and nutraceutical purposes. The plant is made of elongated and pointed leaves. (Ni *et al.*, 2004) Moreover, *A. vera* is a succulent plant and also xerophytes, which are adapted to live in areas of limited water supply and are characterized by possessing a large water storage tissue. Therefore *A. vera* is the water storage tissue. (Reynolds and Dweck, 1999; Vfizquez *et al.*, 1996)

The components of *A. vera* is categorized into the group of vitamins, enzymes, minerals, sugar, anthraquinones, lignin, saponins, fatty acids, salicylic acid, and amino acids (Atherton, 1997)

The fresh leaves of *A. vera* are used to obtain two components, including exudate and mucilaginous gel. Exudate is a bitter yellow juice with high content of 1,8 dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used for their cathartic effects. Most compounds are of phenolic group. The exudate is extracted from the vascular bundles at the junction between the rind and the fillets. Mucilaginous Aloe gel (MAG) has been described using several other terms including inner gel, mucilaginous gel, inner clear pulp from leaf parenchyma. It is a clear pulp and from the parenchymatous tissue, which has been used for topical treatment of skin burns and wounds. There are various polysaccharides in the mucilaginous gel, mainly mannan, galactan, arabinan, arabinorhamnogalactan, pectic substance, and glucuronic acid-containing polysaccharide. Acemannan, acetylated glucomannan, is the majority of the MAG. Acemannan has been studied and found to have effect wound closure in chronic wounds, aphthous ulcers, and reduction of dry socket associated with third-molar extraction sites. Moreover, acemannan has been shown to inhibit AIDS virus replication *in vitro*, and also found to be of significant benefit in FIV-infected cats. In addition, protein is the one component which has biological activities such as Aloctin which was studied in *Aloe arborescens*.

The study of *A. vera* is very popular and it is interested in the use of the gel which has increased dramatically. In many countries it is now a familiar ingredient in a range of healthcare and cosmetic products widely available and advertised in shops. The preserved but otherwise untreated gel is also sold as a therapeutic agent in its own right as are various concentrated, diluted and otherwise modified products.

The benefits of gel are quite a lot so the research concerned with application of MAG and its efficiency are reported continuously. Most of the scientists try to determine the alternative applications due to many gel constituents, their possibilities of usage could be broader. Moreover, the MAG has beneficial effect on wound healing, burn healing, various internal inflammatory conditions (treatment of peptic ulcers), antidiabetic activity, anticancer activity, antiinflammation activity, and antimicrobiological activity. (Reynolds and Dweck, 1999)

A. vera has antiinflammation activity resulted from bradykinase, enzyme, which helps to reduce excessive inflammation when applied to the skin topically and therefore reduces pain, whereas others help digest any dead tissues in wounds. Lipases and proteases which break down foods and aid digestion are present. (Atherton, 1997)

There are many reports shown the positive antibacterial effects of MAG on the growth of a wide range of bacterial such as *Streptococcus pyogenes, Citrobacter sp., Serratia marcescens, Enterobacter aerogenes, Enterobacter sp., Bacillus subtilis, Klebsiella pneumoniae, Klebsiella sp., Candida albicans phagocyte, Mycobacterium tuberculosis, Corynebacterium xerose, Salmonella paratyphi, Pseudomonas aeruginosa S., Proteus vulgaris, Streptococcus faecalis* while *Staphylococcus aureus* and *Escherichia coli* were still have controversy. (Reynolds and Dweck, 1999)

MAG can be prepared in many ways, including: the first method, MAG was harvested from the green leaves, minced, homogenized, dried at 35°C and weighed. (Vfizquez *et al.*, 1996). Second, the leaves were weighed and cut then MAG was scraped out. Subsequently, this jelly tissue was homogenized in a blender for 1 minute. Each leaf produced about 115 ml of gel. The resulting mixture was centrifuged for 30 min at 6400 g at 4°C to remove dense debris. The supernatant (native gel) was dialysed (molecular weight cut-off value 10,000) against water in a 1:10 volume ratio at 4°C for 24 hours. The dialysable material (low molecular weight fraction, LMWF) was collected, lyophilized and kept at -20°C. The non-dialysable fraction (purified gel) was refrigerated at 4°C. (Avila *et al.*, 1997): and heat during pasteurization is one of the stresses imposed on the gel and there are advantages in using high temperatures for short times preferably with the addition of an antioxidant such as ascorbic acid. Mucopolysaccharide integrity during storage was found to be preserved by the addition of other natural polysaccharides which act synergistically. (Reynolds and Dweck, 1999)

There are some tips for using *A. vera* in the production of cosmetic gel, suggested by Sanooksan (2000). The first is aseptic condition which is required for preparing uncontaminated MAG from extraction step until packing. Secondly, *A. vera* cosmetic gel should be prepared with mixer under vacuum condition (vacuum mixer) in order to reduce bubble formation which can cause gel instability. The last tip suggested is *A. vera* cosmetic gel should be packed under vacuum condition and the surface of gel should not be in contact with air because aerobic condition will encourage microbial growth.

The percentage of *A. vera* of in the cosmetic products should be more than 20%. The levels more than 20% *A. vera* can enhance the function of the product. If the product contains less than 5% *A. vera*, it is really immaterial. Most products containing *A. vera*, consist of *A. vera* between 20%-40% although the percentage of *A. vera* gel nearing 100% are both stable and highly effective. (Sanooksan, 2000, and Anonymous, 2006[d])

Samrong (Hoonsaard, 2005)

There are many names for Samrong such as *sterculiaceae*, *scaphium macropodum beaum*, and *sterculia lychnophora*. The characteristic of Samrong seed is 2-3 cm long and 1-1.5 cm in diameter. Apex obtuse-rounded, base somewhat acute and oblique, bearing a pale and round hilum. It is brown or dark brown with irregular wrinkles. The seed swells to spongy-like when treated with water. The seeds are harvested when the fruit ripens and cracks in April to June. The seeds are dried in the sun for later use. The source of Samrong is in the Eastern part of Thailand, Vietnam, Malaysia, Indonesia, and Southern part of China.

Samrong seed consists of four layers which are skin, mucilage layer, coated shell, and endosperm. Skin, fresh fruit is pale green and it will be brown, shrunk, and attach the mucilage layer after roasting or drying under sunlight. Mucilage has light brown layer with the black spot and fragile in the dried seed. It forms spongy structure like jelly after rehydration. Coated shell is a thin, brown color layer attached to inner seed endosperm. It does not absorb water. Seed endosperm is yellow brown color.

Samrong has many medicinal properties such as removing heat from the lung, curing sore throat, counteracting toxicity, and relaxing the bowels. It is usually used as dessert which is obtained by soaking whole fruit in the water for a few hours or overnight, and mucilage is mixed with sugar or syrup.

Moreover, the antimicrobial activity of Samrong was discovered by Hoonsaard in 2005. The flesh, which is the mucilage part, of Samrong could inhibit the growth of *S. aureus* but had no antimicrobial activity when tested with *Escherichia coli*. Hoonsaard studied the antimicrobial property of Samrong using two extraction methods. She had also compared the antimicrobial activity of Samrong treated with different boiling time and different cultivation years. The extraction method was that Samrong seed was treated by soaking in distilled water at seed to water ratio of 3.75: 100 (w/v) for 30 minutes. The mixture was boiled for

90 minutes. Then the samples were filtered with cheesecloth to separate liquid and flesh. The result showed that the boiling time and cultivation years did not affect the antimicrobial property of Samrong.

ASAG could be the alternative cosmetic product for consumers. The information of consumer acceptance and preference is required for development of ASAG's formula to satisfy the consumer. Therefore the sensory analysis of ASAG is conducted in this project in order to know the direction of formulation. Moreover, the sensory analysis can present the possibility of ASAG success in the market.

The word natural has penetrated every aspect of everyday life, including cosmetics. Natural means growing without human care; not cultivated; existing in or produced by nature: not artificial; relating to or being natural food; relating to, produced by or according to nature, to the natural world or human nature; and provided by or based on nature as described by House (2003). The cosmetics market place and the focus groups demonstrate that the natural trend is here to stay.

The key opportunities for natural products were revealed in four groups of people. First, older affluent women, whose children had left home, respond to a natural and luxurious positioning, particularly if it carries a promise of making them feel better and hence look better. The second group was women of all ages who were into their 'lifestyle'. A natural and holistic approach attracts these women, especially where there is an interest in complementary health and in overall well being. Younger consumers (15-18) were the third group. These teenagers enjoy an 'on the go' natural approach. The forth group was natural milimalists, who seek out designer minimalism and ultimate quality with a natural image. (House, 2003)

The objectives of the experiment were to examine antimicrobial properties of *A. vera* and Samrong on *S. aureus*; to formulate the *A. vera* and Samrong acne gel (ASAG); to examine antimicrobial activity of ASAG; and to examine the sensory analysis of ASAG.

Equipments and reagents

I. Equipments

- Analytical balance (Ohause, Analytical plus AP 210s)
- Autoclave (Hirayama, Model HA 300 M II)
- Incubator (Jouan, EB 280)
- Laminar Flow (Dwyer Mark II, "Clean" Model H2)
- Microscope (Nikon, SMZ-1)
- Spectrophotometer (Spectronic, GENESYS 5, Milton Roy)
- Water bath shaker (Clifton)

II. Reagents

- Nutrient agar (NA)
  - Agar powder
  - Beef extract
  - Peptone
- Nutrient broth (NB)
  - Beef extract
  - Peptone

III. Microorganisms

- *Staphylococcus aureus*

IV. Plants

- *Aloe vera* (*Aloe barbadensis Miller*)
- Samrong (*Sterculiaceae*)

Procedure

Experiment 1: Raw material preparation and antimicrobial testing

1.1 *A. vera*

Preliminary experiment in *A. vera*

Preliminary experiment was conducted in order to determine the proper sanitizer and its concentration for reducing native microorganisms. *A. vera* leaf was treated with one of the following sanitizers: 70% Ethanol for 5 minutes and 5% Clorox for 10 minutes following the standard method in tissue cultures treatment for sterilizing leaves. After treating with sanitizer, leaf was washed twice with water.

