ANTIBACTERIAL EFFECT OF SINAW SINAW (*Peperomia pellucida*) LEAF EXTRACT ON *Staphylococcus aureus*

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ABSTRACT

This study was conducted to determine the effectiveness of Sinaw sinaw (*Peperomia pellucida*) leaf extract as an antibacterial against *Staphylococcus aureus*. The study was conducted at the Central Laboratory of West Visayas State University at La Paz, Iloilo on January 6, 2018.

Three experimental treatments were used: 4 milliliters (100 %) of Sinaw sinaw (*Peperomia pellucida*) leaf extract; (75%-25%) three (3) milliliters of Sinaw sinaw (*Peperomia pellucida*) leaf extract and one (1) milliliter of distilled water; and (50%-50%), two (2) milliliters of Sinaw sinaw (*Peperomia pellucida*) leaf extract were placed in a beaker together with the two (2) milliliters of distilled water. Clindamycin, an antibiotic used to treat certain serious bacterial infections is the positive control and distilled water was used as the negative control. The study was replicated thrice using Duncan's Multiple Range Test with five treatments. Based on the result of the study, Sinaw sinaw (*Peperomia pellucida*) Leaf Extract has an antibacterial effect on *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) 24 hours after incubation. There is a significant difference among the antibacterial effect of the different concentrations of Sinaw sinaw (*Peperomia pellucida*) Leaf Extract on *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) twenty-four hours after the incubation. The results showed that the most effective treatment is the 100% pure Sinaw sinaw *(Peperomia pellucida)* leaf extract. The researchers recommend to use the Sinaw sinaw plant as an antibacterial against the diseases caused by *Staphylococcus aureus* and they also recommend to test its anticancer and antifungal effect for further studies

CHAPTER I

PROBLEM AND ITS SCOPE

Background of the Study

The frequency of life threatening infections caused by pathogenic microorganisms is increased worldwide. Although huge numbers of antimicrobial agents have been discovered, the pathogenic microorganisms are developing resistance against these agents. In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases. Research in the field of indigenous plant is a significant aspect to develop a safer antimicrobial principle through isolation, characterization, identification and biological studies. (Khan, A., 2010)

According to the Clinical Microbiology Reviews, *Staphylococcus aureus* is both a commensal bacterium and a human pathogen. Approximately 30% of the human population is colonized with *Staphylococcus aureus*. Simultaneously, it is a leading cause of bacteria and infective endocarditis (IE) as well as skin and soft tissue, pleuropulmonary, and device-related infections. The past 2 decades have witnessed two clear shifts in the epidemiology of *Staphylococcus aureus* infections: first, a growing number of health care-associated infections and prosthetic device infections, and second, an epidemic of community-associated skin and soft tissue infections driven by strains with certain virulence factors and resistance β-lactam antibiotics.

Doctors and researchers continue to develop several types of antibiotics to treat *Staphylococcus aureus* infections. These developed medicines are used to kill this type of bacteria but only few were proven effective. So the researchers came up with an idea to

use Sinaw sinaw (*Peperomia pellucida*) as an organic treatment instead of using chemically-made medicines to treat *Staphylococcus aureus* infections.

Pansit-pansitan (*Peperomia pellucida*) is a common fleshy shallow rooted herb that has been used as food item as well as a medicinal herb. According to Manila Medical Society *Peperomia pellucida* is used to relieve arthritic pains, but can cause depression. Evaluations of antibacterial, anti-inflammatory and analgesic activities were reported for this plant. A study has isolated a compound called patuloside A, a xanthone glycoside from *Peperomia pellucida* that is found to have broad spectrum antibacterial activity.

Statement of the Problem

Generally, this study aimed to determine the effectiveness of Sinaw sinaw (*Peperomia*

pellucida) leaf extract as an antibacterial against *Staphylococcus aureus.*

Specifically, this study answered the following questions:

1. What is the antibacterial effect of the different concentrations of Sinaw sinaw (*Peperomia pellucida*) leaf extract on *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) 24 hours after incubation?
2. Is there a significant difference in the zone of inhibition (in millimeters) of *Staphylococcus aureus* as affected by the different concentrations of Sinaw sinaw

(*Peperomia pellucida)* leaf extract and Clindamycin (positive control) after 24 hours of application?

3. Which treatment is the most effective as an antibacterial indicated by the zone of inhibition (in millimeters) after 24 hours of application?

Hypothesis

There is no significant difference in the zone of inhibition (in millimeters) of *Staphylococcus aureus* as affected by the different concentration of Sinaw sinaw (*Peperomia pellucida*) leaf extract and Clindamycin (positive control).

