COMPARATIVE STUDY OF PARAGIS (*Eleusine indica*) AND GUAVA (*Psidium guajava*) METHANOLIC LEAF EXTRACT AGAINST *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

This study was intended compare the antimicrobial properties of Paragis (*Eleusine indica*) and Guava (*Psidium guajava*) Methanolic Leaf extracts against Escherichia coli and Staphylococcus aureus that were investigated using standard microbiological methods. The 300g powdered Paragis (*Eleusine indica*) and 300g Guava (*Psidium guajava*) leaves were macerated with 900 ml of methanol each plant sample for three (3) days and occasionally shaking for seven (7) days. These macerated samples were extracted using rotary evaporator. The efficacy of these extracts was tested against Escherichia coli and Staphylococcus aureus using disc diffusion method. The findings showed that the methanolic leaf extract of Paragis (*Eleusine indica*) has inhibitory effects against gram-positive bacteria Staphylococcus aureus with mean zone of inhibition of 46 mm and the gram negative bacteria Escherichia coli with mean zone of inhibition of 22 mm, whereas Guava (*Psidium guajava*) also showed an inhibitory activity against Staphylococcus aureus with mean zone inhibition of 19 mm; however, it is not capable to produce an inhibitory effect to Escherichia coli. On the basis of the present findings, the researchers conclude that Paragis methanolic leaf extract may also be a good natural antimicrobial agent. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties.

Key words: *Eleusine indica, Psidium guajava, Staphylococcus aureus, Escherichia coli*

INTRODUCTION

Asia is among the regions with the highest prevalence rates of healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) and community-associated methicillin-resistant *S. aureus* (CA-MRSA) in the world (Elsevier, 2018). A total of 94 cases from 2010 to 2012 were diagnosed to have *Staphylococcus aureus* infection using conventional bacteriologic methods. From these cases, 38 (40.6%) were identified as MRSA and 37 (39.4%) were inducible clindamycin resistant. Wounds and abscesses were considered to be the most common specimens with MRSA infections having 71.05% while blood was the least with 5.3%. The researchers concluded that less than 20% of *S.*

aureus which was isolated mostly from the wounds and abscesses of admitted patients, of a tertiary hospital in Bacolod City (Juayang, Delos Reyes, de la Rama, and Gallega, 2014).

Escherichia coli causes 80% of community-acquired infections while some strains show resistance as a result of wide and frequent inappropriate use of antibiotics (Vranic and Uzunovic, 2016). Many herbal products, such as Paragis (Eleusine indica), have traditional uses that are now being studied to produce evidence base that will lead to the involvement in general medical practice. Paragis (Eleusine indica) or goosegrass, generally considered an adventitious species, is native in the tropics and subtropical regions (Al-Zubairi, et al., 2011). Some of the secondary metabolites of Paragis are flavonoids, tannins, alkaloids, cardiac glycosides, anthracene glycosides and anthraquinones (Morah & Otuk, 2015).

The Guava (Psidium guajava) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. In this study, the researchers aimed to evaluate the total extracts of P. guajava leaves, growing at Fort Valley State University, using various aqueous and organic solvents to establish if it is effective against killing or inhibiting the growth of foodborne bacterium Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, and Bacillus cereus which can cause foodborne illness and spoilage (Biswas, Rogers, Mclaughlin, Daniels, & Yadav, 2013).

Therefore, this study aimed to compare the antimicrobial property of Paragis (Eleusine indica) and Guava (Psidium guajava) utilizing methanolic leaf extracts against Escherichia coli and Staphylococcus aureus. The researchers' aim was only limited in determining if the Paragis (Eleusine indica) and Guava (Psidium guajava) can be susceptible against Escherichia coli and Staphylococcus aureus.