After cleaning leaf shell was peeled and separated MAG which was chopped manually and filtered by cheesecloth. The retentate was called *A. vera* Fraction I (AFI) and the filtrate fraction was called *A. vera* Fraction II (AFII). Both fractions were tested in agar diffusion (NA) and broth culture (NB) methods.

Agar diffusion, the culture was swabbed onto NA. Then, 2g AFI was placed onto NA while the 6mm diameter filter disk was dipped in AFII and placed onto NA. It was incubated at 37°C for 24 hours. The clear zone was checked.

Broth culture, the culture of *S. aureus* was inoculated into 5ml double strength NB with either 2g AFI or 2ml AFII. It was incubated at 37°C for 48 hours and OD_{650} was measured at 0, 24, and 48 hours.

Experiment 1.1: Variation of condition to prepare MAG

After treating with sanitizer, MAG preparation was done with three methods include manual chopped (treatment 1), blended with blender (treatment 2), and blended and pasteurized at 75°C for 15 seconds (treatment 3). Each sample was filtered by cheesecloth and collected AFII to determine the antimicrobial activity by NA and NB. This experiment was done in five replications and three repeats in each replication.

1.2 Samrong

Preliminary experiment in Samrong

3.75g Samrong was soaked in 100ml distilled water for 30 minutes. It was boiled at 100°C for 90 minutes, and then was filtered by cheesecloth. The retentate was called Samrong fraction I (SFI). The clear filtrate solution was called Samrong fraction II (SFII). Each fraction was tested with NA and NB.

Experiment 1.2: Variation of Samrong concentration

SFI was filtered again in order to remove impurities and the filtrate in this step was called Samrong fraction III (SFIII), which like mucilaginous Samrong gel, for improving the absorption ability when apply anti-acne gel on skin. Four levels of Samrong concentration: 25%, 50%, 75%, and 100% (v/v) was studied. SF III of each concentration was tested with agar diffusion and broth culture but checked OD_{650} at 0, 24, 36, and 48 hours. This experiment was done in triplicate and three repeats in each replication.

Experiment 2: Gel formulation and antimicrobial testing

2.1. Plain gel formulation

Experiment 2.1: Variation of water in plain gel.

The standard formula of gel, mentioned in literature review, was used. Carbopol 940 was mixed with glycerine until carbopol was clear, and then water was gradually added. After the carbopol was completely dissolved in water, 1.86ml triethanolamine was added drop by drop with continuous stirring and adjusted pH to 7. The variation of water was varied into four levels which were 40, 60, 80, and 100 ml.

Then, the quality of plain gel was tested by using sensory analysis. The 30 panelists were asked to evaluate three important attributes which were viscosity, absorbency, and overall appearance of 4 different plain gel by using affective test (9-point hedonic scale). The collected data were analyzed by multivariate analysis

2.2 *A. vera* and Samrong gel formulation

Experiment 2.2.1: Variation of Samrong concentration in ASAG

ASAG was processed as described in experiment 2.1 but 20% (v/v) AFII was added with 5% (formula 1), 15% (formula 2), or 25% (formula 3) (v/v) SFIII, instead of water. This experiment was done in triplicate and three repeats were in each replication. All samples measured the antimicrobial properties by NA and NB.

Experiment 2.2.2: Determination of the effect of parabens

This experiment was done in the same way as in experiment 2.1.1 with an additional of 0.2% parabens in all samples. This experiment was repeated twice and three repeats were done in each replication. All samples were measured their antimicrobial properties by NA and NB and sensory analysis. The 30 panelists were asked to evaluate three important attributes which were brown color, spreadability, absorbency, stickiness, and overall appearance of three different ASAG by using affective test (9-point hedonic scale). The collected data were analyzed by multivariate analysis.

Notes:

- The aseptic condition was required in all experiments.
- The culture preparation was done following the method described by Hoonsaard (2005) but the water bath shaker was used instead of room temperature shaker at the step of culture reactivation.
- The sensory analysis of gel was done by applying gel on the skin in limited area (0.5 cm in diameter). The gel was circulated for 15 times after that each attribute was evaluated by preference testing.

Results and discussion

The experiment was conducted in order to study the antimicrobial activity and formulation of ASAG. The methods were described as NA and NB. The antimicrobial activity of NA will result in visible clear zone in agar diffusion. The antimicrobial activity in NB was shown as number of colony forming unit (CFU/ml) over time (h) from broth culture.

Experiment 1: Raw material preparation and antimicrobial testing

1.1 *A. vera*

Preliminary experiment in *A. vera*

The microbial growth of both fractions from sample treated with 70% (v/v) ethanol was higher than control (*S. aureus*) (Figure 1). This indicated that 70% (v/v) ethanol was not effective to reduce native microorganisms. 5 % (v/v) Clorox was also used as it is commonly use for sanitizing leaf in tissue culture procedure. However, 5% (v/v) Clorox showed contamination in AFII (Figure 2) which could be contaminated from the chopping or filtering steps. In contrast, AFI showed slightly inhibition of microbial growth. However, the inhibition still cannot eliminate contamination. Then 15% Clorox was chosen for treating the leaf to ensure the sterility of samples.

Experiment 1.1: Variation of condition to extract MAG

According to NA result (Figure 3), AFII of all treatments did not show clear zone around the disks, which indicate inability to inhibit growth of *S. aureus*. The reason could be that the amount of active ingredients in *A. vera* on the disks might not be enough to inhibit the growth of *S. aureus*. Therefore, NB method was used and measured by single or double strength media, which increased the amount of active ingredients of *A. vera* when compared with NA method (Figure 4). The AFII result from NB showed that the control set (no inoculation) of treatment 1 which was prepared by manual chopping, had the least microbial growth while the control of treatment 3 had the highest microbial growth. Therefore, treatment 1 had the least contamination compared to the other two extraction methods. The result of *S. aureus* + treatment 1 mixture had the lowest microbial growth while the mixture of *S. aureus* + treatment 2 and treatment 3 had same microbial growth. Treatment 2 and 3 may be contaminated from blender or during the transfer of sample from blender to beaker. However, in later experiment the sterility of blender was done more carefully until the contamination

was reduced. Yet, there were no significant different in the growth of *S. aureus* in all treatments. Therefore, treatment 2 was chosen to prepare *A. vera* in gel formulation experiment because it gave the AFII quickly, highly and effectively when compared to all samples.

A. vera was the one of the two raw materials which was interesting to study its antimicrobial activity in cosmetic product. Samrong, another material of ASAG was also studied the antimicrobial activity too.

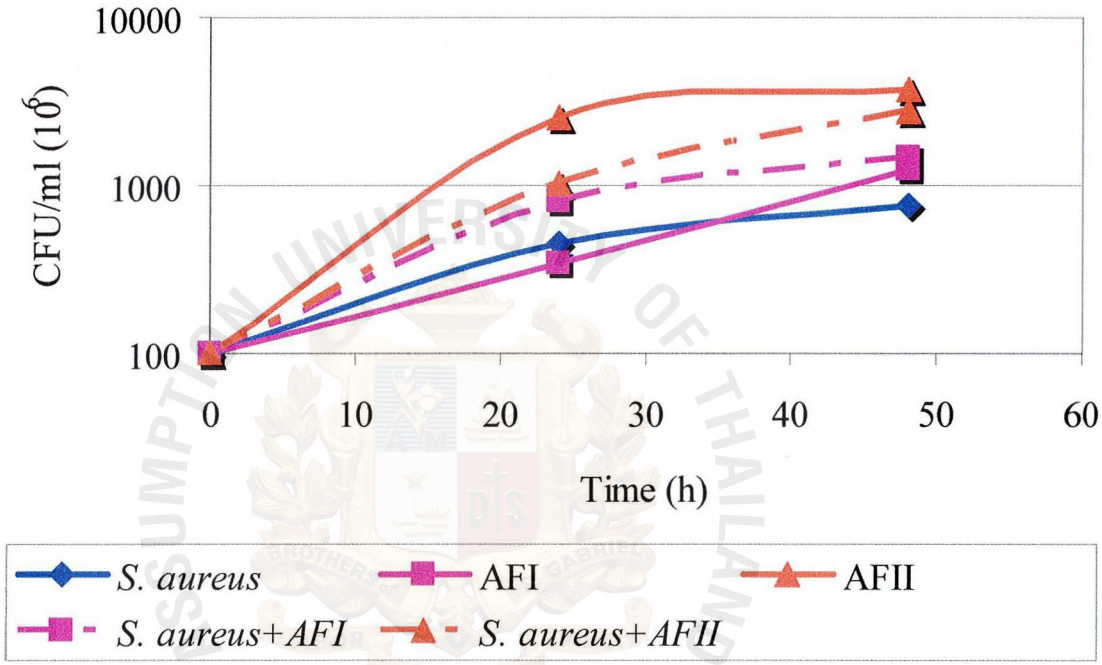


Figure 1: Growth of *S. aureus* in the NB methods using two fragments of *A. vera*, AFI and AFII, from sample treated with 70% ethanol.