Conceptual Framework

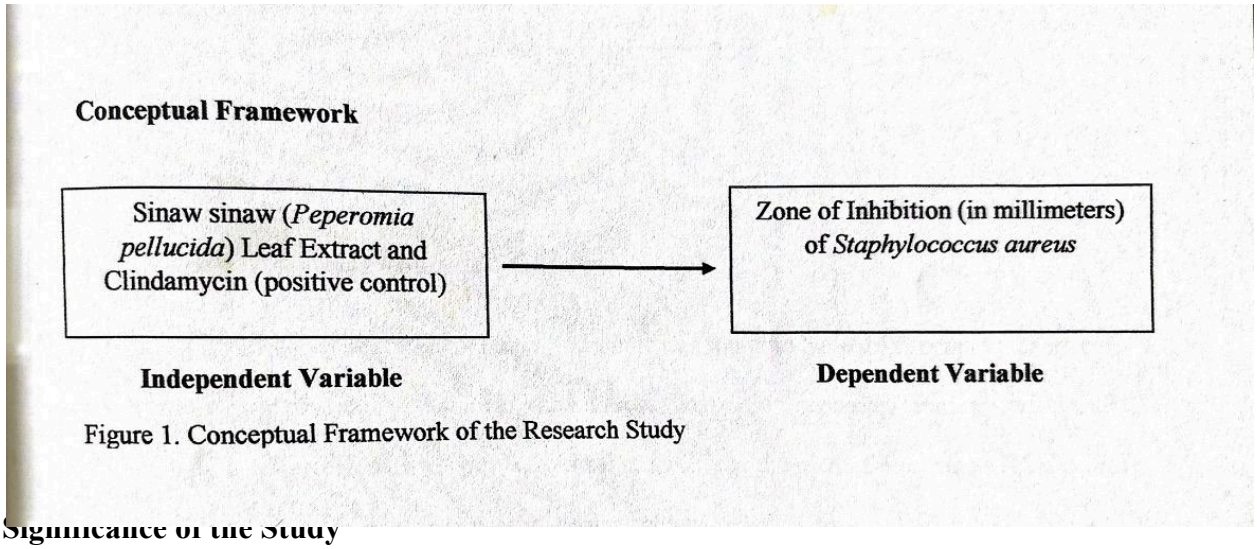

Figure 1. Conceptual Framework of the Research Study

Significance of the Study

The accomplishment of this study can be beneficial to the following:

People in the community. They can have a cheaper and more affordable medicine for

skin condition caused by *Staphylococcus aureus.*

Medical specialists and dermatologists. Doctors could make use of Sinaw sinaw leaf extract as a cure for other skin diseases caused by other bacteria. They could also make other formulations out of the herbal medicine extract.

Future researchers/Scientist. This study could help future researchers and soon to be scientists to discover other uses of benefits of Sinaw sinaw (*Peperomia pellucida*) plant.

Scope and Delimitations

This study was conducted on February 6, 2018 at Central Laboratory of West Visayas State University. This study was limited only to the use of Sinaw sinaw (*Peperomia pellucida*) plant. *Staphylococcus aureus* was provided by the West Visayas State University. In addition, the apparatus needed to perform this study was borrowed from the said institution.

CHAPTER II

REVIEW OF RELATED LITERATURE

Sinaw sinaw (*Peperomia pellucida*) plant

The plant Sinaw sinaw (*Peperomia pellucida*) was found to have different phytochemical constituents. The presence of alkaloids, cardenolides, flavonoids, saponins, tannins, steroid and triterpenoid were revealed on the phytochemical screening of the plant. Sinaw sinaw (*Peperomia pellucida*) is reported to posse antipyretic, analgesic, anti-inflammatory, antimicrobial, refrigerant and CNS activity. People were accustomed to use this plant for medicinal purposes such as medicine for headache, fever, eczema, abdominal pains, and convulsions. Recently, isolation of antifungal and anticancer constituents from this plant was also reported. (Majumder, P.., Abraham, P., Satya V. 2011)

Sinaw sinaw (*Peperomia pellucida*) is a shiny bush or silver bush, belonged to family Piperacee. It is an herbal plant, commonly found in many South American and Asian countries. This kind of specie grows in moist areas and usually develops on rainy seasons and in humid soils under the shade of trees. Each part of this plant has been widely used for different medicinal purposes. Despite being a folk medicine, there is just few scientific documentation accessible on its chemical constituents and as well as its pharmacological and biological activities. (Majumder, et al.. 2011)

Sinaw sinaw (*Peperomia pellucida*) is an annual, shallow rooted herb with succulent stems. It may reach 40 cm high and has an alternate, heart-shaped and turgid leaves. Its heart shaped leaves, measuring about 1.5 cm in diameter, are shiny, watery and can be easily destroyed when matured gradually. Numerous tiny seeds drop off when

matured and grow easily in damp areas. It can be reproduced through seeds and through cuttings. (Teovisio, 2014)

Sinaw sinaw (*Peperomia pellucida*) also known as Silver bush, belongs to the family Piperaceae. It is an herbaceous plant and can be seen in many South American and Asian countries. It has a history of ethno- medicinal uses such as treatment for abdominal pain, abscesses, acne, boils, colic, fatigue, gout headache, renal disorders, rheumatic pain and to treat breast cancer, impotence, measles, mental disorders and small pox. However, Sinaw sinaw (*Peperomia pellucida*) dosing has not yet been validated in clinical trials. Patients with known hypersensitivity reactions to any of the components of the plant species should avoid using it. This species also has contraindications for nursing mothers because it interferes with prostaglandin synthesis. This plant has been used to lower cholesterol level in Guyana and has been used in the Amazon region to suppress cough, diuretic, emollient and treatment of cardiac arrhythmia. Numerous chemical investigations has been conducted and found that Sinaw sinaw (*Peperomia pellucida*) possess essential oils. One study identified 71 compounds from the essential oils of 10 piperaceae species. Flavonoids and phytosterols such as acacetin, apigenin, isovitexin, pellucidatin campesterol and stigmasterol, substituted styrenes and pellucidin A have also been documented. Other compounds like the peperomins with in vitro cytotoxic or anticancer activity and arylpropanoids such as the apiols with antifungal activity have been isolated from Sinaw sinaw (*Peperomia pellucida*) species. (Majumder, et al., 2011)