Research Questions

This study aimed to determine the antimicrobial property of Paragis *(Eleusine indica*) and Guava *(Psidium guajava)* methanolic leaf extract against *Escherichia coli and Staphylococcus aureus.* Specifically, this study attempted to answer the following:

1. What are the phytochemical constituents of the extract of Paragis *(Eleusine indica) and* Guava (*Psidium guajava)?*

2. What are the zones of inhibitions produced by the Paragis *(Eleusine indica)* and Guava (*Psidium guajava),* positive *(amoxicillin)* and negative controls (*distilled water)* when tested against *Escherichia coli and Staphylococcus aureus?*
3. Is there a significant difference in the antimicrobial property of the positive control *(amoxicillin)* and the experimental controls Paragis (*Eleusine indica*) and Guava *(Psidium guajava*) against *Escherichia coli*?

4. Is there a significant difference in the antimicrobial property of the positive control *(amoxicillin)* and the experimental controls Paragis (*Eleusine indica*) and Guava (*Psidium guajava)* against Staphylococcus aureus?

5. Which of the two experimental controls is more sensitive to the two microorganisms?

Hypotheses

1. There is no significant difference in the antimicrobial property of the positive control (*amoxicillin)* and the experiment control of Paragis (*Eleusine indica*) and Guava (*Psidium guajava)* against *Escherichia coli.*

2. There is no significant difference in the antimicrobial property of the positive control *(amoxicillin)* and the experimental controls Paragis (*Eleusine indica*) and Guava (*Psidium guajava)* against *Staphylococcus aureus.*

3. There is no significant difference in the zone of inhibition produced by the Paragis *(Eleusine indica)* and Guava (*Psidium guajava),* positive (amoxicillin) and negative controls when tested against*, Escherichia coli and Staphylococcus aureus.*

Significance of the Study

This study was conducted primarily to inform how beneficial Paragis (Eleusine indica) and Guava (Psidium guajava) are to the community especially to those individuals where medical health services are difficult to avail. This research study could help the researchers to discover new drug formula using Paragis (Eleusine indica) and Guava (Psidium guajava) for treating diseases in the Philippines since there is already an increasing rate of bacterial resistance in

certain pharmaceutical drugs. As an alternative to resistant drugs, it will also help people who cannot afford to buy such expensive antibiotics. This study will contribute to the advancement of the database in discovering new ways to treat certain diseases and in order for the Medical Technology students to practice their theoretical learnings into a knowledge that may be used in the laboratory set up for their future profession.

Literature Review

Ethnobotany of *Eleusine indica*

Paragis or goosegrass as its common name is generally considered as an adventitious species. It may be seen in tropical and subtropical areas especially in waste places, along river banks, roads, and settled areas in the Philippines. *Eleusine indica* in the Philippines is one of the medicinal plants that have been traditionally used for the treatment of several diseases like kidney problems, diabetes, and gastrointestinal diseases and as a diuretic. It is commonly known as "Bila-bila", “Bakis-bakisan” and “Parag-is” (Responte, Dacar, Nuñeza, and Uy, 2015).

It has a broad tolerance to a wide range of environmental conditions, but its vegetative growth is significantly reduced during dry seasons. The root also has been reported in Malaysia with a depurative, diuretic, febrifuge and laxative effect, and hence used as a treatment for influenza, hypertension, oliguria, and urine retention. The seed, on the other hand, is sometimes used as a famine food and also used as a treatment for liver diseases (Al-Zubairi, *et.al.,* 2011).

Eleusine indica is also used as herbal medicine in different countries. The plant is examined with low in protein (2.21%) but rich in minerals with an ash content of 8.40%. The total carbohydrate is 80.19% with fiber content accounting for 27.5%. The leaf is very rich in fiber and may be a good source of cellulose for the paper industry. The phytochemical constituents of Paragis (*Eleusine indica*) are Flavonoids, Tannins, Saponins, Alkaloids, Cardiac glycosides, Anthracene, glycosides, Anthraquinones (Morah, and Otuk, 2015).

Ethnobotany of *Psidium guajava*

Guava (Psidium guajava) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. Many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Biswas, et. al., 2013).

It also evaluates the effectivity against killing or inhibiting the growth of foodborne bacteria Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, and Bacillus cereus. Results indicates that the plant extracts have no antibacterial effect on the gram negative bacteria particularly E.coli and B. cereus showing that they do not contain active ingredients against the organisms but show inhibitory effects on the gram positive organisms S. aureus and S. enteritidis (Biswas, et. al., 2013).