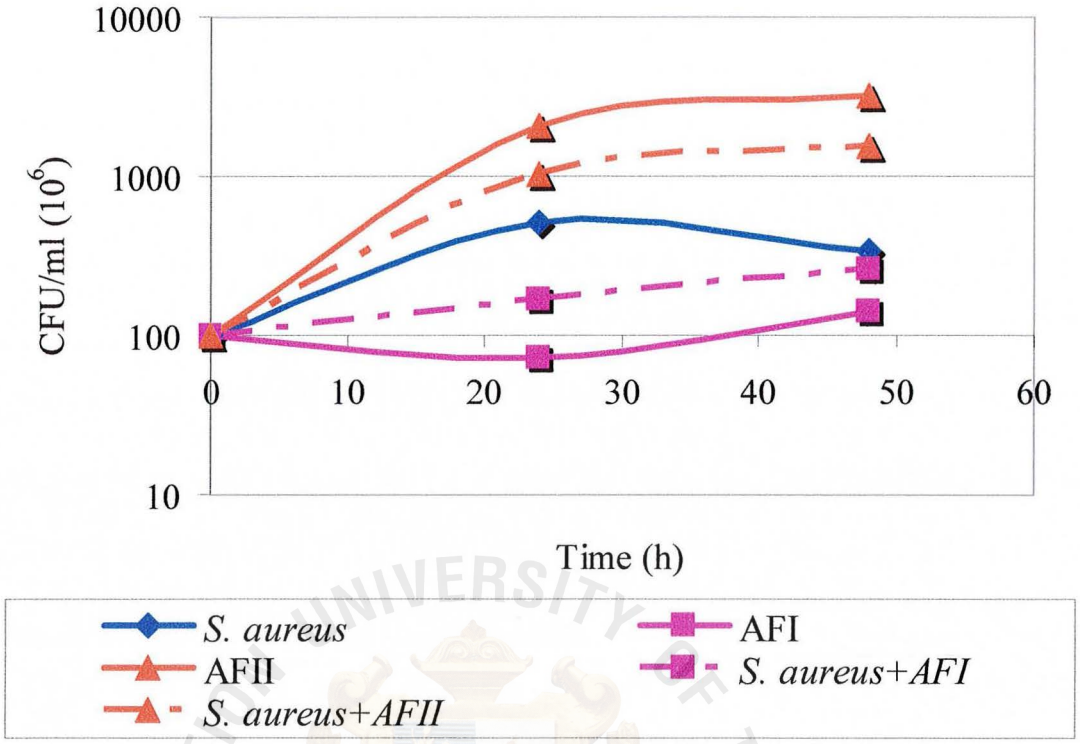
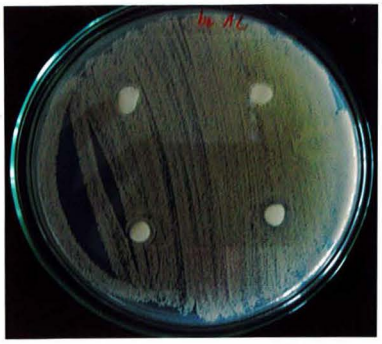
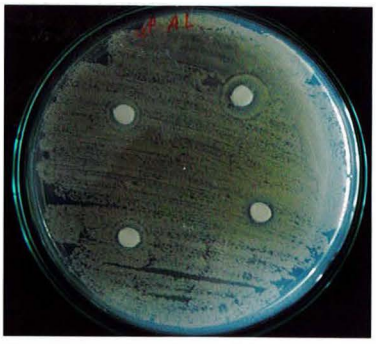


Figure 2: Growth of *S. aureus* in the NB methods using two fragments of *A. vera*, AFI and AFII, from sample treated with 5%Clorox.



Treatment 1: Manual chopped



Treatment 2: Blended by blender

Treatment 3: Blended by blender and pasteurized

Figure 3: Growth of *S. aureus* in the NA methods using three treatments of *A. vera*, AFII. Treatment 1-manual chopped, Treatment 2-blended by blender, and Treatment 3-blended and pasteurized.

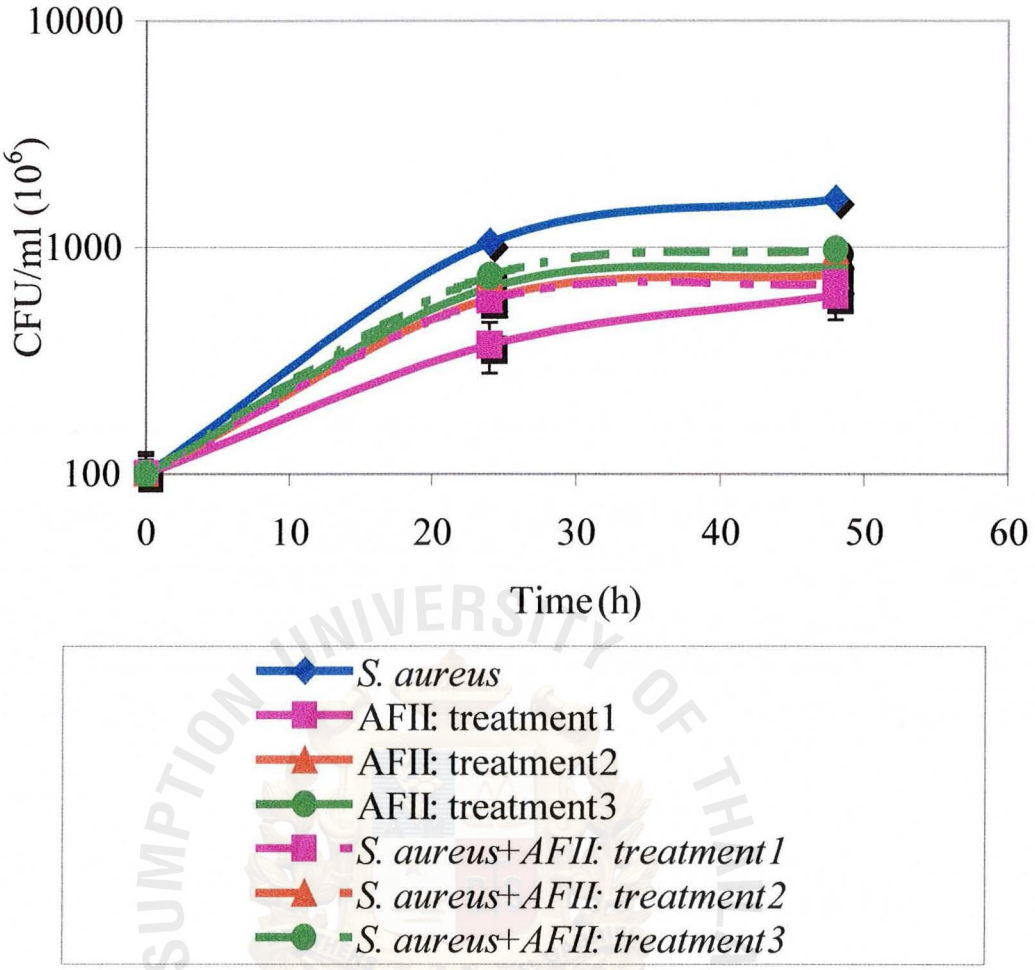


Figure 4: Growth of *S. aureus* in the NB methods using three treatments of *A. vera* AFII. Treatment 1-manual chopped, Treatment 2- blended by blender, and Treatment 3-blended and pasteurized.

1.2 Samrong

Preliminary experiment in Samrong

Both SFI and SFII showed antimicrobial activity on *S. aureus*. The result from SFI showed inhibition in both NA and NB while only NB showed inhibition for SFII. The result of broth culture was represented in figure 5, inwhich the microbial growth of all samples was less than the growth of *S. aureus*. Control of SFI showed constant microbial load while the microbial growth of control of SFII increased continuously. The microbial growth of SFI mixed with *S. aureus* was lower than SFII mixed with *S. aureus*. Therefore, SFI showed better antimicrobial activity toward *S. aureus* than SFII. The result from NA corresponded with earlier research (Hoonsaard, 2005) that there is no antimicrobial activity detected by agar diffusion. However, the result in NB suggested that amount of active ingredients using in NA method may not be adequate to inhibit growth of *S. aureus*.

Although, SFI had higher antimicrobial activity than the SFII, SFI was not the suitable form for making gel because it was in a solid form, which could cause problem in mixing step. Then the Samrong preparation was modified in order to get the MSG or SFIII. The Samrong was filtered twice in which the water was separated first and then the jellylike substance was squeezed by hand and designated as SFIII. SFIII was selected to use in further experiment.

Experiment 1.2: Variation of Samrong concentration

Then MSG concentration was varied with water to the final concentration of 25%, 50%, 75%, and 100% (v/v). The varied MSG showed positive result in both NA and NB. The result from NA showed no difference among the size of clear zone from four concentrations of Samrong, 25%, 50%, 75%, and 100% (v/v) (figure 6). Therefore, the conclusion was drawn that different concentration of Samrong did not affect the antimicrobial activity using NA method.

According to figure 7, when tested antimicrobial activity of Samrong using NB, there were significant reductions of microbial growth. The microbial growth of SFIII showed inhibition as followed: treatment 1 and 2 showed the similar growth at 48 hours, treatment 3 showed the least microbial growth, and the microbial growth of treatment 4 was higher than treatment 3 but less than treatment 1 and 2. Samrong concentration at 25% and 50% (v/v) showed similar antimicrobial activity on *S. aureus*.