The Ayurvedic system of medicines had been using Sinaw sinaw (*Peperomia pellucida*) as a drug. It has been reported to posse antipyretic, analgesic, anti-inflammatory, antimicrobial, refrigerant and CNS activity. Traditionally, it is used in

the treatment of headache, fever, eczema, abdominal pains and convulsions. The phytochemical screening of Sinaw sinaw (*Peperomia pellucida*) had shown its various constituents: flavonoids, alkaloids, cardenolides, saponins, tannins, alkaloids, steroid and triterpenoid. The plant's stem also contains alkaloids, tannins, flavonoids and steroids. Its roots had shown the presence of alkaloids, tannins, steroids, carbohydrates and etc. (Majumder, et al., 2011)

The essential oils of Sinaw sinaw (*Peperomia pellucida*) plant were found originally on the medical literature. One study identified 71 compounds from the essential oils of 10 Piperaceae species. Sesquiterpenes appeared to be the major chemical constituents in the essential oils. Carotol (13.41%) was the major hydroxylated sesquiterpene in a chemical analysis of Sinaw sinaw (*Peperomia pellucida*). Flavonoids, phytosterols, arylpropanoids (egapiols), substituted styrenes, and a dimeric ArC2 compound or pellucidin A have been isolated. Antifungal activity has been documented for arylpropanoids such as the apiols. Other compounds, like the peperomins, have cytotoxic or anticancer activity in vitro. Isolated flavonoids include acacetin, apigenin isovitexin, and pellucidatin. Isolated phytosterols include campesterol and stigmasterol. Isolation of antifungal and anticancer constituents from this plant was also reported newly. (Majumder et al., 2011)

Akinibosun, L.A, Akinibosu, F.1, German, BE. (2008) investigated the antibacterial activity of aqueous and ethanolic leaf extract of Sinaw sinaw (*Peperomia pellucida*) on Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa using Agar-well diffusion method. Agar-well diffusion method was used to measure the zone of inhibition. Six Petri-dishes were poured with already sterilized nutrient agar to the level

of obtaining a standard well and allowed to set. The organisms, dissolved in nutrient broth were poured into set petri dishes and uniform distribution was ensured. Sterile cork borer of 10mm diameter was used to punch holes in the agar. Each of the holes (4 in number) in each petri-dish were filled with 0.3ul of the serially diluted extracts and kept in an incubator for 24 hours at 37 OC for the organisms to grow. In this assay the degree of sensitivity was expressed as a measure of the diameter of the inhibition of growth in millimeters. Results showed that E. coli displayed the highest susceptibility in water extract (17.4mm-21.2mm) followed by P. mirabilis (12.4mm-15mm) and least in P. aeruginosa (10.2mm-12.24mm). Conversely, the ethanolic extract showed the highest inhibition in P. aeruginosa (13.4mm-19.6mm) followed by Proteus mirabilis (10.2mm-182mm) and the least was in Escherichia coli (0.0mm-12.2mm). The results revealed that ethanol is better than water as solvent for extraction of Sinaw sinaw (*Peperomia pellucida*) for it to show its highest inhibitory activity on Proteus mirabilis and P. aeruginosa while water is the best solvent for extraction of Sinaw sinaw (*Peperomia pellucida*) for it to show its highest inhibitory activity on E. coli. The results of this investigation support the claims by local practitioners of ethno medicine in the therapeutic efficacies of this herb. The antimicrobial action of the medicinal herb used in this study has shown that the plants extracts is a potential source of antimicrobial agent against *E. coli, P. mirabilis* and *P. aeruginosa* and could be used in the management of nosocomial infection. (Akinnibosun, H. A., Akinnibosun, F.I., German, B.E., 2008)

Staphylococcus aureus

Staphylococcus aureus is both a commensal bacterium and a human pathogen. It is a major human pathogen that causes numerous cases of clinical infections. Approximately 30% of the human population is colonized with *Staphylococcus aureus*. Contemporarily, it is the leading cause of bacteremia and infective endocarditis (TE), osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. (Tong S., Davis J., Eichenberger E., Holland T., and Fowler Jr. V., 2015).

Staphylococcus aureus belongs to the family *Staphylococcaceae.* It is a type of bacteria, stains Gram positive and is non-moving small round shaped or non-motile cocci and is found in grape-like (staphylo-) clusters. *Staphylococcus aureus* affects all known mammalian species, including humans. Further due to its ability to affect a wide range of species, it can be easily transmitted from one species to another. This includes transmission between humans and animals. (Mandal A., 2012)

According to the article in The Journal of Infectious Diseases (2018), the antimicrobial peptides (AMPs) is very important part of the host defense. Limiting the therapeutic potential of AMPs is the fact that bacteria have developed the resistance of the antimicrobial peptides mechanisms during their co-evolution with humans. However, there is no direct evidence that AMP resistance is important during an infection. The *Staphylococcus aureus* transporter defends the bacteria from killing by important human antimicrobial peptides and elimination by human neutrophils. By showing that Pmt contributes to virulence during skin infection in an AMP-dependent manner, we provide evidence that AMP resistance plays a key role in bacterial infection. (The Journal of Infectious Diseases, 2018)

