FORMULATION OF ANTIBACTERIAL SOAP FROM THE LEAF EXTRACT OF WATER APPLE (*Syzygium samarangense*) AGAINST *Staphylococcus aureus*-CAUSED IMPETIGO

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SCIENCE RESEARCH 2

by

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Fourth Year – Tau

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FORMULATION OF ANTIBACTERIAL SOAP FROM THE LEAF EXTRACT OF WATER APPLE (*Syzygium samarangense*) AGAINST *Staphylococcus aureus*-CAUSED IMPETIGO

prepared and submitted by Jaybayl Abril B. Jayme in partial fulfilment of the requirements in Science Research 2, has been examined and recommended for acceptance and approval.

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Formulation of antibacterial soap from the leaf extract of Water Apple (*Syzygium samarangense*) against *Staphylococcus aureus*-caused Impetigo

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ABSTRACT

The anti-bacterial activity of soaps made from 90% ethanolic extracts of *Syzygium samarangense* leaves was assessed using streak plate method. Soaps composed of 10%, 20%, and 30% extract in base soap were administered with control groups consisting of the soap base for negative control. Measurements of the zone of inhibition made after the streak plate method using a calliper. The 10%, 20%, and 30% non-developed soaps were not observed to have a significant anti-bacterial activity against *Staphylococcus aureus*. Due to the failure of the saponification process, the soaps were not developed as planned. Further investigations on quality control in terms of shelf life, toxicity, etc. should be made before testing on human skin.

Keywords: *Anti-bacterial, Staphylococcus aureus, Syzygium samarangense*

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CHAPTER 1

INTRODUCTION

A. Background of the Study

Impetigo is a highly contagious bacterial skin infection and is most common among pre-school children. Its name is derived from the Latin *impetere* ("assail") and it is sometimes referred to as school sores. People who play close contact sports (e.g. rugby, American football, wrestling, etc.) are also susceptible, regardless of the age. It is primarily caused by the bacterium *Staphylococcus aureus*, though in some cases, it could be caused by the *Streptococcus pyogenes*. The infection is spread by direct contact with lesions or with nasal carriers. This infection generally appears as honey-colored scabs formed from dried serum and is often found on the arms, legs, or of the face (Harborne 1999).

For generations, the disease was treated with an application of the antiseptic gentian violet. Today, topical or oral antibiotics are usually prescribed. Treatment may involve washing with soap and water and letting the infection dry in the air. Mild cases may be treated with bacterial ointment, such as Mupirocin (Kirtiker 1975). More severe cases require oral antibiotics, such as Dicloxacillin, Flucloxacillin, or Erythromycin.

Apart from it being a cure, washing with soap and water is also one way to prevent this kind of infections. One can also prevent this by keeping the areas that have been injured clean and covered.

An antibacterial soap is a cleaning product to which active antibacterial ingredients are added (Mokoka 2013). The chemicals kill bacteria and microbes but they do not kill natural skin bacteria. Many studies have examined the benefits of antibacterial soaps. It has been found that soaps containing antibacterial active ingredients remove more bacteria than simply washing with plain soap. Soaps can penetrate every part of the body easily.

Flavonoid found in *Syzygium samarangense* is proven to be an antibacterial component. It can fight bacteria, such as *Staphylococcus aureus* (Oonmetta-aree 2005).

About 25-30% of the human population of the earth is affected by staph infections. Globally, impetigo affected approximately 140 million people, which is 2% of the overall population. Preventing these infections will be easy with the use of soap, which has the

capability of penetrating every part of the body. Flavonoids in *Syzygium samarangense* (Water Apple) can fight back, *Staphylococcus aureus*, which causes infections (Pandey 2011).

In the Philippines, the prevalence of staph infections was reported to be rate of 17-18% from the years 1999-2003. It is common especially in widely populated urban areas. This study would like to evaluate the coming from *Syzygium samarangense* as to its effectivity in fighting staph infections with the hope that such a study will help in alleviating the problem of staph infection in the Philippines as well as giving an alternate economically-friendly solution to this problem (Rachana 1995).

