ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER AGAINST *STAPHYLOCOCCUS EPIDERMIDIS* AND *STAPHYLOCOCCUS SAPROPHYTICUS*

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Abstract

Bacteria are one of the major causes of urinary tract infection. To ease and prevent the wide spread of it, the researchers test a flowering plant, Hibiscus rosa-sinensis (Gumamela red flower) to fight against Staphylococcus epidermidis and Staphylococcus saprophyticus. The researchers used in vitro testing of gumamela flower ethanolic extract to know its effectiveness against Staphylococcus epdermidis and Staphylococcus saprophyticus. It was tested at different concentrations to identify its capacity to fight against the said causative agent of urinary tract infection Concentration used was 80% and 40% ethanolic extracts. The researcher used the method minimum inhibitory concentration or the MIC as the lowest concentration of an antimicrobial drug that will inhibit the visible growth of microorganism after an overnight incubation in paper diffusion agar. Each ethanolic extract was diluted into 1:1, 1:10, 1:100 and 1:1000 using serial dilution to perform the MIC. Results revealed that gumamela extract is not potent as treatment for the two bacteria. There is no visible zone of inhibition in different concentrations.

Keywords: *Urinary tract infection, Hibiscus rosa-sinensis (Gumamela red flower), Staphylococcus epidermidis, Staphylococcus saprophyticus, Ethanolic extract, Zone of Ihibition*

INTRODUCTION

According to World Health Organization (WHO) 80% of the world population uses herbal medicine as their primary source of medication. The importance of the plants are basically originates due to the presence of specific biological active classes of organic compounds (Joshi and Dhawan 2005). During the last several decades, natural products with antimicrobial effect were investigated in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health. Aromatic plants are known for a very long time and they are used in phototherapy and food preservation. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (ShahlaSahraei, et al. 2014).

Gumamela (*Hibiscus*) flower varies in different color: red, yellow, orange, white, purple, pink, and other color combination. But the researchers are just focusing in the Gumamela red flower, known as the *Hibiscus rosa-sinensis,* the flower is used as the medicinal plant in the Philippines. According to Gilmann(1999), "It is said to be one of the sweetest - smelling flowers in the country. It is also considered as medicinal plant that represents a rich source of antimicrobial agent. *Hibiscus rosa-sinensis* common name is Chinese hibiscus from the family of Malvaceae. It has broadleaf evergreen, flowering pot plant and indoor foliage plant. This popular landscape shrub creates a bold effect with its medium-textured, glossy dark green leaves and vibrantly colored, four to eight-inch-wide, showy flowers, produced throughout the year."

The *Hibiscus rosa-sinensis* known to treat many illness like expectorant, diuretic, anti-infectious, anti-inflammatory, antipyretic anodyne, and refrigerant. It also used as treatment of Bronchitis- as an expectorant, coughs, sore throat, fever- as refrigerant drink, treats dysentery, urinary tract Infection, bladder Infections, High Blood Pressure, prevention of constipation, headaches, boils, swelling, abscesses and mumps, in Venezuela, used to treat tumors, this is according to Tiffany Annetan (2015).

And because *Hibiscus rosa-sinensis is* use as treatment of urinary tract infection, the researcher found out that one of the major causes that can infect urinary tract are the bacteria. The two bacteria that will be used in this study are staphylococcus epidermidis, is often associated with instrumentation

of the urinary tract in a hospital setting, including neonates in the neonatal intensive care unit (M. Keita, et al. 2012), and the other one is the staphylococcus saprophyticus, is the second most common pathogen associated with UTIs, causing 10-20% of all UTIs in sexually active young women (Shikha Paul, et al. 2011). The researcher will be using the pure culture of the said causative agent of urinary tract infection.

Urinary Tract Infections (UTI) are generally one of the most common infections seen in primary care settings. The reason why this study is being created is due to the different urinary tract infections that are commonly encountered now a day. Bacteria are one of the major causes that can infect urinary tract. To ease and prevent the wide spread of it, the researchers will test a flowering plant, *Hibiscus rosa-sinensis* (Gumamela red flower) to fight against this causative agents *Staphylococcus epidermidis* and *Staphylococcus saprophyticus.*

The researchers are focusing in the in vitro testing of gumamela flower ethanolic extract to know its effectiveness against *Staphylococcus epdermidis* and *Staphylococcus saprophyticus*. It will be test on different concentration to identify its capacity to fight against the said causative agent of urinary tract infection.