The article from the Nature Reviews Microbiology 15 (2017) says that although human colonization by facultative bacterial pathogens, such as *Staphylococcus aureus*, represents a major risk factor for infections, these pathogens has remained a neglected area of research. *Staphylococcus aureus* colonizes approximately 30% of the human population and recent studies suggest that the composition of highly variable nasal microbiota has a major role in promoting or inhibiting *Staphylococcus aureus* colonization. Understanding the mechanisms of these pathogens will be crucial for the development of new strategies for the prevention of the facultative pathogens. (Nature Reviews Microbiology 15, 2017)

In addition, *Staphylococcus aureus* is a bacterium that infects the human nares and skin,is a frequent cause of soft tissue and bloodstream infections. A hallmark of staphylococcal infections is their frequent recurrence, even when treated with antibiotics and surgical medication, which demonstrates the bacterium's ability to adapt the immune responses. (Nature Reviews Microbiology 13, 2017)

According to Sampedro and Wardenburg (2017) in their article The Journal of Infectious Diseases (2017), *Staphylococcus aureus* is the leading cause of infection in the critical illness and injury. This bacterium causes life-threatening infection in otherwise healthy individuals and complicates the clinical course of patients requiring intensive care as a result of their medical or surgical disease processes. *Staphylococcus aureus* infection in the intensive care unit (ICU) most commonly manifests as sepsis, pneumonia, and infection of surgical sites and indwelling medical devices. With the epidemic spread of methicillin-resistant *Staphylococcus aureus*, many cases of staphylococcal infection in the ICU are now classified as drug resistant, prompting hospital-based screening for this

kind of bacterium and implementation of both isolation practices and decolonization strategies in the ICU patients. (Sampedro, et.al. 2017)

The genetic adaptability of *Staphylococcus aureus*, remains enigmatic and suggesting a need to define a disease classification subtypes that inform disease progression and therapy, *Staphylococcus aureus* infection in the ICU not presents a unique opportunity for individualized risk stratification coupled with the investigation to mitigate this disease. Given the increasing knowledge of the molecular pathogenesis of *Staphylococcus aureus* disease, they suggested that the application of molecular pathological epidemiology to *Staphylococcus aureus* infection can usher a new era of highly focused personalized therapy that may be particularly beneficial in the setting of critical illness and injury. (Sampedro, et.al. 2017)

Rotary evaporation

Rotary evaporation is the process of reducing the volume of a solvent by distributing it as a thin film across the interior of a vessel at elevated temperature and reduced pressure. This promotes the rapid removal of excess solvent from less volatile samples. Most rotary evaporators have four major components: heat bath, rotor, condenser, and solvent trap. Additionally, a vacuum pump needs to be attached, as well as a bump trap and round bottom flask containing the sample to be concentrated. (LibreTexts, 2017)

Clindamycin

According to the article from the Everydayhealth (2014), clindamycin is the generic name of the prescription drug Cleocin, which is an antibiotic used to treat certain serious bacterial infections. It belongs to a group of medicines known as lincosamide or lincomycin antibiotics. It works by stopping bacteria from producing the protein they need to reproduce and spread infection in the body. Clindamycin was first approved by the Food and Drug Administration (FDA) under the brand name Cleocin in 1970, and was manufactured by Pharmacia and Upjohn (now Pizer), It could be used to prevent an infection in the heart before a dental procedure, too, especially for people who may be allergic to take penicillin. It is also prescribed to treat babesiosis, an infection of the blood caused by ticks that have been infected with a specific parasite. (Everydayhealth, 2014)

Clindamycin is used to treat variety of bacterial infections. It is an antibiotic that works by stopping the growth of bacteria. These antibiotic treats only bacterial infections. It will not work for virus infections like common, cold, and flu. Unnecessary use or misuse of any of these antibiotics can lead to its decreased effectiveness. (WebMD, 2018)

Clindamycin is an antibiotic that fights bacteria in the body. It is used to treat serious infections caused by bacteria. It is also used to treat babesiosis and other related diseases. (Drugs.com, 2012)

Agar Well Diffusion Assay

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate surface is inoculated by spreading a volume of the microbial inoculum cover the entire agar surface. Then, a hole with diameter of six to eight millimeters is punched with a sterile cork borer or a tip, and a

volume (20-100L) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then agar plates are incubated under suitable conditions depending upon the test of microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of microbial strain tested. (Balouiri, 2016)

The agar diffusion assay allows bacteria to screen in a routine, economical and easy way for the detection of resistance (Narins, 2003).

Extraction of Phenolic Compounds Using Solvents

According to Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. (2017), the scientists have already studied and analyzed the impact of several types of solvents, such as methanol, hexane, and ethyl alcohol, for the antioxidant extraction from various plants parts, such as leaves and seeds. To extract different phenolic compounds from plants with a high degree of accuracy, various solvents of different polarities must be used. Moreover, scientists have discovered that highly polar solvents, such as methanol, have a high effectiveness as antioxidants. Anokwuru et al. reported that acetone and dimethylformamide (DMF) are highly effective at extracting antioxidants, while Koffi et al. found that methanol was more effective in at a large amount of phenolic contents from walnut fruits when compared to ethanol. (Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.., 2017)

It has been reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol. Multiple solvents have been commonly used to extract phytochemicals, and scientists usually use dried powder of plants to extract bioactive compounds and eliminate the water at the

same time. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the different number of analogous compounds in the desired yield. (Altemimi, A., et. Al. 2017)

Definition of Terms

Sinaw sinaw (*Peperomia pellucida*) **leaves**. In this study, the Sinaw sinaw (*Peperomia pellucida*) leaves were extracted and used as the experimental treatment to test its effectivity against *Staphylococcus aureus.*

Clindamycin. Clindamycin is used as the positive control in this research study.