Escherichia coli

Although most strains of *Escherichia coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, *Escherichia coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (Centers for Disease Control, n.d).

The resistant strains of the bacterium, which can cause urinary tract infections and blood poisoning, are harder to treat and more deadly than non-resistant *Escherichia coli.* Today, of the 30,000 cases of *E. coli* blood infections reported each year, 10% is thought to be resistant (Gallgher, 2013). *Escherichia coli* causes 80% of community-acquired infections while some strains show resistance as a result of wide and frequent inappropriate use of antibiotics (Vranic, and Uzunovic, 2016).

Researches show that *Escherichia coli* is resistant to the ethanol extract, but becomes susceptible to the methanol extract (Morah, and Otuk, 2015). Only Gram-positive bacteria, *Bacillus cereus,* and *Staphylococcus aureus* were susceptible to the Guava *(Psidium guajava)* methanolic and ethanolic extracts, while neither of the Gram-negative bacteria, *Salmonella enteritidis* and *Escherichia coli* showed any inhibition (Biswas, et. al., 2013). E. coli is resistant to the ethanol extract but becomes susceptible to the methanol

Staphylococcus aureus

Staphylococcus aureus is one of the main causes of human infections. It can cause diseases ranging from minor infections such as pimples and boils to serious systemic fatal infections. *Staphylococcus aureus* strains have been effectively able to adhere to and colonize the skin and mucosa of nares, to invade the bloodstream, to evade host immunological responses, to form protective biofilms, and to develop resistance to several antibiotics. Consequently, despite the availability of many antibiotics with activity against wild-type strains, *S. aureus* is a highly successful and increasingly clinically important gram-positive pathogen (Naber, 2009).

The guava leaf extracts and essential oil are very active against *S. aureus*, thus making up important potential sources of new antimicrobial compounds. Only Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* were susceptible to the Guava *(Psidium guajava)* methanolic and ethanolic extracts, with the zones of inhibition with 10 mg/50 μL, the methanol extract had a slightly higher antibacterial activity with mean zones of inhibition 8.27 and 12.3 mm than ethanol extract with mean zone of inhibition 6.11 and 11.0 mm against B. cereus and S. aureus, respectively. Antibacterial screening has been done selectively by many researchers in guava essential oil and solvent extract (Biswas,et.al, 2013).

Amoxicillin

Amoxicillin is a widely-used antibiotic drug. It belongs to the penicillin group of drugs and is prescribed to treat certain infections that are caused by bacteria.

The widespread use of antibiotics in the past 80 years has saved millions of human lives, facilitated technological progress and killed incalculable numbers of microbes, both pathogenic and commensal (Langdon, Crook, and Dantas, 2016). The researchers provide evidence that hla and α-toxin expression and total hemolysis do not differ significantly when amoxicillin, gentamicin, or moxifloxacin is added to in vitro cultures of strain Staphylococcus aureus 8325-4 and that these antibiotics are well-suited for the treatment of localized chronic Staphylococcus aureus infections, when they are used at even sub-inhibitory concentrations, because they reduce the level of hemolysis by decreasing bacterial numbers (Dieter, et al., 2001).

Amoxicillin is an amino-penicillin, created by adding an extra amino group to penicillin, with the goal of battling antibiotic resistance. Amoxicillin covers a wide variety of gram-positive bacteria, with some added gram-negative coverage compared to penicillin. Similar to penicillin, it covers most Streptococcus species and has improved coverage of Listeria monocytogenes and Enterococcus. It also has coverage over Haemophilus influenzae, Escherichia coli, Actinomyces, Clostridialspecies, Salmonella, Shigella, and Corynebacteria. Amoxicillin is FDA approved for the treatment of genitourinary tract infections, ear, nose, and throat infections, lower respiratory tract infections (Akhavan, & Vijhani, 2018).

Traditional and Alternative Medicine Act Of 1997

Using herbal medicine was a practice a long time ago. The community utilizes their available resources in order to prevent and manage their diseases such as infections. Herbal medications are not only considered for their effective and cost-effective way of preventing or managing infections but, also, due to their constant availability in the community.