Staphylococcus epidermidis is part of human skin flora, a commensal one. It can also be found in the mucous membranes and in animals. *Staphylococcus epidermidis* is not usually pathogenic but patients with compromised immune systems are often at risk for developing an infection. These infections can be both nosocomial and community acquired. The ability to form biofilms on plastic devices is a major virulence factor for *Staphylococcus epidermidis*. Strains are often resistant to antibiotics. *Staphylococcus epidermidis* causes biofilms to grow on plastic devices placed within the body (intravenous catheters, medical prostheses). *Staphylococcus epidermidis* has become a leading cause of nosocomial infections. Its ability to produce a thick, multilayered biofilm allows *Staphylococcus epidermidis* to colonise polymer surfaces and consequently a wide range of implanted medical devices, while also providing a degree of resistance to both antibiotics and the host's immune system. Therefore, while not possessing the wide range of toxins and virulence factors of its close relative, *Staphylococcus aureus*, *Staphylococcus epidermidis* remains a clinically important pathogen.

Staphylococcus saprophyticus is considered an important causative agent of urinary tract infection (UTI) in young women, and some earlier studies have reported up to approximately 40% of UTIs in this patient group being caused by *Staphylococcus saprophyticus*. *Staphylococcus saprophyticus* is

identified as belonging to the Staphylococcus genus using the Gram stain and catalase test. It is identitified as a species of coagulase negative staphylococci (CoNS) using the coagulase test. Lastly, Staphylococcus saprophyticus is differentiated from *Staphylococcus epidermidis*, another species of pathogenic CoNS, by testing for susceptibility to the antibiotic novobiocin. *Staphylococcus saprophyticus* is novobiocin-resistant, whereas *Staphylococcus epidermidis* is novobiocin-sensitivy.

The main purpose of the study is to know the antibacterial activity of *Hibiscus rosa-sinensis* ethanolic extract against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. If this would be possible, it will contribute to the knowledge of fellow researchers and to other medical practitioners, and will give a social impact to the readers. It will also lead to a newest and one of the cheapest ways to prevent the spread of *Staphylococcus saprophyticus* and decontamination of *Staphylococcus epidermidis* on catheter.

Review of Related Literature

As we are in the modern days herbal medicine are still in the highest rate of medication. Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care (Kamboj, 2000).

Hibiscus rosa-sinensis is from the well-known family of Malvaceae, it grows as an evergreen herbaceous plant in tropical and sub-tropical regions. Popularly cultivate as an ornamental plant. The specification of plant are very typical i.e. roots are cylindrical of 5-15 cm length, leaves are simple ovate or ovatelancolate about 7-12 cm with coarsely toothed at the apex, flowers are pedicillate with 5 petals of red color (Lalit 2012).According to Rummel (2005), the flowers contain substantial quantities of flavonoids and pro-anthocyanidins, which are good antioxidant, antipyretic (fever-reducing), analgesic (pain-relieving), and spasmolytic (spasm-inhibiting) activities. Other compounds that have been isolated from hibiscus are: quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. The leaves can also apply to boils and the roots were placed on carbuncles, (Andre Lee Garcia, MD and Alvin Florentino, MD (2005).

Gumamela scientifically known as (*Hibiscus rosa- sinensis Linn*) is a shrub that usually grows from 1 meter up to 4 meters high and usually culminated as ornamental plant in the Philippines. Gumamela (*Hibiscus*) flower varies in different color: red, yellow, orange, white, purple, pink, and other color combination. The *Hibiscus* with 5 petals is noted to have medicinal properties. The flowers are considered as astringent, the roots contain

mucilage that is calming on the mucous membranes of the digestive and respiratory tracts. It has also the following medicinal use: expectorant, diuretic, anti-infectious, anti-inflammatory, antipyretic anodyne, and refrigerant. Used as treatment of Bronchitis- as an expectorant, coughs, sore throat, fever- as refrigerant drink, treats dysentery, urinary tract Infection, bladder Infections, High Blood Pressure, prevention of constipation, headaches, boils, swelling, abscesses and mumps, in Venezuela, used to treat tumors in Dominican Republic, used to treat hematomas, and myocardial Injury.

Staphylococcus saprophyticus infections are more commonly seen in young women and elderly men. *Staphylococcus epidermidis* urinary tract infection in healthy pediatric patients is rare and only a few cases have been reported in preadolescent children (M. Keita, et al. 2012). *Staphylococcus epidermidis* is known as the major cause of medical implant device infections such as peripheral or central intravenous catheters (CVCs) (Sara Bastarahang, et al.2014). *Staphylococcus epidermidis* commonly causes infections associated with indwelling central venous catheters, cerebrospinal fluid shunts, prosthetic heart valves, and peritoneal dialysis catheters. When *Staphylococcus epidermidis* is isolated from blood or body fluids in patients without predisposing factors, it is often considered a contaminant. Urinary tract infections caused by *Staphylococcus epidermidis* is often associated with instrumentation of the urinary tract in a hospital setting, including neonates in the neonatal intensive care unit (M. Keita, et al. 2012)