When comparing results from both methods of antimicrobial testing, NA and NB, and the brown color of Samrong, 25% (v/v) Samrong was selected to use in ASAG formulation. The color of 25% Samrong was suitable because the intensity of brown color was less than the other four concentrations of Samrong; 25%, 50%, 75%, and 100% (v/v). The color of gel cosmetic product should be clear and light, which are commonly used and accepted. The dark color of gel was not attractive to buy and use.

After the antimicrobial activity from two raw materials was examined, the gel formulation was conducted and the sensory analysis was performed.

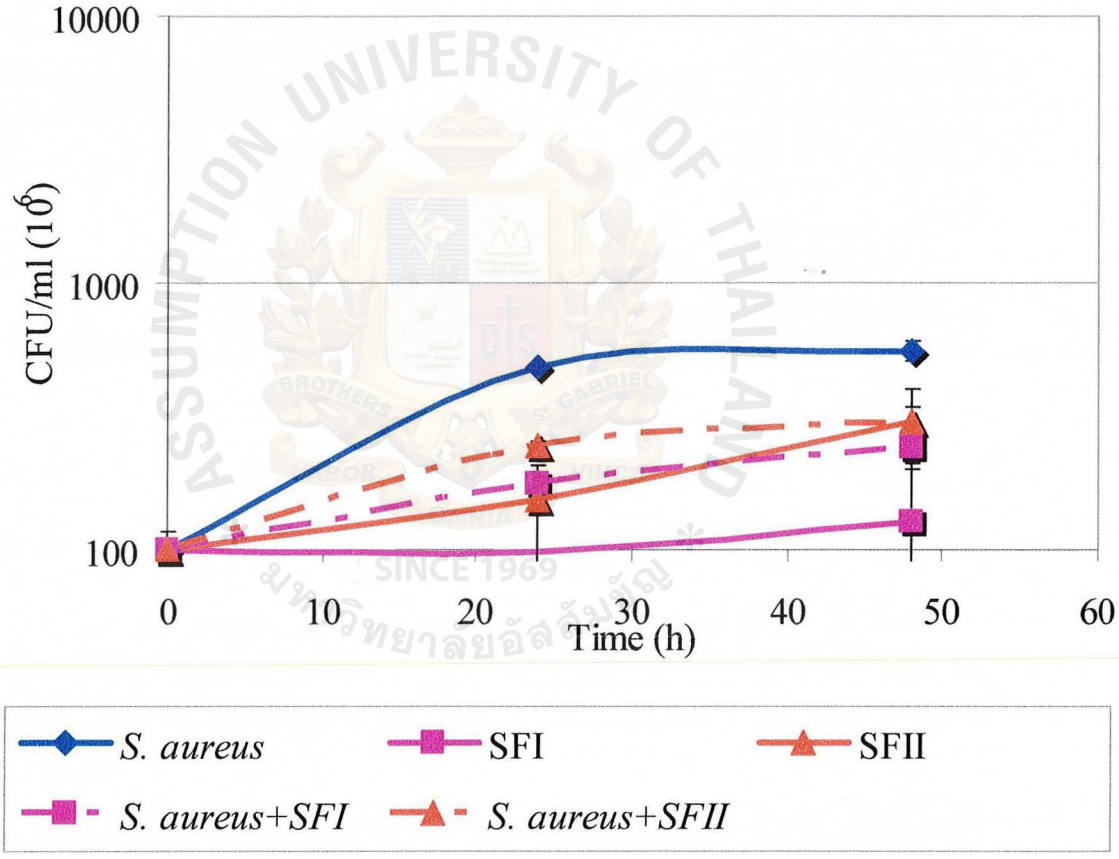


Figure 5: Growth of *S. aureus* in the NB methods using two fragments of Samrong, SFI and SFII.

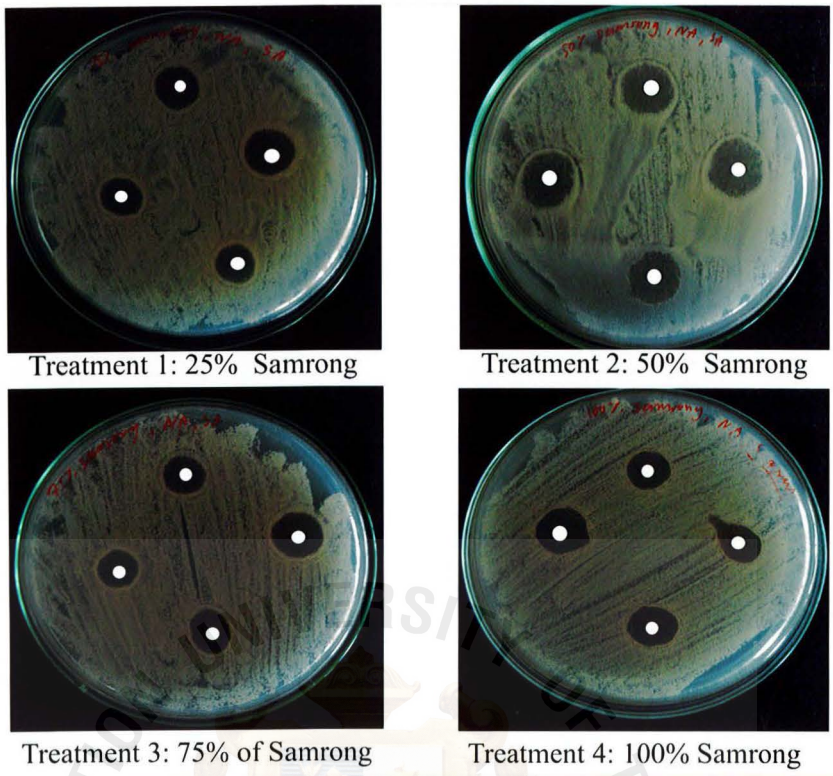


Figure 6: Growth of *S. aureus* in the NA methods using four treatments of Samrong, SFIII. Treatment 1-25% SFIII, Treatment 2-50% SFIII, Treatment 3-75% SFIII, and Treatment 4-100% SFIII.

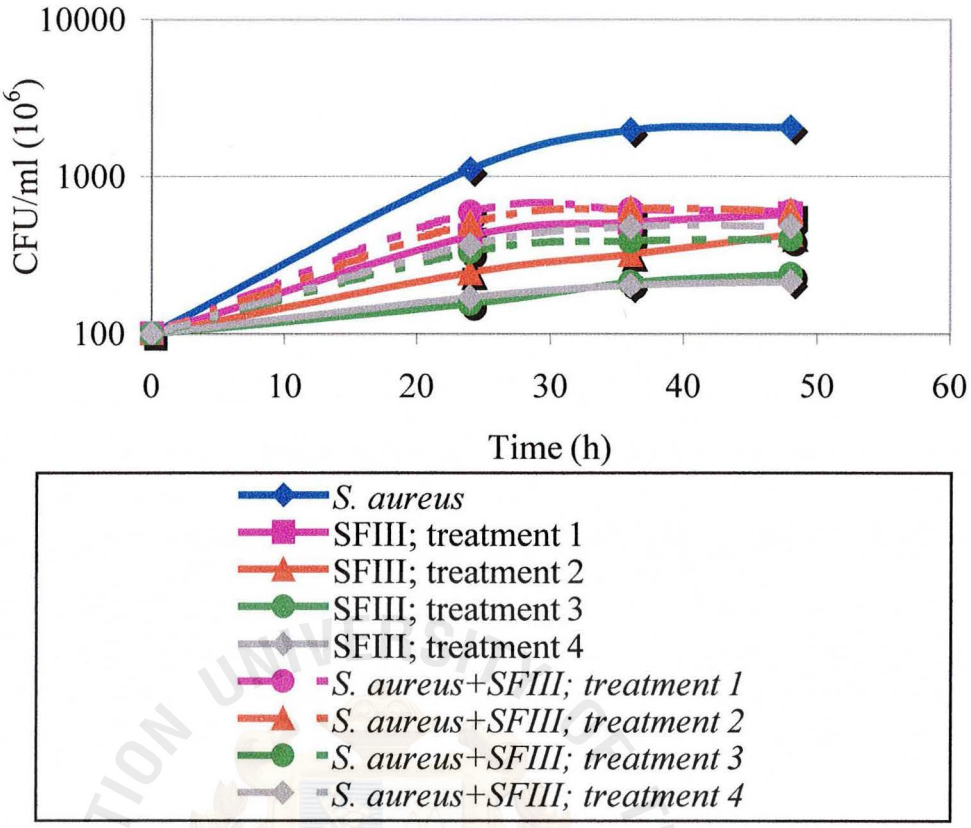


Figure 7: Growth of *S. aureus* in the NB methods using four treatments of Samrong, SFIII. SFIII:treatment 1 was 25% of SFIII, SFIII: treatment 2 was 50% of SFIII, SFIII: treatment 3 was 75% of SFIII, SFIII: treatment 4 was 100% of SFIII.

Experiment 2: Gel formulation and antimicrobial testing

Experiment 2.1: Variation of water in plain gel.

The plain gel formulation was studied before formulating the ASAG in order to be used as basic formula. The variation of water content in the gel was studied. The formula was evaluated by 30 panels.