Republic Act 8423 (RA 8423) also known as the Traditional and Alternative Medicine Act of 1997 is focused on developing different traditional health-related management in the country. Drugs for prevention, cure, lessening signs and symptoms, diagnosis and maintaining a healthy lifestyle with lower price are needed to explore and develop. The alternative medications undergo methods of proper compounding (Nolledo, 2015).

This law encourages the indigenous people to share their traditional medicines and for people to study more about the safety and effectiveness of these alternative medicines. The healthcare professionals should become aware of these alternative medications and promote to their patients. By this, our countrymen would encounter more alternative medicines coming from that cost much lesser than existing drugs. The cheaper the medicines get, the more patients will comply with medication (Nolledo, 2015).

Research Paradigm

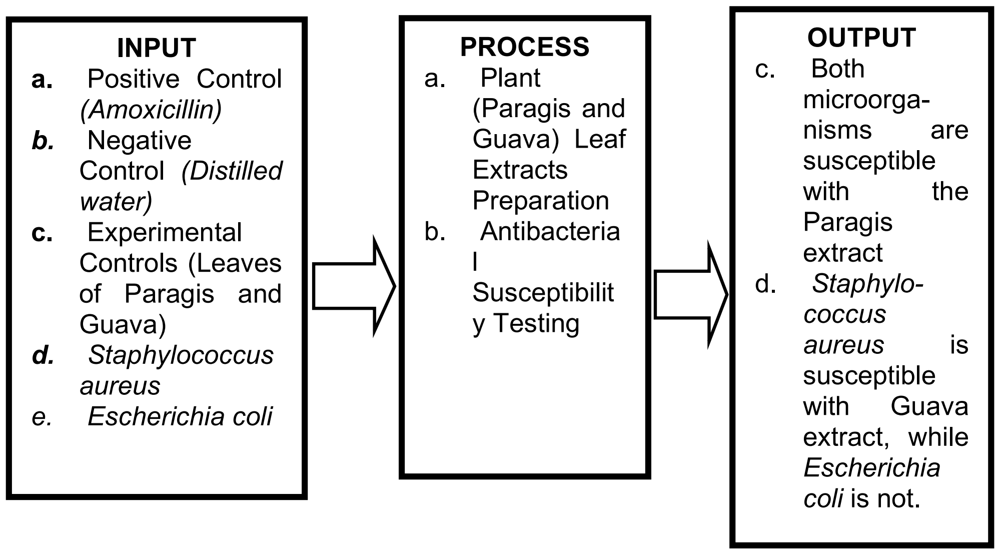

Figure 1. *Research Paradigm*

Figure 1 describes the concept of the whole study wherein the input consists of the following: Positive Control *(Amoxicillin)*, Negative Control *(Distilled water)*, Experimental Controls Paragis *(Eleusine indica)*, and Guava *(Psidium guajava)*, it also includes *Escherichia coli*, and *Staphylococcus aureus*.

On the other side, the process being used is through extraction of samples (leaves of Paragis and Guava were macerated), and Antibacterial Susceptibility Testing. The output is the antibacterial activity of both extracts against the tested organisms.

The researchers wanted to find out the antibacterial activity of the Paragis (Eleusine indica), and Guava (Psidium guajava) methanolic leaf extracts against Escherichia coli, and Staphylococcus aureus.

METHODS

Research Design

This study used an experimental design with the procedures that are related to Medical Technology profession, using Paragis (Eleusine indica) and Guava (Psidium guajava) methanolic leaf extract; utilized 300g sample immersed methanol for three (3) days with occasional shaking of seven (7) days. This study also used positive (amoxicillin) and negative control (distilled water) to compare the results of the study. The study was focused and limited only to the in vitro analysis of antimicrobial property of Paragis (Eleusine indica) and Guava (Psidium guajava) leaf methanolic extract against Escherichia coli and Staphylococcus aureus. The other parts of Paragis plant and other bacterial species and microbes are beyond the researchers' concern.