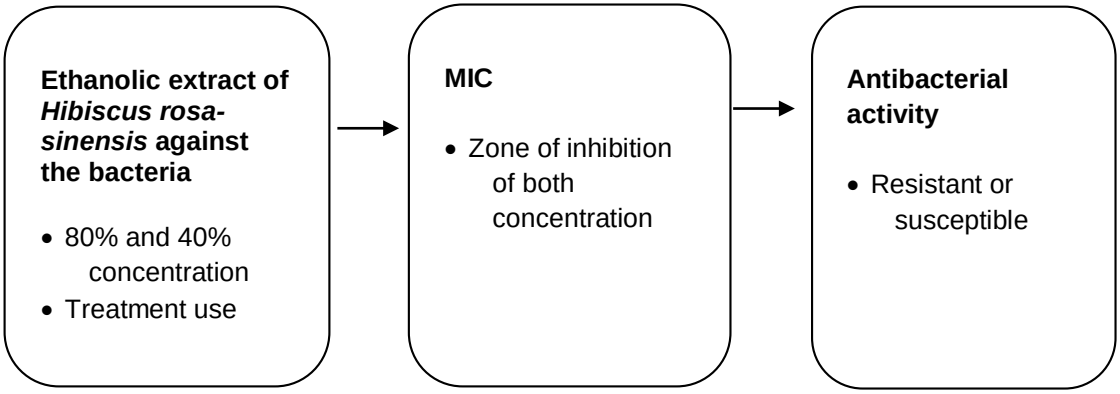
The emergence and spread of vancomycin resistance among staphylococci, although rarely described in Japan to date, are major clinical concerns. We describe a case of central venous catheter-associated bloodstream infection in which *Staphylococcus epidermidis* intermediately resistant to vancomycin (minimum inhibitory concentration, 8 μg/ml) was isolated. The patient fully recovered with removal of the intravenous catheter (Jun Nakajima, et al. 2013)

Staphylococcus saprophyticus is the second most common pathogen associated with UTIs, causing 10-20% of all UTIs in sexually active young women. Like other Staphylococci, *Staphylococcus saprophyticus* is globular and resembles clusters of grapes it colonizes in the urinary tract of humans and is isolated from urine samples. Young women are more susceptible to colonization in the urinary tracts and sexual intercourse promotes its spread. *Staphylococcus saprophyticus* is not normally present in the body. It is also isolated from the carcasses of dead animals. *Staphylococcus saprophyticus* is second only to *E. coli* as the most frequent causative organism of uncomplicated UTI in women. The vast majority of infections occur in young sexually active women. It is referred to as "honeymooner's" UTI due to its

association with intercourse. There are also several case reports of infections in young girls (Shikha Paul, et al. 2011)

Since both of the microorganism used in the study belongs to the genus Staphylococci, vancomycin antibiotic will serve as a control. Vancomycin is the only cell wall active agent that retains activity and is the alternative drug of choice for resistant strains. High-level resistance to vancomycin has not been described in clinical staphylococcal isolates, but strains with minimal inhibitory concentrations in the intermediate range have been encountered. (Diagnostic Microbiology- Bailey and Scotts, 11thed.)

Procedural Framework



Objectives of the study

The general objective of the study is to determine the antibacterial activity of ethanolic extract of *Hibiscus rosa-sinensis* against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The study also aims to determine the activity of *Hibiscus rosa-sinensis* using 80% and 40% concentration of ethanolic extract and to know the relationship of *Hibiscus rosa-sinensis* to *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* by measuring the zone of inhibition expressed in millimeters.

MATERIALS AND METHOD

Gathering Gumamela Flower and Extract

The flowers were bought and authenticated from University of the Philippines- Los Banos. The researchers multiply the growth of the plant until

they gathered the amount needed. The ethanolic extract 500g of fresh flowers of *Hibiscus rosa-sinensis* was done in the University of the Philippines- Los Banos using rotary evaporation.

Agar Preparation

Mueller Hinton Agar is a solid medium originally designed for the isolation of pathogenic Neisseria species, now widely used for antibiotic susceptibility testing (including sulfonamides). It is used in antimicrobial susceptibility testing by the paper diffusion method. This formula conforms to Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS). The prepared media was stored below 8°C, protected from direct light. The dehydrated powder was stored in a dry place, in tightly-sealed containers at 2-25°C. For preparation of the agar 38 g of powder was suspended in 1 liter of distilled water, brought to a boil to dissolve the medium completely and was sterilized by autoclaving at 121°C for 15 minutes. Cultural characteristics were observed after 24 hours incubation at 35°C. (J.H. Müller, J. Hinton, Proc. Soc. Exptl. Biol. 48, 330).