B. Objectives

B.1. General Objective

To develop water apple soap and evaluate its antibacterial activity.

B.2. Specific Objectives

1. To measure the zone of inhibition of soap solutions with varying concentrations (0%, 10%, 20%, and 30%) of leaf extracts against *Staphylococcus aureus*.
2. To compare the zone of inhibition of soap solutions with varying concentrations (0%, 10%, 20%, and 30%) of leaf extracts against *Staphylococcus aureus*.

C. Significance of the Study

This study was given additional knowledge to the people who are suffering from staphylococcal infections. This study was given basic information to those with staphylococcal infections, the knowledge on how to prevent impetigo, a *Staphylococcus aureus*-caused skin infection.

D. Scope and Delimitation

Syzygium samarangense leaves were obtained from Tigbauan, Iloilo, and were stored in Jaro, Iloilo City, Iloilo. The samples used were limited to an amount of approximately three kilograms (3 kg) in fresh weight only.

The researcher will consider one species of Syzygium, namely *Syzygium samarangense*. The extraction of the *Syzygium samarangense* leaves will be performed at the University of San Agustin in Iloilo City, Iloilo.

The experimental phase of the study was conducted in the laboratory vicinities of Philippine Science High School – Western Visayas located in Bito-on, Jaro, Iloilo City, Iloilo.

This study was conducted on the middle of the month of January 2014 and spanned until March 2014. The soap solutions were tested on the cultures of *Staphylococcus aureus*.

E. Definition of Terms

Antibacterial Soap – It is any cleaning product to which active antibacterial ingredients have been added. These chemicals kill bacteria and microbes, but are no more effective at deactivating viruses than any other kind of soap or detergent, and they also kill non-pathogenic bacteria.

Bacteria (*Staphylococcus aureus*) – It has been estimated that 20% of the human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages. *S. aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages. *S. aureus* can cause range illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis (Somchit 2003).

Bacterial Culture – It allows bacteria to reproduce on a culture medium in a controlled environment. This process involves spreading bacteria across an agar plate and allowing them to incubate at a certain temperature for a period of time (Umar 2011).

Extraction – It is the process of obtaining juices from specific fruits or parts from plants for other purposes.

Impetigo – A highly contagious bacterial skin infection most common among preschool students (Reddy 2012). Sometimes referred to as school sores and comes from the Latin word *impetere*, meaning assail.

Saponification – It is a process that produces soap, usually from fats and lye. It involves base (caustic soda, NaOH) hydrolysis of triglycerides, which are esters of fatty acids, to form the sodium salt of carboxylate. In addition to soap, such traditional saponification processes produces glycerol (Rammiden 2007).

Syzygium samarangense –Thin-skinned and shining, the fruit varies from white, to light-red to red, is pear-shaped with a narrow neck and broad apex; 5/8 to ¾ in long, 1 to 1 1/3 in wide. The apex is concave; bears the thick calyx segments and the protruding, slender, bristle-like style

(Rajendra 2011). The flesh is white or pink, mildly fragrant, dry or juicy, crisp or spongy, and usually of sweetish but faint flavour. There may be 3 to 6 small seeds, frequently only 1 or 2, but generally the fruits are seedless.

CHAPTER 2

REVIEW OF RELATED LITERATURE

A. Impetigo

A contagious infection caused by staphylococcal and streptococcal bacteria and characterized by blisters from yellow-brown scabs.

A.1. Cause and Factors of Impetigo

Bacterial infection caused by a bacterium labelled as *Staphylococcus aureus*, a Gram-positive bacteria which includes species that can cause a wide variety of infections in humans and other animals through the production of toxin. When the skin is punctured or broken for any reason, staph bacteria can enter the wound and cause an infection (Cushnie 2005). People can get staph infections from contaminated objects, but staph bacteria often spread through skin-to-skin contact.