Staphylococcus aureus. In this study the commercially cultured bacteria, *Staphylococcus aureus* is where the Sinaw sinaw (*Peperomia pellucida*) leaf extract is being applied.

CHAPTER II

METHODOLOGY

This chapter presents the materials and procedure employed in this study.

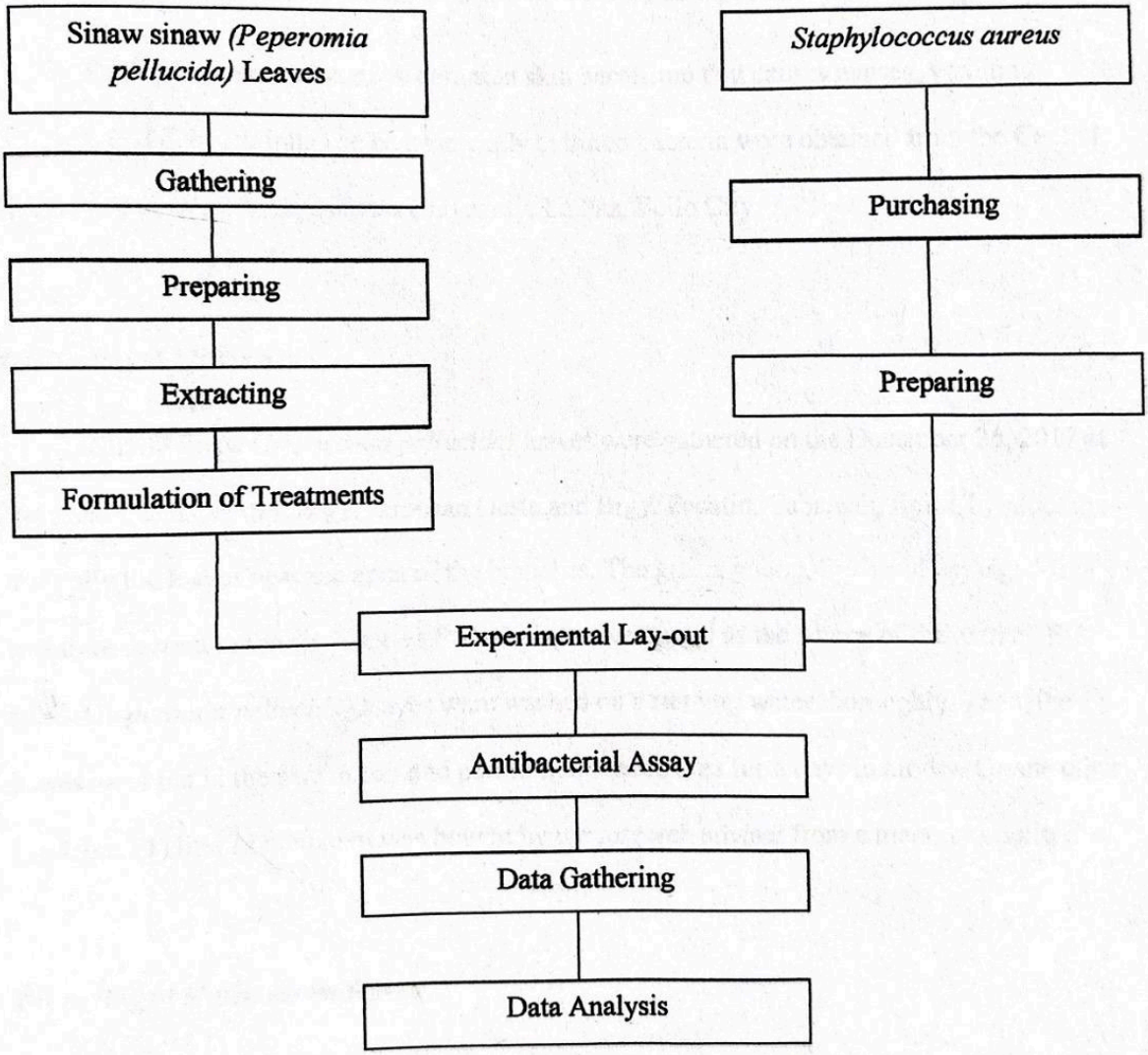

Figure 2.1 Schematic Diagram of the Research Procedure

Description of the Study Variables

Sinaw sinaw plant. A common fleshy shallow rooted herb that grows to about 15cm to 45cm in height in damp and lightly shaded areas. The green, young, fresh and damaged-free leaf from second to the sixth leaves from the apex were used as the source of extract.

Staphylococcus aureus. A common skin bacterium that causes nausea, vomiting, diarrhea, and dehydration. The commercially cultured bacteria were obtained from the Central Laboratory of West Visayas State University, La Paz, Iloilo City.

Gathering of Materials

Sinaw sinaw (*Peperomia pellucida*) leaves were gathered on the December 26, 2017 at Brgy. Janipaan Central, Brgy. Janipaan Oeste and Brgy. Pacatin, Cabatuan, Iloilo, by plucking manually the leaves near the apex of the branches. The green, young, fresh and damaged-free leaf from second to the sixth leaves from the apex were used as the source of the extract. Sinaw sinaw (*Peperomia pellucida*) leaves were washed on a running water thoroughly. Then, the leaves were put in the screen bag and placed in a shaded area for 8 days to air dry. On the other hand, one (1) liter of methanol was bought by the research adviser from a market in Iloilo City.