There was no any significant difference among four treatments in the terms of viscosity, absorbency, and overall at $p>0.05$. Four samples were hardly perceived the differences by panels which show significant difference among the replications at $p\leq0.05$. The 40ml water treatment showed that the panels felt neither like nor dislike score in all attributes because gel was sticky and slowly absorption when apply on the skin. (Anonymous, 2006[j]) Whereas, the liking score of 60 and 80ml water treatment was similarly. The panelists felt slightly like score in all attributes. The 100ml water treatment showed lower liking score of viscosity when comparing with 60 or 80ml water. However, the absorbency and overall were similar to 60 or 80ml water. Besides, the antimicrobial activity of anti-acne gel is the major properties. The texture of anti-acne gel stills concern. Both *A. vera* and Samrong have their own gel-like texture. The solubilization is also concerned in anti-acne gel because the solubilization property allows the higher concentrations of the active ingredients. The increasing of water concentration leads to a reduction in viscosity of the formulation, facilitating the application or spreading of the gel by the panels, while providing a better cosmetic appearance. Therefore the higher water content results in good moisturizing properties. (Anonymous, 2006[j]) In addition, after applying gel on the skin, gel is easily absorbed and then water evaporates so the panels feel cool but not oily.

Therefore, 100 ml water was chosen for experiment 2.2, ASAG formulation, based on two reasons. The higher water content should increase solubilization properties which will lead to higher amount of ingredients. Another reason is both gel-like texture of *A. vera* and Samrong increased proper viscosity and provide good moisturizing and spreading properties in anti-acne formula when increase water content.

Table 2: Sensory analysis of plain gel formulation with varying the water content

Water content (ml)	Viscosity	Absorbency	Overall
40	5.37±1.90	5.77±1.89	5.92±1.62
60	6.07±1.31	5.93±1.68	6.20±1.30
80	5.90±1.49	5.77±1.30	6.10±1.09
100	5.70±1.64	5.93±1.95	6.10±1.77

$p > 0.05$

Experiment 2.2: *A. vera* and Samrong gel formulation

Experiment 2.2.1: Variation of Samrong concentration in ASAG.

The concentration of Samrong, SFIII part, was varied into three levels which were 5%, 15%, and 25% (v/v). The percentage of AFII was fixed at 20% (v/v) because most *A. vera* product in the market contains 20% (v/v) AFII (Sanooksan, 2000, and Anonymous, 2006[d]). If the content is less than 20% (v/v) AFII, AFII's abilities is not effective. The experiment was done in series begun with the ASAG without parabens, then ASAG with parabens, and the last was sensory analysis of three formulas of ASAG.

ASAG without parabens showed the negative result, no clear zone, when tested by NA (figure 8) while the clear zone, positive result, was represented by ASAG with parabens (figure 10). The clear zone was suspected to result from the activity of parabens, which was the preservative. Another explanation may be drawn from the fact that the gel might not distribute on agar well, then the active compound in gel was not work effectively. Besides that the amount of active compound in ASAG was not enough to inhibit the growth of *S. aureus*. The concentrated *A. vera* and Samrong, such as dried form, may be used in order to receive the antimicrobial activity efficiently.

The result from experiment 2.2.1 on NA was different from NB. ASAG containing 5%, 15%, and 25% (v/v), SFIII could reduce $1.2x10^9$ (52%), $9.94x10^8$ (44%), $1.04x10^9$ (46%) CFU/ml of *S. aureus* respectively but there was no significant different in antimicrobial activity on *S. aureus* among these three formulas at $p>0.05$ (the result was not shown). The result of ASAG without parabens when tested with NB was shown in figure 9. The microbial growth of Gel: treatment 1 control was constant. Gel: treatment 2, the microbial growth increased until 24 hours and after that it was stable. The control of Gel: treatment 3

and 4 increased in the microbial growth but the microbial growth of Gel: treatment 3 was higher than Gel: treatment 4. The microbial growth of mixture of *S. aureus* and all gel treatments was closed together and showed slightly lower microbial growth with no significant different to the positive control. Moreover, the result of the plain gel added with *S. aureus* had no growth because the plain gel may not have nutrient for microorganism, it was composed of the chemical agent only. The condition of plain gel did not stimulate the growth of microorganisms, while the *A. vera* and Samrong have the nutrient such as vitamins and sugar. The growth of microbes in the condition with ASAG may result from trace nutrients available in the ASAG itself from plant raw materials.

Although the antimicrobial activity from plain gel and ASAG was similar, the benefit from plain gel was probably less than ASAG. Even though *A. vera* does not have antimicrobial activity, it is well known as moisturing and antiinflammation properties. (Reynold and Dweck, 1999)

The skincare product always contains preservative to prevent the microbial growth so ASAG with parabens was studied in experiment 2.2.2. Parabens affected the growth of *S. aureus* on NA as shown in figure 10, which ASAG could inhibit the growth of *S. aureus* indicated by clear zone around the gel. The result of the microbial growth from NB (figure 11) was similar to the result from NA (figure 10). Control of Gel: treatment 1 and 2 showed the increasing in microbial growth and then the microbial growth was stable after 24 hours. At the initial time of incubation until reaching 24 hours, the microbial growth of the control Gel: treatment 3 and 4 was similar to Gel: treatment 1 and 2, after 24 hours, the microbial growth of Gel: treatment 3 and 4 decreased continuously. Mixture of *S. aureus* and all gel treatments showed closed level of microbial growth. Therefore, the effect of parabens to ASAG and its antimicrobial activity were not very effective. Parabens was used to control the contamination because their ability to lower microbial load, compared with the control of gel without parabens.

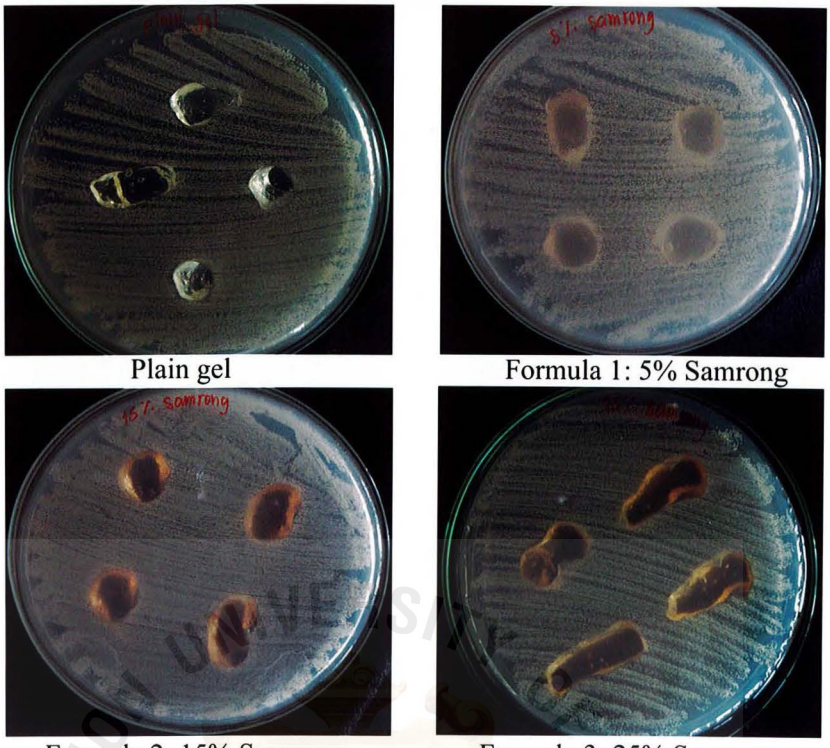
The expectation of ASAG was that the combination should reduce the growth of *S. aureus* more than with one ingredient but the result was not show as assumption. ASAG with parabens containing 5%, 15%, and 25% (v/v) Samrong could reduce 6.73×10^8 (69%), 7.15×10^8 (67%), and 5.46×10^8 (74%) CFU/ml of *S. aureus* respectively. The active compound of *A. vera* and Samrong might be lost during gel making, especially in *A. vera*.

The antimicrobial activity of ASAG was not only checked but the physical attributes were also evaluated by 30 panels.

According to figure 12, the intensity of brown color of ASAG was different obviously. The gel containing 5% (v/v) Samrong was the lightest brown color which was quite clear and the darkest brown was the gel containing 25% (v/v) Samrong. All formulas had the small brown particles thoroughly which should be from Samrong. After applying the gel on the skin, the particle will remain for a short period of time. After gel was dry, this particle disappeared. Then the sensory analysis was conducted in order to know the direction to develop the gel formula.

According to table 3, there was no any different between formula 1and 2 in term of the color, absorbency, and overall ($p>0.05$) but the color, absorbency, and overall appearance of formulas 1 and 2 were different to formula 3 ($p\leq0.05$). There were significantly different in the spreadability between formula 1 and 3 at $p\leq0.05$ while the spreadability of formula 2 was similar to the other formulas. There was no any different in the stickiness among three formulas ($p>0.05$). There were significantly different among replication at $p\leq0.05$.

All attributes affected the liking score of ASAG containing 5%, 15%, and 25% (v/v) Samrong, except the stickiness. The panels also affected the liking score. The lightest color was the most preferred and it affected the liking score of the other attributes because formula 1 received the highest liking score from all attributes. This might be a result because the panels were familiar with clear anti-acne gel in market. In additional, some panelists commented that the darker color would be accepted because there are many cosmetic products which are dark at this moment such as brown from tamarind, black from algae, and yellow from tumeric. If they could not compare the color, they might not perceive the difference in color. The product should be served one at the time, not all at once to avoid such bias. As described above, MAG and MSG have their gel-like characteristics. The panels like slightly the spreadability and absorbency of ASAG: formula 1 and 2. The increasing MSG content would increase the mucous of the gel which affected the spreadability and absorbency of ASAG. The increasing to 25 (v/v) of Samrong: formula 3 was decreased in spreadability, absorbency and stickiness score because the higher small particle remained on the skin and affected on the preference score on those attributes.