A.2. Preventions and Treatments of Impetigo

Being aware of some common-sense precautions can help lower the risk of developing staph infections. These precautions include proper hygiene like the frequent use of soap during the washing of the hands or the body. Many common skin infections will require incision and drainage of the infected site and infections may require antibiotics (Hanumantharaju 2010). Some serious infections typically require hospitalization and treatment with intravenous antibiotics. Globally, impetigo affected approximately 140 million people.

B. Bacteria (*Staphylococcus aureus*)

It has been estimated that 20% of the human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis (Harborne 1999). The colonies are

often surrounded by a clear zone of hemolysis due to production of hemolysins; species specificity. The culture has characteristics such as Hardy microbes, with stand heat at 60°C for 30 min, grow in media containing 7.5% to 10% NaCl, remain viable for months on agar plates stored at 4°C, grows at 6.5°C to 46°C, and grows at pH 4.2 to 9.3.

C. Antibacterial Soap and its Advantages and Disadvantages

An antibacterial soap is any cleaning product to which active antibacterial ingredients have been added (Khandaker 2012). These chemicals kill bacteria and microbes, but are no more effective at deactivating viruses than any other kind of soap or detergent, and they also kill non-pathogenic bacteria.

Studies have found that soaps containing anti-microbial active ingredients remove more bacteria than simply washing with plain soap and water. Though washing thoroughly with plain soap is sufficient to reduce bacteria and is as also effective against viruses, it is best to concern oneself with more precautious processes of prevention against foreign dangers (Kirtiker 1975).

D. Water Apple (*Syzygium samarangense*)

The tree may reach 10 or even 32 ft. has a short, crooked trunk branching close to the ground, and a non-symmetrical, open crown. The opposite leaves, on very short, thick petioles, are obviate- or elliptic-oblong, chordate at the base and clasping the twig; blunt and notched or short-pointed at the apex; 2 to 10 in long, 1 to 6 3/8 in wide; dull, light-green above, yellowish-green beneath; leathery; not aromatic or only slight so when crushed (Moghimipour 2010). Flowers, faintly fragrant, are home in loose terminal or axillary clusters of 3 to 7, mostly hidden by the foliage. The 4-parted calyx and 4 petals are pale-yellow, yellowish-white or pinkish and there are numerous concolorous stamens to ¾ in long. Thin-skinned and shining, the fruit varies from white, to light-red or red, is pear-shaped with a narrow neck and broad apex; 5/8 to ¾ in long, 1 to 1/3 in wide. The apex is concave; bears the thick calyx segments and the protruding, slender, bristle-like style (Mokoka 2013). The flesh is white or pink, mildly fragrant, dry or juicy, crisp or spongy, and usually of sweetish but faint flavour. There may be 3 to 6 small seeds, frequently only 1 or 2, but generally the fruits are seedless.

Figure 1.*Syzygium samarangense* fruits

D.1. Availability or Range of *Syzygium samarangense*

The water apple occurs naturally from southern India to eastern Malaysia. It is commonly cultivated in India, south-eastern Asia, and Indonesia. In the Philippines, it grows as though wild in the provinces of Mindanao, Basilan, Dinagat, and Samar (Okoko 2011). It has never been widely distributed but is occasionally grown in Trinidad and Hawaii. It was introduced into Puerto Rico in 1927 but survived only a few years. In Malaya, there are two crops a year, one in the spring and a second in the fall. In Indonesia, the tree frequently blooms in July and again in September, the fruits ripening in August and November.

D.2. Uses of *Syzygium samarangense*

The water apple is mainly consumed by children, the appeal being largely its thirst-relieving character (Oonmetta-aree 2005). In Indonesia, the fruits are sold in markets in piles or skewered on slender bamboo sticks. Superior types are sometimes served sliced in salads. According to early writings, a water apple salad is a ceremonial dish for new mothers. The wood

is hard and is fashioned into small pieces of handicraft. In the studies, the water apple has been proven to have an anti-bacterial property against *S. aureus* and various bacteria (Pandey 2011). Water apple is available throughout Southeast Asia and the fruit production is non-seasonal. The anti-bacterial property of *Syzygium samarangense* is effective against *S. aureus*. There is a bigger chance that the leaf extracts of water apple that will be used to develop an anti-bacterial soap to prevent *S. aureus* infections (Rachana 1995).