Preparing of Sinaw sinaw leaves

The Sinaw sinaw (*Peperomia pellucida*) leaves were brought at Cabatuan National Comprehensive High School after 8 days of air- drying for soaking. The leaves were weighed and were cut into tiny pieces. Then, a 210 g of Sinaw sinaw leaves were divided into the two Erlenmeyer flask and 725 ml of methanol was poured into the two flask. The flasks were fully-covered with carbon paper and were kept safely in the science laboratory.

After 72 hours of soaking, the mixture was filtered using a Worthon paper. The filtrate produced was placed in a bottle and safely sealed. Then, it was brought to West Visayas State University (WVSU) Central Laboratory to undergo rotary evaporation.

Formulation of Treatments

Five treatments including the positive and negative control were used in this study: In treatment A (100 %), four (4) milliliters of Sinaw sinaw (*Peperomia pellucida*) leaf extract was prepared to obtain 100%-0% concentration. In treatment B (75%, three (3) milliliters of Sinaw sinaw (*Peperomia pellucida*) leaf extract and one (1) milliliter of distilled water was placed in a beaker to obtain 75%-25% concentration. In treatment C (50%), two (2) milliliters of Sinaw sinaw (*Peperomia pellucida*) leaf extract were placed in a beaker together with the two (2) milliliters of distilled water to obtain 50%- 50% concentration. In treatment D, Clindamycin was used as a positive control.

The 300mg capsule was dissolved in 100ml of distilled water. In treatment E, distilled water served as the negative control.

Experimental Lay-out

The forty petri dishes of bacteria that the researchers used were divided: nine (9) petri dishes were given to each of the three (3) experimental treatments and the positive control, while only four (4) petri dish were set aside for the negative control. Randomized Complete Block Design (RCBD) was the experimental layout used in the study. The petri dishes were assigned to the treatments using lottery method.

Figure 2.2. Experimental Lay-out

Treatment A

T_1R_1	T_1R_2	T_1R_3
T_2R_1	T_2R_2	T_2R_3
T_3R_1	T_3R_2	T_3R_3

Treatment B

T_1R_1	T_1R_2	T_1R_3
T_2R_1	T_2R_2	T_2R_3
T_3R_1	T_3R_2	T_3R_3

Treatment C

T_1R_1	T_1R_2	T_1R_3
T_2R_1	T_2R_2	T_2R_3
T_3R_1	T_3R_2	T_3R_3

Treatment D

T_1R_1	T_1R_2	T_1R_3
T_2R_1	T_2R_2	T_2R_3
T_3R_1	T_3R_2	T_3R_3

Treatment E

T_1R_1	T_1R_2	T_1R_3	T_1R_4

The figures show the layout of the set-ups using RCBD Experimental Design.

Antibacterial Assay

Agar Well Diffusion was used in assessing the antibacterial activity of the treatments on *Staphylococcus aureus*. A loopful of pure *Staphylococcus aureus* cultured in the microbiology laboratory of the West Visayas State University was placed in 20 milliliters nutrient broth. The turbidity of the mixture was adjusted to 0.5 latex McFarland Standard. One hundred microliters of 1.5x 10% CFU/ml suspensions of Staphylococcus aureus were placed into the solidified Mueller-Hinton Agar in each petri dish using a micropipetor. A sterile bent glass rod was used to evenly spread the bacterial inoculum.

A ten milliliter (10 mm) sterile cork borer was used to make a hole on the solidified Mueller- Hinton Agar plates. Four milliliters of different treatments were dropped into each of the hole of the assigned petri dishes using a micropipetor. The inoculated plates were placed in the incubator at 37+2° C degrees Celsius for twenty-four (24) hours.

Data Gathering

After twenty- four (24) hours of incubation, the researchers measured the zone of inhibition of the different concentrations of Sinaw sinaw extract as well as the positive and negative control using the Vernier's Caliper. The researchers located the widest part of the inhibition zone of the treatments in order to get a more accurate result.

Data Analysis

The gathered data were analyzed electronically using statistical tool ANOVA. Analysis of Variance (ANOVA) was employed using Microsoft Excel Analysis Tool Pak in order to determine if there is a significant difference on the Sinaw Sinaw methanolic leaf extract to the *Staphylococcus aureus.* Upon getting data from the said observations, the researchers analyzed the results.

Data of the antibacterial assay undergone single factor Analysis of Variance at 0.05 level of significance with the use of Microsoft Excel Tool Pak in order to find out if there is a significant difference among the zone of inhibition produced by the treatments. Duncan's Multiple Range Test was solved manually and electronically by the researchers to find out which treatment is significantly higher or lower with the other treatments.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results of the study on the antibacterial effect of Sinaw sinaw (*Peperomia pellucida*) leaf extract on *Staphylococcus aureus.*

Zone of Inhibition

Results showed that all the experimental treatments formed a zone of inhibition on *Staphylococcus aureus* and were higher compared to that of the negative control. This means that the experimental treatments possess antibacterial effect against *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) twenty-four hours after incubation.

The experimental treatments A and B have wider zone of inhibition compared to that of the positive control. This means that Treatments A and B are more effective compared to the positive control.