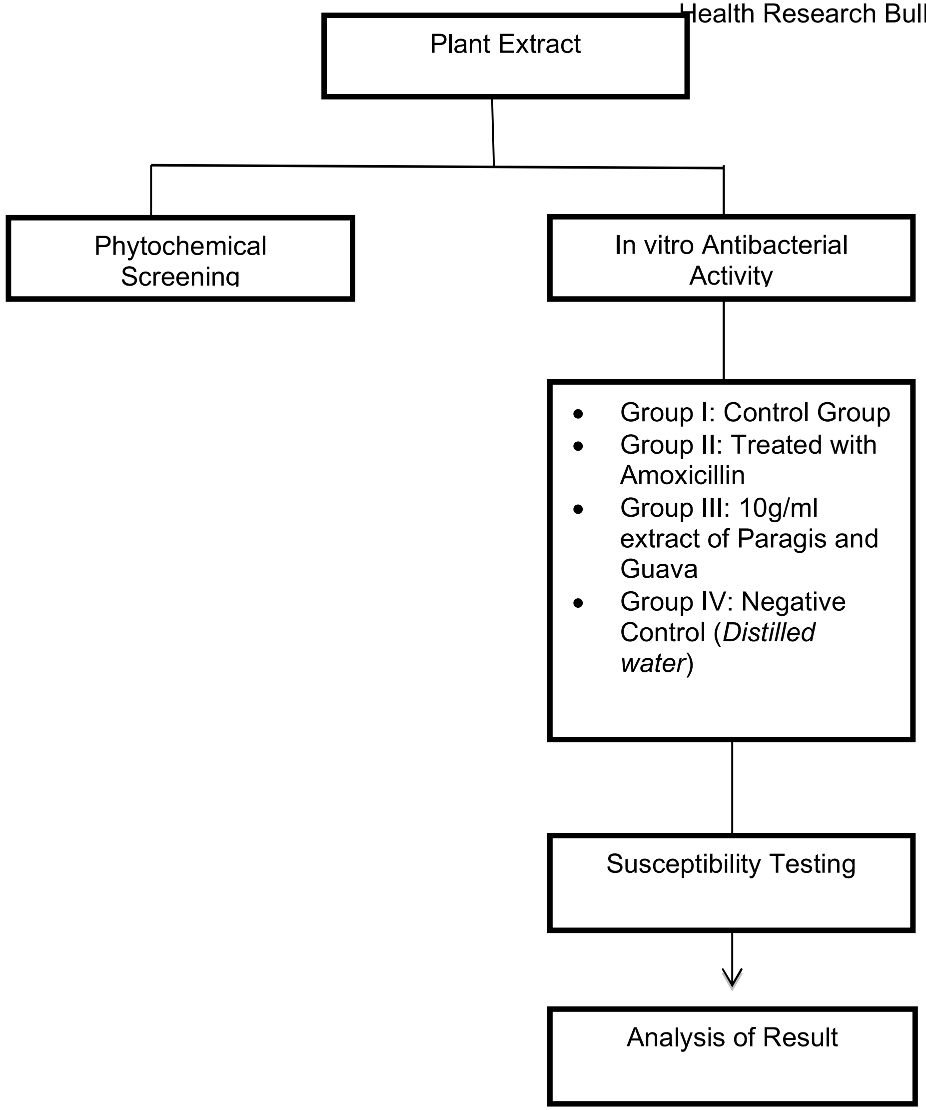

Figure 2. *Methodological Work Flow*

Locale of the Study

The study was conducted at the Medical Technology Laboratory of the School of Health Sciences of the University of Saint Louis (USL), Tuguegarao City, Cagayan and Department of Science and Technology Regional Office No.

02 (DOST RO2) Regional Standards and Testing Laboratory, Bagay Road, San Gabriel, Tuguegarao City, Cagayan.

Subject of the Study

The study utilized Paragis (Eleusine indica) and Guava (Psidium guajava) methanolic leaf extracts immersed for three (3) days with occasional shaking of seven (7) days. The researchers' criteria in selecting Paragis plant include the following: a) The Paragis (Eleusine indica) and Guava (Psidium guajava) should be fresh with green colored leaves, and b) It should be free from withered leaves before collection.