D.3. Anti-bacterial Property (Flavonoids)

Flavonoids are ubiquitous in photosynthesizing cells and therefore occur widely in the plant kingdom. They are found in fruit, vegetables, nuts, seeds, stems, and flowers as well as tea, wine, propolis, and honey, and represent a common constituent of the human diet. In the US, the daily dietary intake of mixed flavonoids is estimated to be in the range 500 to 1000 mg, but this figure can be as high as several grams for people supplementing their diets with flavonoidsor flavonoid-containing herbal preparations (Raganathan 2000). They have been reported to possess many useful properties, including anti-microbial property (Rajendra 2011). Flavonoids can withstand a temperature of 97°C and lower (Ramidden 2007).

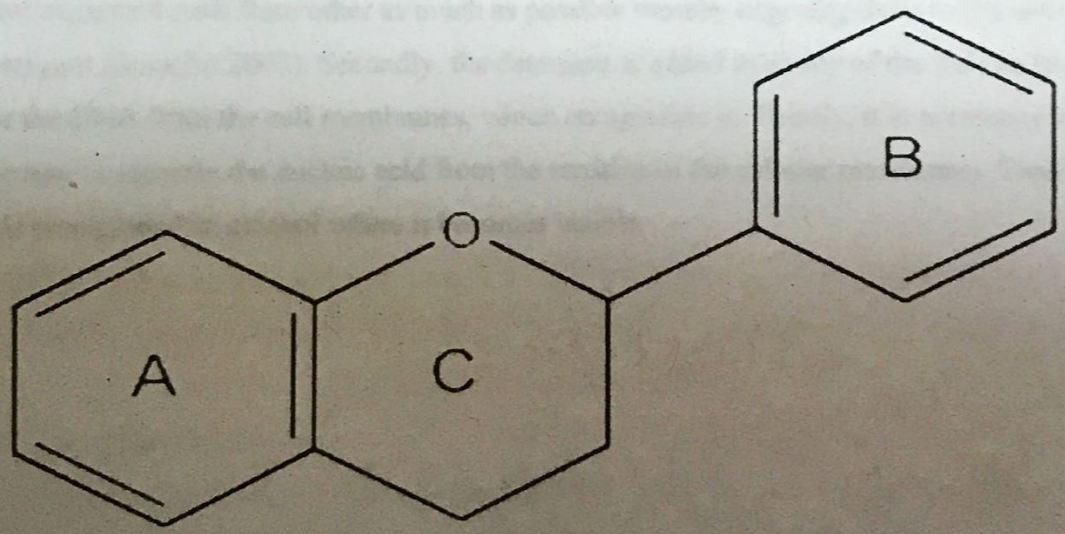

Figure 2. Flavonoid structure present in *S. samarangense*

E. Bacterial Culture

Bacterial culture streaking allows bacteria to reproduce on a culture medium in a controlled environment. This process involves spreading bacteria across an agar plate and allowing them to incubate at a certain temperature for a period of time. Bacterial streaking can be used to identify and isolate pure bacterial colonies from a mixed population. It is used to identify microorganisms and diagnose infections (Reddy 2011).

The materials needed in the process are petri dishes, desired amount of bacteria, inoculating loop, and some filter papers or disks.

Microbiological cultures can be grown in petri dishes of differing sizes that have a thin layer of agar-bases growth medium. Once the growth medium in the petri dish is inoculated with the desired bacteria; the plates are incubated at the best temperature for the growing of the selected bacteria.

F. Extraction

The materials needed in the process of extraction are leaves to be extracted, conical tube for extraction solution, flip-top, test tubes, funnel, thermometer, and ethanol or isopropyl.