Plain gel | Formula 1: 5% Samrong

Formula 2: 15% Samrong | Formula 3: 25% Samrong

Figure 8: Growth of *S. aureus* in the NA methods using plain gel and three formulas of ASAG without parabens. Formula 1-5% SFIII, Formula-15% SFIII, and Formula 3-25% SFIII.

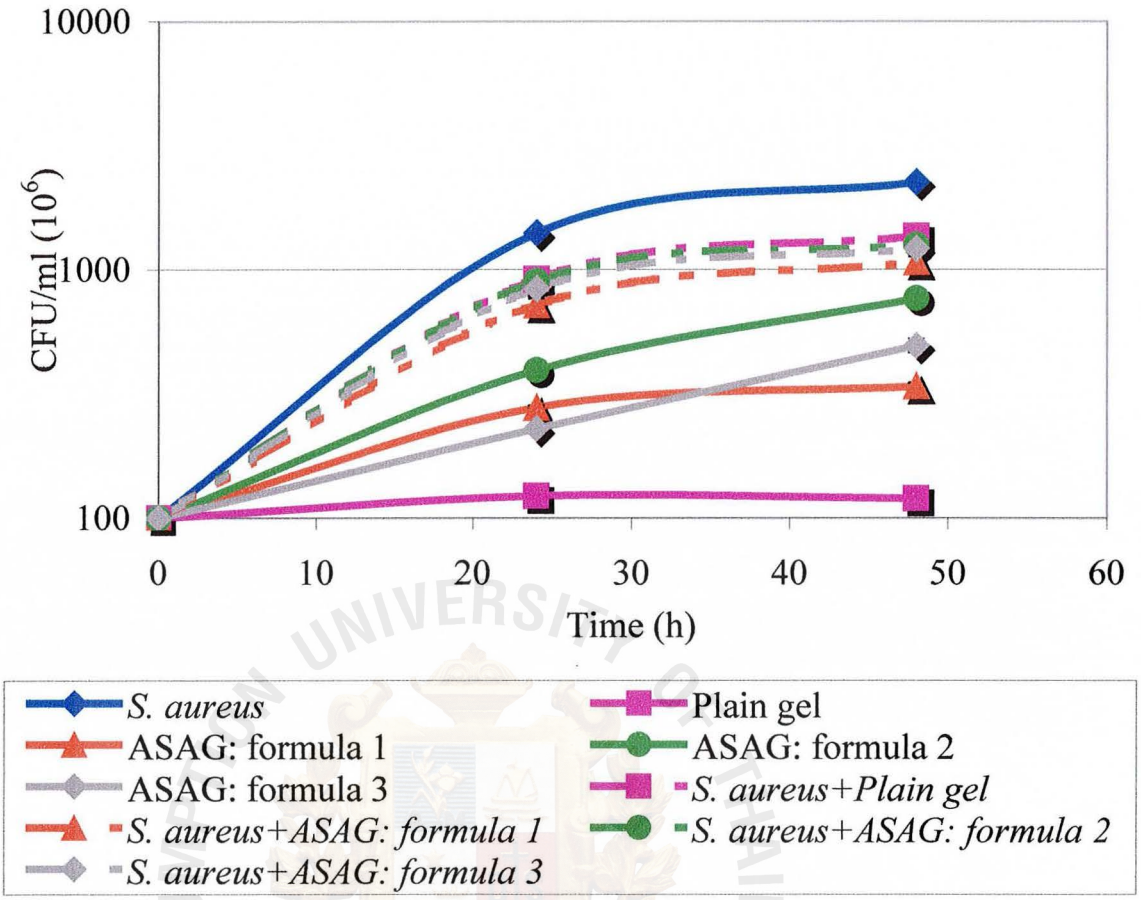


Figure 9: Growth of *S. aureus* in the NB methods using plain gel and three formulas of ASAG without parabens. Formula 1-5% SFIII, Formula-15% SFIII, and Formula 3-25% SFIII.

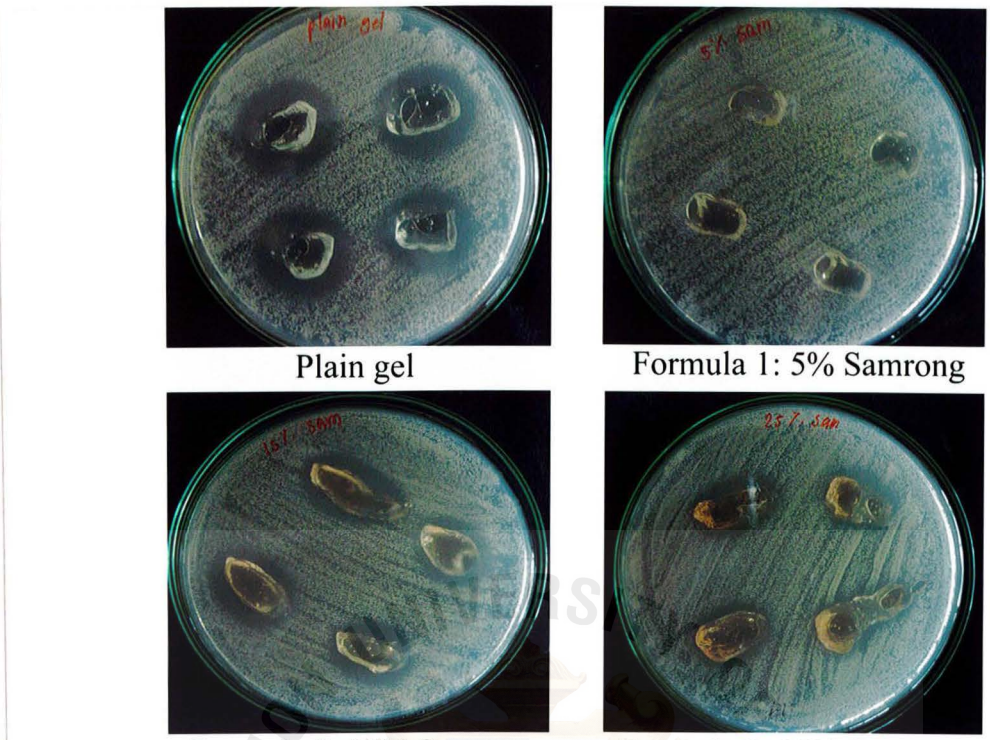


Figure 10: Growth of *S. aureus* in the NA methods using plain gel and three formulas of ASAG with parabens. Formula 1-5% SFIII, Formula 2-15% SFIII, and Formula 3-25% SFIII.

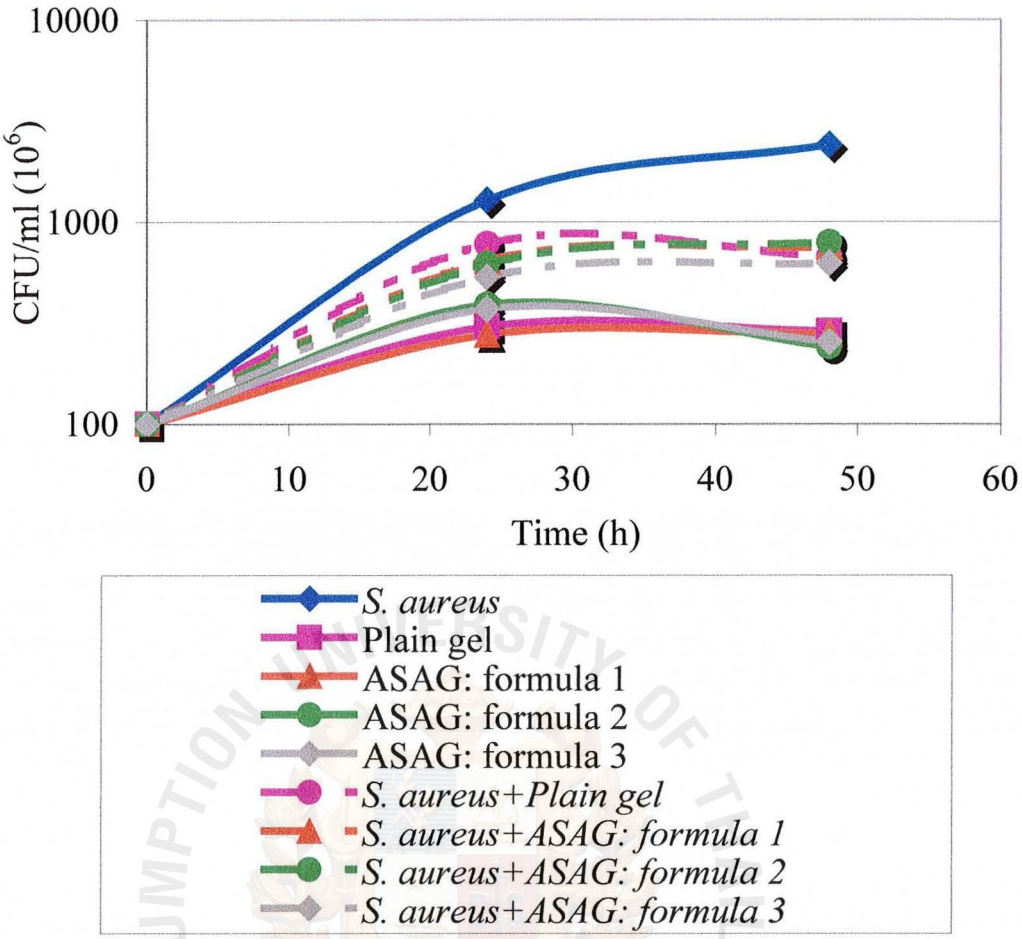
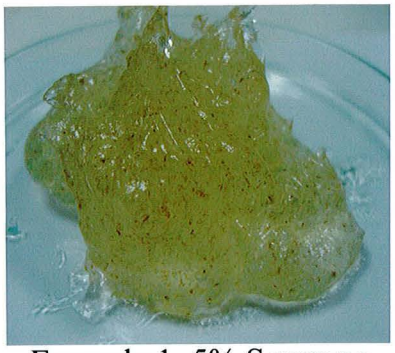
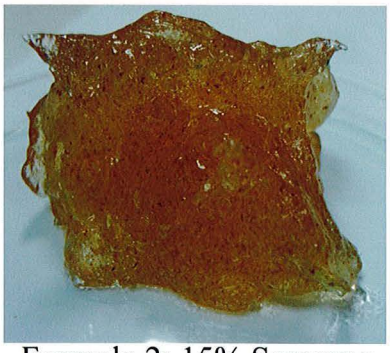