The study also utilized the Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli, for the evaluation of the antimicrobial property of plant extracts.The bacterial strains were obtained from the DOST RO2 Regional Standards and Testing Laboratory at Bagay Road, San Gabriel, Tuguegarao City, Cagayan.

Data Gathering Procedure

1. Collection and Preparation of Plant Materials

The plant materials utilized in the study were fresh Paragis *(Eleusine indica)* and Guava *(Psidium guajava*) leaves. *Eleusine indica* leaves were collected from a cultivated garden at Gumarueng, Piat, Cagayan and the Guava *(Psidium guajava)* at Naganacan, Cauayan City, Isabela. These were transported to the Medical Technology Laboratory of the School of Health Sciences of the University of Saint Louis (USL), Tuguegarao City, Cagayan.

2. Plant Extraction. The following procedures were based on the study of Al-Zubairi, *et.al.,* (*2*011).

2.1. The plant were washed using tap water and distilled water, respectively.
2.2. The rinsed samples were then air dried.
2.3. Leaves were powdered using a clean blender before cold maceration for 72 hours.
2.4. The extraction process for the two (2) experimental controls were conducted using powdered leaves with the solvent Methanol in order to increase the polarity and to dissolve the components of the leaves.
2.5. The extraction took seven (7) days of occasional shaking.
2.6. The solution was filtered through Whatman No. 41 filter paper.

2.7. Finally, rotary evaporator was used to remove the methanol from the two (2) plant extracts. The extracts were stored in a refrigerator at 4°C until these were required to be used in the experiment

3. Phytochemical screening: It was done by the DOST RO2 Regional Standards and Testing Laboratory at Bagay Road, San Gabriel, Tuguegarao City, Cagayan. (Guevarra et.al, 2005).

3.1 Detection of saponins

Froth test. Extracts will be diluted with the 20 ml distilled water and this will be shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

3.2 Detection of tannins

Gelatin Test. To the extract, 1% gelatin solution containing sodium chloride will be added. Formation of white precipitate indicates the presence of tannins.

3.3 Detection of Flavonoids

About 5 ml of each aqueous extracts will be added with 1% NH_3 solution. A positive test result will be confirmed by the formation of a yellow coloration or turbidity.

4. Antibacterial Assay. The following procedures are based from Guevara, 2005, that used by the DOST RO2 Regional Standards and Testing Laboratory.

4.1 Equipment/Materials

The following are the important materials and equipment needed for the susceptibility testing: Incubator set at 35°C-37°C ambient air, refrigerator (approximately at 2°C-8°C), autoclave set at 121°C, hot air oven (50-70°C for 5-10 minutes), top loading balance, Erlenmeyer flask, magnetic stirrer, biosafety cabinet level 2, pH meter, Petri dishes, test tubes with screw-capped, sterile cotton swab, stirring rod, forceps, puncher, cork, Whatman filter paper no. 41, parafilm, nutrient agar, normal saline solution (NSS), Amoxicillin (positive control) and distilled water (negative control).

4.2 Sterilization of materials

To ensure that no contamination will occur, all the materials that are necessary for the sterilization process were autoclaved at 121 degrees Celsius for 30 minutes at 15 psi of pressure.

4.3 Reagent for Disc Diffusion Method

Mueller Hinton medium was poured with 25-30 ml into 100 mm plates or 60-70 ml into 150 mm plates on a level surface to a uniform depth of 4 mm. To avoid the collection of moisture, the plates with agar were left slightly open and if some of the plates have moisture, incubate plates at 35°C for 10-30 minutes or at room temperature (22-24°C) for 1 hour before use. The medium was allowed to harden.

The final pH of the medium should be 7.2 to 7.4. The sample plates were incubated in 30-35 °C overnight to check the sterility. The plates were used immediately or it may be refrigerated to be used within seven (7) days if medium shows no sign of dehydration.