The first operation in this procedure is to break-up the fruit into pulp or mush so that the cells are separated each from other as much as possible thereby exposing them to the action of the detergent (Somchit 2003). Secondly, the detergent is added to a pulp of the fruit so as to release the DNA from the cell membranes, which encapsulate it. Thirdly, it is necessary to filter the mixture to separate the nucleic acid from the remains of the cellular membranes. Finally, the DNA is precipitated in alcohol where it becomes visible.

CHAPTER 3

METHODOLOGY

A. Overview of the Study

This study aimed to develop an anti-bacterial soap from the leaf extracts of *Syzygium samarangense*.

The Minimum Inhibitory Concentration of the *Syzygium samarangense* leaf extracts was measured in this study. An anti-bacterial soap was formulated from the leaf extracts of the said plant which may stunt the growth of *Staphylococcus aureus*.

B. Research Design

There were four treatments: Control Group with 0% Extracts in Soap Concentration, 10% Extracts in Soap Concentration, 20% Extract in Soap Concentration, and 30% extract in Soap Concentration. Total trials will be 12 and in each treatment, there were 3 trials. The study was conducted at Philippine Science High School – Western Visayas Campus, Microbiology Laboratory, Jaro, Iloilo City, Iloilo, Philippines. The study was conducted on the month of January in the year 2014.

C. Materials and Equipment

C.1. Extraction

A. *Syzygium samarangense* leaves (3.9 Kg)

B. 80% Aqueous Ethanol (1 L)

C. Digital Weighing Scale

D. 10 mL Pipette

C.2. Culturing Bacteria

A. *Staphylococcus aureus* broth (WVSU)

B. Funnel

C. Glass Container

D. Inoculating Loop

E. Strainer

C.3. Formulation of Soap

A. Lye or NaOH Solution
B. Mineral Oil
C. Distilled Water
D. Beakers
E. Ring or Ring Stand
F. Wire Screen
G. Tongs
H. Burner
I. Stirring Rod

D. Preparation of Media

D.1. Acquiring of Bacteria

Staphylococcus aureus was ordered from West Visayas State University – Microbiology Laboratory. Other equipment needed for culturing bacteria, such as agar bottles, was requested from Philippine Science High School – Western Visayas. The specimen was incubated at a temperature of 37°C for storage.

D.2. Gathering of Leaves

Syzygium samarangense was gathered with the use of scissors and sack. The scissors were used for cutting the leaves from the plant. The sack served as storage of the leaves. The leaves stored were brought to Philippine Science High School – Western Visayas Campus.

E. Extraction

E.1. Soaking of *Syzygium samarangense* leaves

The leaves were air dried. The duration of the drying process was a week long. After the process, the dried leaves were powdered with the use of a blender. The leaf powder was placed in a plastic container and was stored for the soaking process. The powder was measured to be 1 kilogram. The powder was soaked solution was filtered with the use of a filter paper folded into a cone shape and placed on top of a fifty millilitre beaker. The solution was poured in the 50 mL beaker and was placed in a sealed plastic container. The container was placed in a refrigerator for storage.

E.2. Extraction Proper

The solution was sent to the Microbiology Laboratory of the University of San Agustin. And the extraction process was performed by the in-charge professionals of the said laboratory.

F. Formulation of Soap

F.1. Preparation of Chemicals

Mineral oil was purchased in SM Supermarket. The mineral oil was stored in room temperature. Nine hundred grams (900 g) of mineral oil was placed in a separate bowl. Lye, or NaOH solution, was purchased in RS Fragrance. One hundred and thirty millilitres (130 mL) of lye was placed in a container. Distilled water was purchased SM Supermarket. Three hundred millilitres (300 mL) of distilled water was placed in a container and was stored in room temperature. The one hundred and forty grams (140 g) of leaf extracts was prepared.

F.2. Formulation Proper

Lye and water were combined by pouring the lye into the water and stir liquid lye was completely dissolved. The liquid was caustic and not to be touched in anyway. The outside of the bowl was extremely hot as well. Then add the lye mixture to the extracts then to the coconut oil. Stir the mixture for 3-5 minutes until mixture is thick. Spoon soap mixture into molds and allow soap to cool and harden for 24 hours. After the cooling, cut the soap into bars.