Among the experimental treatments, the 100% Sinaw sinaw (*Peperomia pellucida*) leaf extract has the highest zone of inhibition.

Table 2.1 Zone of Inhibition (in mm) of *Staphylococcus aureus* Affected by the Different Treatments Twenty-four Hours After Application

Treatments	Replications									Treatment Total	Treatment Mean
	I			II			III				
A (100% *Peperomia pellucida* leaf extract)	59.31	60.26	57.18	65.01	62.05	55.10	87.39	62.28	66.10	574.68	63.85[a]
B (75% *Peperomia pellucida* leaf extract and 25% distilled water)	58.02	55.35	37.38	58.02	32.22	60.09	38.15	58.40	66.14	463.77	51. 53[b]
C (50% *Peperomia pellucida* and 50% distilled water)	30.34	30.21	32.28	34.07	30.21	38.02	33.35	44.21	34.30	306.99	34.11[d]
D (+) (Clindamycin)	33.35	55.06	67.34	54.40	40.04	25.25	34.44	30.36	57.15	397.39	44.15[c]
E (-) (Distilled water)	0	0	0	0	0	0	0	0	0	0	0[e]
Grand Total										1742.83	
Grand Mean											38.73

Note: Similar superscripts are not significantly different

ANOVA showed that there is a highly significant difference among the antibacterial effect of the different treatments in terms of the zone of inhibition formed against *Staphylococcus aureus.*

Table 3.1 Analysis on Variance of Zone of Inhibition (in milliiters) of *Staphylococcus aureus* Twenty-four Hours After Application of Different Treatments

Source of Variation	SS	df	MS	F	P-value	F crit	Omega Sqr.
Between Groups	4906.64	4	1226.66	80.09	0.0001	5.19	0.97
Wihin Groups	76.58	5	15.32				
Total	4983.22	9					

Furthermore, DMRT revealed that all the experimental treatments are significantly higher compared to the negative control, and thus was proven to be effective as an antibacterial against *Staphylococcus aureus*. Among experimental treaments, treatment A (100% *Peperomia pellucida* leaf extract) and treatment B (75% *Peperomia pellucida* and 25% distilled water) have highly significant difference compared to the positive control.

It was also revealed that there is no significant difference between the treatment means of 50% *Peperomia pellucida* leaf extract and the positive control (Clindamycin). This signifies that they have the same level of effectiveness against *Staphylococcus aureus*.

CHAPTER V

SUMMARY, CONCLUSION AND RECOMMENDATIONS

This chapter presents the summary of the whole study, its conclusion and recommendation based on the results.

Summary

This study aimed to determine the antibacterial effect of Sinaw sinaw (*Peperomia pellucida*) Leaf Extract on *Staphylococcus aureus.* This study was conducted at West Visayas State University Central Laboratory last February 6, 2018.

There were five treatments made, Treatment A: 100% *Peperomia pellucida* leaf extract, Treatment B: 75% *Peperomia pellucida* leaf extract and 25% distilled water, Treatment C: 50% *Peperomia pellucida* leaf extract and 50% distilled water, Treatment D: the positive control (Clindamycin) and Treatment E: the negative control (Distilled Water). The different treatments were assayed using Agar-well Diffusion Method and the zone of inhibition was measured 24 hours after incubation.

Results revealed that the nutrient agar filled with experimental treatments had a significantly higher zone of inhibition than that of the negative control and the positive control.

Conclusion

Sinaw sinaw (*Peperomia pellucida*) Leaf Extract has an antibacterial effect on *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) 24 hours after incubation.

There is a significant difference among the antibacterial effect of the different concentrations of Sinaw sinaw (*Peperomia pellucida*) Leaf Extract on *Staphylococcus aureus* in terms of zone of inhibition (in millimeters) twenty-four hours after the incubation. Among all the treatments, the results showed that the most effective treatment is the 100% pure *Peperomia pellucida* leaf extract. The 75% *Peperomia pellucida* is also effective than the Clindamycin which is the positive control.

Recommendations

The researchers recommend to use Sinaw sinaw (*Peperomia pellucida*) leaf extract against the diseases caused by Staphylococcus aureus. The use of the whole Sinaw sinaw (*Peperomia pellucida*) plant for further study is also recommended by the researchers. They can use the stem or the roots of the plants to make new studies and discoveries that can benefit the people in the community. Addition of different levels of Sinaw sinaw (*Peperomia pellucida*) treatments are also recommended to prove more the effectiveness of this plant.

The researchers also recommend to test the antibacterial and antifungal effect of the different levels of concentration of Sinaw sinaw (*Peperomia pellucida*) extract on other bacteria and to further explore the phytochemicals of this plant.

The result of this study can help the people in the community to achieve affordable and organic medications for the diseases caused by *Staphylococcus aureus*. Some authorized people can also make other formulations out of this herbal plant. Since the Sinaw sinaw (*Peperomia pellucida)* plant can grow anywhere especially to the rural areas, it is very convenient to study and to make experiments on it.

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APPENDIX A

COMPUTATIONS

Table 1. Zone of Inhibition of Staphylococcus aureus 24 Hours after Application of Treatments

Treatment	Total	Treatment mean
A	574.68	63.85^{a}
B	463.77	51.53^{b}
C	306.99	34.11^{d}
D	397.39	44.15^{c}
E	0	0^{e}

1.