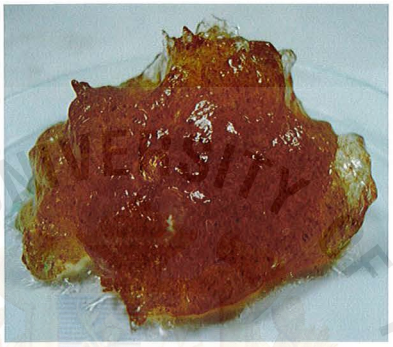
Figure 11: Growth of *S. aureus* in the NB methods using plain gel and three formulas of ASAG with parabens. Formula 1-5% SFIII, Formula-15% SFIII, and Formula 3-25% SFIII.



Formula 1: 5% Samrong



Formula 2: 15% Samrong



Formula 3: 25% Samrong

Figure 12: The characteristic of ASAG containing 5%, 15%, and 25% Samrong.

Table 3: Sensory analysis of ASAG formulation with varying the Samrong concentration.

Formula	Brown color	Spreadability	Absorbency	Stickiness	Overall
5% Samrong	7.10 ± 1.18^{a}	6.93 ± 1.7^{a}	6.90 ± 1.58^{a}	6.33 ± 1.84	7.00 ± 1.34^{a}
15% Samrong	6.63 ± 1.30^{a}	6.70 ± 1.37^{ab}	6.83 ± 1.42^{a}	6.37 ± 1.38	6.57 ± 1.23^{a}
25% Samrong	4.57 ± 1.79^{b}	6.13 ± 1.91^{b}	5.83 ± 1.86^{b}	6.20 ± 1.49	5.90 ± 1.26^{b}

p=0.05

For further experiment, the other forms of *A. vera* and Samrong should be used such as dried and concentrated form. *A. vera* should be carefully extracted, concentrated and using freezed drying techniques to maintain their antimicrobial activities. Samrong should be further developed in extraction method, bleaching and concentration techniques. It was a challenge to meet both requirements, which are to reduce the small brown particle and the intensity of brownness but to still remain their antimicrobial activities. The antimicrobial activity at higher than 20 % (v/v) *A. vera* and at lower than 25% (v/v) Samrong should be studied. The ASAG should be developed to have a characteristic of a clear brown gel and improve their physiochemical properties when apply on skin. The development of ASAG formulation should include the addition of fragrance. The antimicrobial activity should not be checked by *in vitro* only, but the *in vivo* should also be done in order to indicate the properties of ASAG

Conclusion

1. Clorox at 15% (v/v) was the most suitable agent to treat *A. vera* leaves.
2. *A. vera* was prepared by blending with blender and filtering with cheesecloth, AFII: treatment 2, was used to prepare *A. vera* in *A. vera* and Samrong anti-acne gel formulation.
3. Both *A. vera* and Samrong could not inhibit the growth of *S. aureus*.
4. Plain gel contained 2g Carbopol, 4.4ml Glycerine, 1.86ml Triethanolamine based on water 100ml, formula 4 of plain gel, was the standard formula for *A. vera* and Samrong anti-acne gel.
5. *A. vera* and Samrong anti-acne gel could not inhibit the growth of *S. aureus*.
6. *A. vera* and Samrong anti-acne gel containing 5% Samrong was the most acceptable formula by 30 panelists.

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Appendices

Appendix A

Media formulation; based on 1000 ml distilled water

Nutrient agar

Peptone	5 g
Beef extract	3 g
Agar powder	15 g

Nutrient broth

Peptone	5 g
Beef extract	3 g

Double strength nutrient broth

Peptone	10 g
Beef extract	6 g

Autoclave at 121°C for 15 minutes.

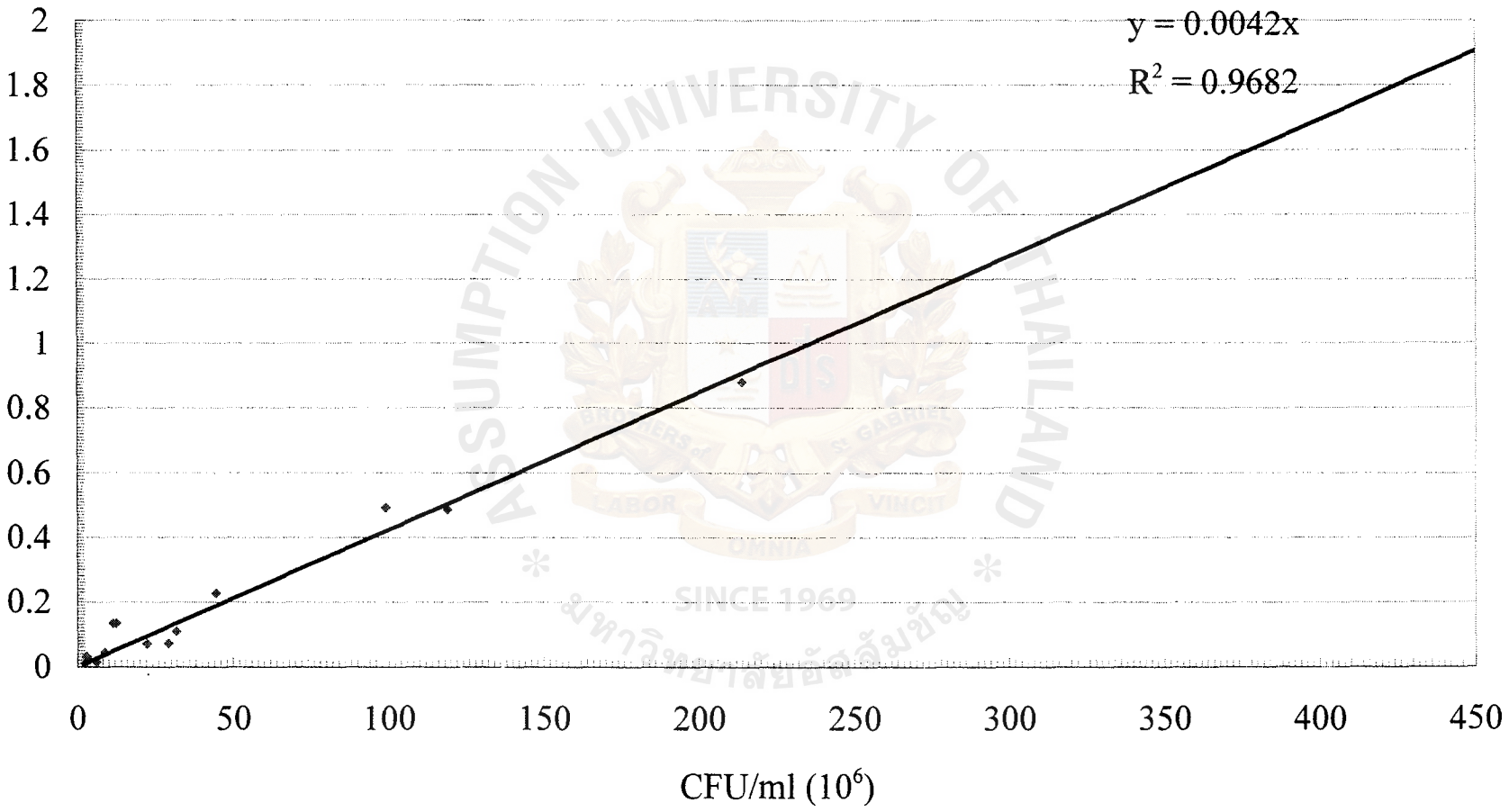

Figure 13: The standard curve of the concentration of *S. aureus* at each optical density at 650 nm(A).

Table 4: The average microbial growth (x 10^6 CFU/ml) of three preparation methods of *A. vera*, AFII.