G. Experimental Procedure

G.1. Sub-culturing of Bacteria

G.1.1. Formulation of Agar Solution

Ten grams of Mueller-Hinton agar was weighed using a weighing scale and was dissolved with 250 mL distilled water. The agar solution was boiled in one minute until complete dissolution. The solution was transferred to a media bottle and was sterilized in an autoclave at 121°C or 15 psi for 15 minutes. The prepared medium was stored at room temperature.

G.1.2. Transfer of Agar to Petri Dishes

The solution was obtained from the autoclave. Four 100 mm petri dishes were prepared. Each petri dish was filled with the agar solution at about 2/3 full. After filling, the lids of each petri dish were closed. The solution was left to dry until agar will appear jelly-like. For smooth streaking, the agar was observed that no bubble will form. The plates were allowed to cool at room temperature at duration of 24 hours. After the cooling, the plates were stored at a temperature of 4°C.

G.1.3. Streak Plate Method

The stored plates were prepared. The sterile inoculating loop was dipped in a broth culture and was streaked across the plates. The dish was streaked completely. After the streaking process, the dishes were allowed to dry for five minutes. The dried agar was incubated at 35°C.

H. Determining of the Zone of Inhibition

H.1. Preparation of Filter Disks

With the use of a puncher, 12 holes were punched on a filter paper. The filter disks were obtained with the use of tweezers. Each filter disk was dipped in different concentrations of *Syzygium samarangense*-based soap (0%, 10%, 20%, and 30%). The filter disks were used immediately.

H.2. Placement of the Filter Disks

The filter disks with different concentrations were placed on the plate culture. Each disk placed has an equal distance with other filter disks. The disks placed have its entire surface touching the culture. The plates were inverted and placed in an incubator for 24 hours at a temperature of 37°C.

H.3. Measurement of the Zone of Inhibition

The plates were prepared after incubation. With the use of a standard ruler, zone of inhibition of each disk was measured to the nearest millimetre. The data were recorded.

CHAPTER 4

RESULTS AND DISCUSSION

This study aimed to develop an anti-bacterial soap containing 90% ethanolic extracts of *Syzygium samarangense* leaves. Three different *Syzygium samarangense* extract concentrations (10%, 20%, and 30%) on soaps were used for the study with base soap as control. Anti-bacterial activity was evaluated through streak plate method. Zone of inhibition was measured from the cultures of *Staphylococcus aureus*. Zone of inhibition values were analysed through one-way analysis of variance (ANOVA).

A. Soap Development

Base soaps were not fully formed using the base soap containing (a) 10%, (b) 20%, and (c) 30% of the *Syzygium samarangense* ethanolic extracts. The soaps were not fully formed due to the lye solution. The NaOH used in this study was the liquid form of NaOH. The non-developed soaps exhibited a darkish brown coloration. It was observed that with increasing *Syzygium samarangense* extract concentration, the darker the coloration of the non-developed soaps become.

Due to the duration of the time given; the soaps were not developed as processed. The heating process was not done properly as in the process in saponification. In the process of saponification, the mixture of the soap ingredients should have been heated and stirred at the same time. In this study, the heating process of the method was not done.

In the process of saponification, the lye solution and the water must be mixed first. The solution will be hot due to the reaction of the lye to the water. After mixing the lye-water solution, the solution should be heated whilst mixing the other ingredients which are the extracts and the mineral oil.

While heating the soap solution, the mixture must be stirred. In this study, the lye-water solution, extracts, and mineral oil were mixed before the solution was heated. The solution for the soap did not harden and formed soap. The soap tested on the bacterial culture was dripping and oily. There were visible areas that were not stirred and heated properly.

B. Anti-Bacterial Testing

Zone of inhibition of each trial was measured with the use of a calliper. The zones were not measured accurately, due to the streaking. The streaking was not spread properly. Each trial was streaked in a sterilized area. The 10% non-developed soap showed the lowest value of the zone of inhibition, followed by the 20% non-developed soap, and the 30% non-developed soap showed the highest value of the zone of inhibition.