A – 63.85

B – 51.53

D – 44.15

C – 34.11

E – 0

$$C.V. = \frac{\sqrt{\text{error MS}}}{GM} \times 100\%$$

$$C.V. = \frac{\sqrt{15.32}}{38.73} \times 100\%$$

$$C.V. = 10.11\%$$

2.

$$S_x = \sqrt{\frac{EMS}{r}}$$

$$S_x = \sqrt{\frac{15.32}{3}}$$

$$Sx = 2.26$$

3.

p	R_P
2	3.151
3	3.293
4	3.376
5	3.430

4.

Treatment Mean	$S_x \cdot rp$	Rp	Treatment Mean - Rp	Difference
63.85	(3.23) (3.151)	10.18	63.85 - 10.18	53.67
51.53	(3.23)(3.293)	10.64	51.53 - 10.64	40.89
34.11	(3.23)(3.376)	10.90	39.76 - 10.90	28.86
44.15	(3.23)(3.430)	11.08	44.15 - 11.08	33.07
0				

APPENDIX B

PICTORIALS

Plate 1: Plucking of *Peperomia pellucida* leaves

Plate 2: Washing of *Peperomia pellucida* Leaves

Plate 3: Air-drying of *Peperomia pellucida* Leaves

Plate 4: Cutting of *Peperomia pellucida* Leaves

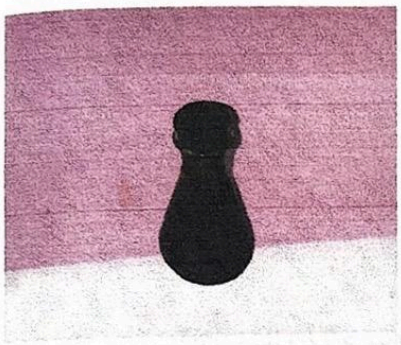

Plate 5: Soaking

Plate 6: Filtration

Plate 7: Rotary Evaporation

Plate 8: Exracted *Peperomia pellucida*

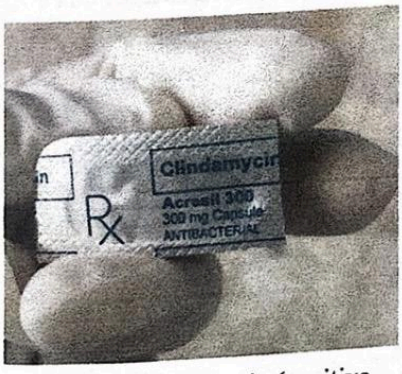

Plate 9: Clindamycin (positive control)

Plate 10: Petri Dishes with agar

Plate 11: Making well on the agar

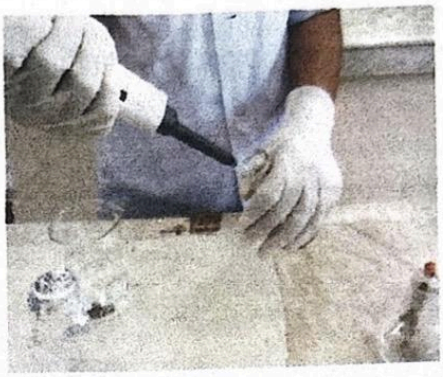

Plate 12: Measuring of solution

Plate 13: Different concentrations of treatments

Plate 14: Applying of treatments

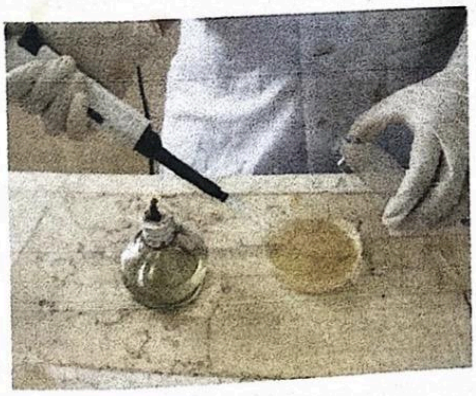

Plate 15: Putting *Staphyloccocus aureus* on agar plates

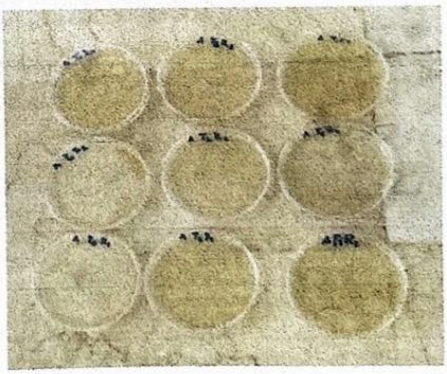

Plate 16: Agar plates with *Staphyloccocus aureus*

Plate 17: Spreading the inoculums on the plates

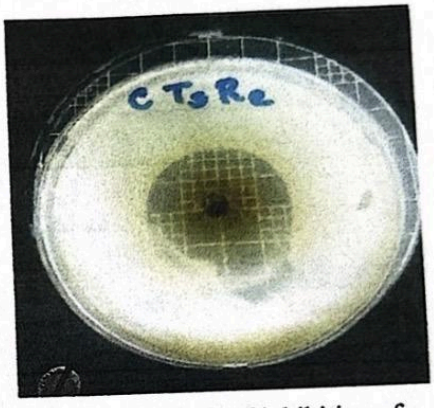

Plate 18: Zone of inhibition of *Staphyloccocus aureus*

Plate 19: Measuring the zone of inhibition of *Staphyloccocus aureus*