Time(h)	*S. aureus*	AFII;treatment1	AFII;treatment2	AFII;treatment3	*S.aureus* +AFII; treatment 1	*S.aureus* +AFII; treatment 2	*S.aureus* +AFII treatment 3
AVE 0	11.03	34.50	30.20	26.71	37.88	27.44	27.08
SD 0	3.97	25.35	3.66	15.71	21.78	11.88	12.55
%growth 0	100.00	100.00	100.00	100.00	100.00	100.00	100.00
AVE 24	120.24	110.56	156.99	189.83	239.09	217.60	212.34
SD 24	20.34	80.64	76.95	111.03	55.32	40.80	35.67
%growth 24	1089.90	320.43	519.87	710.79	631.22	792.99	784.02
AVE 48	186.79	192.27	203.90	236.85	280.48	283.29	275.06
SD 48	17.19	135.56	99.31	120.06	29.00	65.74	44.53
%growth 48	1693.20	557.25	675.19	886.85	740.49	1032.40	1015.60

Remarks; AFII; treatment 1 was MAG was chopped manually and then filtered by cheesecloth.

AFII; treatment 2 was MAG was blended by blender and then filtered by cheesecloth.

AFII; treatment 3 was MAG was blended by blender and after filtered, it was pasteurized.

Table 5: The average microbial growth (x 10^6 CFU/ml) of four concentrations of Samrong, SFIII.

Time(h)	*S. aureus*	SFIII; treatment 1	SFIII; treatment 2	SFIII; treatment 3	SFIII; treatment 4	*S. aureus* +SFIII; treatment 1	*S. aureus* +SFIII; treatment 2	*S. aureus* +SFIII; treatment 3	*S. aureus* +SFIII; treatment 4
AVE 0	11.35	11.19	24.33	43.97	56.47	20.91	29.25	56.63	65.95
SD 0	2.75	6.17	18.13	11.51	7.66	6.75	11.86	6.62	5.86
%growth 0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
AVE 24	126.63	46.75	59.72	68.49	96.59	124.37	148.45	188.73	242.54
SD 24	19.31	7.92	11.61	12.66	23.96	7.50	13.77	7.29	23.13
%growth	1115.70	417.73	245.51	155.78	171.05	594.69	507.60	333.29	367.75
AVE 36	223.85	57.18	78.21	93.14	115.08	128.77	183.21	220.83	317.34
SD 36	77.28	18.75	23.10	14.80	12.72	15.63	41.50	27.45	48.72
%growth 36	1972.40	510.99	321.53	211.82	203.79	615.75	626.46	389.98	481.17
AVE 48	232.74	64.64	104.37	105.04	121.31	125.79	176.90	226.63	320.00
SD 48	70.45	24.61	22.11	18.28	11.31	12.53	55.69	33.90	49.96
%growth 48	2050.70	577.66	429.04	238.90	214.83	601.52	604.88	400.21	485.20

Remarks; SFIII; treatment 1 was 25% of Samrong with 75% of steriled water.

SFIII; treatment 2 was 50% of Samrong with 50% of steriled water.

SFIII; treatment 3 was 75% of Samrong with 25% of steriled water.

SFIII; treatment 4 was 100% of Samrong.

Table 6: The average microbial growth (x 10^6 CFU/ml) of plain gel and three formulas of ASAG without parabens.

Time	*S. aureus*	Gel; treatment 1	Gel; treatment 2	Gel; treatment 3	Gel; treatment 4	*S. aureus* +gel; treatment 1	*S. aureus* +gel; treatment 2	*S. aureus* +gel; treatment 3	*S. aureus* +gel; treatment 4
AVE 0	9.72	12.38	8.49	6.27	8.97	16.35	23.53	19.76	20.60
SD 0	2.02	4.06	4.03	2.24	1.73	3.00	7.24	4.96	4.25
%growth 0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
AVE 24	136.63	15.20	23.69	24.72	20.56	150.12	170.20	177.82	173.17
SD 24	33.76	5.13	4.14	6.37	1.61	15.34	43.45	36.51	37.09
%growth 24	1405.30	122.76	278.97	394.30	229.20	918.20	723.27	899.80	840.85
AVE 48	218.77	14.84	28.81	48.10	44.37	222.70	251.90	248.29	249.80
SD 48	22.15	3.1286	10.32	15.26	30.41	22.01	57.19	57.70	52.65
%growth 48	2250.20	119.87	339.25	767.09	494.69	1362.10	1070.50	1256.40	1212.90

Remarks; Gel was ASAG, which its components was Carbopol 940, glycerine, triethanolamine, AFII, and SFIII. The samrong was varied into 3 levels while the others were constant.

Plain gel was gel without AFII and SFIII.

Gel treatment 2 was composed of 5% SFIII.

Gel treatment 3 was composed of 15% SFIII.

Gel treatment 4 was composed of 25% SFIII.

Table 7: The average microbial growth (x 10^6 CFU/ml) of plain gel and three formulas of ASAG with parabens.

Time	*S. aureus*	Gel; treatment 1	Gel; treatment 2	Gel; treatment 3	Gel; treatment 4	*S. aureus* +gel; treatment 1	*S. aureus* +gel; treatment 2	*S. aureus* +gel; treatment 3	*S. aureus* +gel; treatment 4
AVE 0	8.6111	6.9048	8.6111	8.0159	8.5714	14.405	14.96	17.262	20.437
SD 0	1.5823	2.8372	4.6277	3.1967	2.9814	2.9383	4.039	4.916	4.3576
%growth 0	100	100	100	100	100	100	100	100	100
AVE 24	110.52	20.992	23.929	31.23	31.905	112.3	96.111	107.06	109.37
SD 24	15.462	10.505	8.5233	20.131	13.195	89.184	14.617	19.067	14.197
%growth 24	1283.41	304.023	277.8802	389.604	372.2222	779.6143	642.4403	620.2299	535.1456
AVE 48	207.62	19.921	24.048	19.365	21.984	97.46	112.7	137.22	128.02
SD 48	8.3136	4.2347	5.1508	2.025	0.8362	5.2937	5.8531	16.89	8.3379
%growth 48	2411.06	288.5057	279.2627	241.5842	256.4815	676.584	753.3156	794.9425	626.4078

Remarks; Gel was ASAG, which its components was Carbopol 940, glycerine, triethanolamine, parabens, AFII, and SFIII. The samrong was varied into 3 levels while the others were constant.

Plain gel was gel without AFII and SFIII.

Gel treatment 2 was composed of 5% SFIII.

Gel treatment 3 was composed of 15% SFIII.

Gel treatment 4 was composed of 25% SFIII.

Appendix B

Questionnaire of Sensory analysis

1. Plain gel

Hedonic scaling test

Name:.....
Date.....

Product: Plain gel

Instruction

1. Please apply the gel on the back-handed skin in the circular direction with the providing space for 15 times.
2. Please test the samples in the order presented, from left to right
3. Please rate whether the level of a sensory attribute from most preferred to least preferred using the following numbers:

1 = dislike extremely 2 = dislike very much

3 = dislike moderately 4 = dislike slightly

5 = neither like nor dislike 6 = like slightly

7 = like moderately 8 = like very much

9 = like extremely

Sample	_____	_____	_____	_____
1. The viscosity of gel during coating the skin	_____	_____	_____	_____
2. The absorption on skin	_____	_____	_____	_____
3. Overall	_____	_____	_____	_____

2. ASAG

Hedonic scaling test

Name:.....
Date.....

Product: ASAG

Instruction

1. Please apply the gel on the back-handed skin in the circular direction with the providing space for 15 times.
2. Please test the samples in the order presented, from left to right
3. Please rate whether the level of a sensory attribute from most preferred to least preferred using the following numbers:

1 = dislike extremely
2 = dislike very much
3 = dislike moderately
4 = dislike slightly
5 = neither like nor dislike
6 = like slightly
7 = like moderately
8 = like very much
9 = like extremely

Sample			
1. Brown color	_____	_____	_____
2. Spreadability	_____	_____	_____
3. Absorbency	_____	_____	_____
4. Stickiness (after rubbing on skin)	_____	_____	_____
5. Overall	_____	_____	_____

Table 8: Statistical analysis of sensory analysis of plain gel formulation.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
TRT	Viscosity	8.158	3	2.719	1.238	.301 ns
	Absorbency	.833	3	.278	.113	.952 ns
	Overall	1.256	3	.419	.248	.863 ns
BLOCK	Viscosity	106.742	29	3.681	1.676	.035 *
	Absorbency	130.300	29	4.493	1.825	.017 *
	Overall	103.060	29	3.554	2.101	.004 **
Error	Viscosity	191.092	87	2.196		
	Absorbency	214.167	87	2.462		
	Overall	147.181	87	1.692		
Total	Viscosity	4285.000	120			
	Absorbency	4452.000	120			
	Overall	4686.250	120			

p = 0.05

Table 9: Statistical analysis of sensory analysis of *A. vera* and Samrong gel formulation.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
TRT	Brown color	109.067	2	54.533	37.834	.000**
	Spreadability	10.156	2	5.078	4.025	.023*
	Absorbency	21.422	2	10.711	5.793	.005**
	Stickiness	.467	2	.233	.235	.791ns
	Overall appearance	18.422	2	9.211	10.494	.000**
REP	Brown color	99.433	29	3.429	2.379	.003**
	Spreadability	126.456	29	4.361	3.456	.000**
	Absorbency	123.789	29	4.269	2.309	.003**
	Stickiness	160.900	29	5.548	5.593	.000**
	Overall appearance	93.156	29	3.212	3.660	.000**
Error	Brown color	83.600	58	1.441		
	Spreadability	73.178	58	1.262		
	Absorbency	107.244	58	1.849		
	Stickiness	57.533	58	.992		
	Overall appearance	50.911	58	.878		
Total	Brown color	3641.000	90			
	Spreadability	4117.000	90			
	Absorbency	4081.000	90			
	Stickiness	3791.000	90			
	Overall appearance	3952.000	90			

p = 0.05