The streaking process should be accurate as possible. The force between each streak should be approximately equal. The gaps between each streak must be equal as possible. In this study, the zone of inhibition is not relevant as it is, due to the streaking method. The plates were not properly streaked in a constant force. The gaps between each streak were not equal. The zones did not have an equal distribution of radius, due to the dripping disks placed in each trial.

In the study of Reddy (2011), *Syzygium samarangense* leaves have an anti-bacterial effect on *Staphylococcus aureus*. Whilst in this study, the soap concentration of the extracts of *Syzygium samarangense* leaves gave no effect against *Staphylococcus aureus*. The chemicals used in the soap must have reacted with the flavonoids that caused the non-effectivity of the anti-bacterial property against *Staphylococcus aureus*. In the process of mixing the soap ingredients, the reaction of the lye solution and the water made the mixture hot. Flavonoids cannot be heated. The heat in the lye-water solution has caused the flavonoids to inactively affect *Staphylococcus aureus*. Due to the inactive antibacterial property of the flavonoids, the zone of inhibition gave no significant difference.

Table 1. Results of One-Way Analysis of Variance (ANOVA).

	Sum of Squares	df	Mean Square	F	P-value	Significance
Between Groups	14	2	7	0.3981	**0.6829**	**Not Significant**
Within Groups	158.25	9	17.5833			
Total	172.25	11				

The results display that the extracts of *Syzygium samarangense* in soap did not fully exhibit an anti-bacterial activity against *Staphylococcus aureus*.

CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

This study aimed to develop an anti-bacterial soap containing 90% ethanolic extracts of *Syzygium samarangense* leaves. Anti-bacterial activity was evaluated through streak plate method. Zone of inhibition was measured.

A. Summary of Results

The findings of the study are as summarized as follows:

1. The soaps were not developed as followed in the process of saponification due to the duration of time given.
2. One-way analysis of variance (ANOVA) followed that all concentrations showed no significant difference ($p<0.05$).

B. Conclusion

The non-developed soaps containing *Syzygium samarangense* extracts have no bacterial-inhibiting effects comparable to that of base soap. Overall, the soap cannot serve as an effective treatment against skin infections with effects comparable to those of base soaps.

C. Recommendation

The use of cold process of saponification is recommended for faster formation of the soap. Streaking pattern should be followed properly. In saponification, the use of solid NaOH would form the soap. Streaking in the methods should be accurate to the reason that the culture would show a clear measurement. Since the non-developed soaps were found no significant difference in this study, higher concentrations of the plant extracts against *Staphylococcus aureus* can be recommended in future researches.

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APPENDIX A

RAW DATA

Table 1. Measurement of the Zone of Inhibition of each trial in each treatment

Plant Extract with Soap Concentration	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Average (mm)
0% Concentration	7	6	6	6.3333
10% Concentration	6	6	8	6.6667
20% Concentration	8	10	11	9.6667
30% Concentration	10	19	14	14.3333

APPENDIX B

PHOTOS

Photo 1. Dried *Syzygium samarangense* leaves

Photo 2. Powdered *Syzygium samarangense* leaves

Photo 3. Mixing of Soap Ingredients

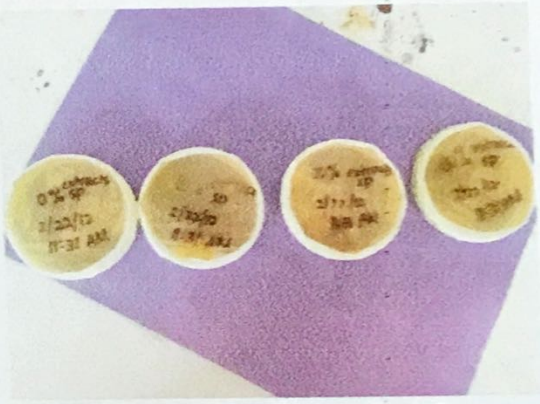

Photo 4.Zone of Inhibition Results