Mueller Hinton Agar is recommended for the disk diffusion method of antibiotic susceptibility testing. Mueller Hinton medium is recommended by FDA, World Health Organization and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria in food and clinical material. The medium shows good batch-to-batch reproducibility. It is low in sulfonamide, trimethoprim, and tetracycline inhibitors and yields satisfactory growth of most non-fastidious pathogens. Paper discs impregnated with certain amount of specific antibiotics are placed on the surface of the medium. The plates are incubated and the zones of inhibition around each disc are measured. Different factors influence the disc diffusion susceptibility tests as inoculum concentration, agar depth, disc potency, medium pH and beta-lactamase production by test organisms.

Mac Farland Turbidity Standard

McFarland Turbidity Standard is an important technique performed in the microbiology laboratory especially when carrying out antimicrobial susceptibility studies. It is a turbid solution that contains a mixture of barium salt, distilled water and tetraoxosulphate (VI) acid (H_2SO_4). McFarland turbidity standard is used to compare and balance the turbidity of both the test and control microorganisms in the microbiology laboratory prior to microbiological analysis.

RESULTS AND DISCUSSION

The aim of this study is to determine the antibacterial activity of ethanolic extract of *Hibiscus rosa-sinensis* against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Thus the study also aims to determine the activity of *Hibiscus rosa-sinensis* using 80% and 40% concentration of ethanolic extract and to know the relationship of *Hibiscus rosa-sinensis* to *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* by measuring the zone of inhibition expressed in millimeters.

Table 1: Results of First Trial of 40% Ethanolic Extract

Serial Dilution	1:1000	1:100	1:10	1:1	Positive Control	Negative Control
Staphylococcus epidermidis	R	R	R	R	S	R
Staphylococcus saprophyticus	R	R	R	R	S	R

R = Resistant; **S** = Susceptible

Table 1shows the results in 40% ethanolic extract for both *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* from dilution 1:1 to 1:1000 shows no zone of inhibition thus graded as resistant.

Table 2: First Trial of 80% Ethanolic Extract

Serial Dilution	1:1000	1:100	1:10	1:1	Positive Control	Negative Control
Staphylococcus epidermidis	R	R	R	R	S	R
Staphylococcus saprophyticus	R	R	R	R	S	R

R = Resistant; **S** = Susceptible

Table 2 shows the results in 80% ethanolic extract for both *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* from dilutions 1:1 to 1:1000 shows no zone of inhibition thus graded as resistant.

Table 3: Second Trial 40% Ethanolic Extract with Diluted Bacterial Suspension

Serial Dilution	1:1000	1:100	1:10	1:1	Positive Control	Negative Control
Staphylococcus epidermidis	R	R	R	R	S	R
Staphylococcus saprophyticus	R	R	R	R	S	R

R = Resistant; **S** = Susceptible

Table 3 shows the result of 40% ethanolic extractfor both *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* from dilution 1:1 to 1:1000 shows no zone of inhibition thus graded as resistant.

Table 4: Second Trial 80% Ethanolic Extract with Diluted Bacterial Suspension

Serial Dilution	1:1000	1:100	1:10	1:1	Positive Control	Negative Control
Staphylococcus epidermidis	R	R	R	R	S	R
Staphylococcus saprophyticus	R	R	R	R	S	R

R = Resistant; **S** = Susceptible

Table 4 shows the result of 80% ethanolic extractfor both *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* from dilution 1:1 to 1:1000 shows no zone of inhibition thus graded as resistant.

CONCLUSION AND RECOMMENDATION

Conclusion

The researchers used *Hibiscus rosa-sinensis* ethanolic extract that is

tested against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The researchers found out that the 80% and 40% extract concentration that are folded into four dilutions are resistant among the two bacteria. It is shown in their experiment proper conducted at the Lyceum of the Philippines-Laguna, that there is no zone of inhibition even in different concentration.

The researchers concluded that the ethanolic extract of *Hibiscus rosa-sinensis* is not potent to *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The researchers found out that *Hibiscus rosa-sinensis* cannot be used as alternative medicine in treating of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* infection.

Recommendation

For the recommendation, the researchers wanted the readers, fellow medical technology students, next researchers, and any other individual capable of doing this kind of study to look for other bacteria other than *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The researchers recommended a flower plant that has a higher antimicrobial activity that can inhibit the production of bacteria. They also suggest the use different parts of the plant such as roots, barks, and leaves in a higher percentage of concentration of extract or even in a use of different method of extraction. The researchers also recommend using other parts of the plant aside from flower, like leaves and its bark.

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