4.4 Preparation of Susceptibility discs

Amoxicillin (positive control) and distilled water (negative control) were maintained under anhydrous storage conditions below 8°C for maximum stability. Replace the desiccant when indicator changes in color. The susceptibility discs were removed from the refrigerator to room temperature 1 to 2 hours before use to minimize the possibility of condensation. Lastly, for the pure extract, the sterilized 6 mm filter paper disc was prepared by immersing it to 20 µL of pure extract

4.5 Inoculum Preparation

The inoculum was only used within 15 minutes of preparation. The cotton swabs were used to touch four to five isolated colonies in Nutrient Broth plate to be tested. The cotton swabs with colonies were suspended in 5 ml of sterile normal salt saline. The saline tubes were whirled to create a smooth suspension.

4.6 Inoculation of MHA Test Plates

The sterile cotton swab was dipped into the inoculum tube. The swab was rotated against the side of the tube (above the fluid level) to ensure that the swab was not dripped wet. Bacterial suspensions of the three clinical isolates were prepared based on the 0.5 McFarland turbidity standard. Each dried surface of an MHA agar plate will be inoculated by streaking the wet swab four to five times over the entire agar surface; the plates were rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. In each plate, the different sterile cotton swabs were used. The swabs were discarded into an appropriate container. The lids of the inoculated plates were left slightly opened. For the surface of the agar plate to dry, the plates were allowed to sit at a room temperature of at least 3 to 5 minutes, but not more than 15 minutes.

The plates utilized in different species were two (2) plates for each control; three (3) replicates of control for each of the plates. One piece of 6 mm soaked filter paper disc was placed gently into respective location as well as to the positive control disc (amoxicillin) and negative control (distilled water). Each disc was gently pressed down with sterile forceps to ensure complete contact with the inoculated plates. For each plate, different forceps were used to avoid contamination. A minimum spacing of 24 mm from disc to the center must be observed to avoid overlapping of the zone of inhibition. After 15 minutes of applying the discs, the inverted plates were aerobically incubated at 35 degrees for 24 hours. After 24 hours, the diameter of complete inhibition was measured in millimeter using caliper.

Data Analysis

The researchers used one-way ANOVA (Analysis of Variance) and T-tests to determine whether there is a significant difference in the different methanolic leaf extract of Paragis *(Eleusine indica)* and Guava *(Psidium guajava)*

Waste Management

At the conclusion of the experiment, all plates were disinfected for safe disposal. The proper way to dispose bacterial cultures was to autoclave the plates in a heat-stable biohazard bag. Wear proper safety equipment when working with the bleach solution because it is known to be corrosive.

Ethical Consideration

For the collection of plants, no specific permits were required for the described field study. For the locations/activities, permission was required. All locations where the plants were collected are not privately-owned or protected in any way.

DOST RO2 Regional Standards and Testing Laboratory was responsible for the disposal of microorganisms. All protocols that were used in conducting this study were subjected for approval by the ethics committee of University of Saint Louis (USL).

RESULTS

Table 1. *Comparison of the Results of Phytochemical screening of Paragis (Eleusine indica) and Guava (Psidium guajava)*

Sample	Parameter	Result
Paragis	Flavonoids	+
	Tannins	+
	Saponins	+
Guava	Flavonoids	+
	Tannins	+
	Saponins	+

Table 1 Illustrates the comparison of the phytochemical screening results yielded by Paragis (Eleusine indica) and Guava (Psidium guajava). Results showed that both Paragis (Eleusine indica) and Guava (Psidium guajava) yielded a positive result to the three (3) secondary metabolites tested namely Flavonoids, Tannins and Saponins which were responsible for the antimicrobial activity of the these plants.

Table 2. *Antibacterial activity of the different treatments against E. coli and S. aureus*

Treatment	***E. coli***		***S. aureus***	
	Mean Zone of Inhibition (mm)	**Qualitative Description**	**Mean Zone of Inhibition (mm)**	**Qualitative Description**
Positive control (Amoxicillin)	36.67 mm	Very active	50.00	Very Active
Negative Control (Distilled water)	6 mm	Inactive	6.00	Inactive
Treatment 1 (Paragis)	22.33 mm	Very active	45.00	Very active
Treatment 2 (Guava)	6 mm	Inactive	17.00	Active

Table 2 shows that the positive control (Amoxicillin) is the most active among the four (4) controls against Escherichia coli as illustrated with the highest mean zone of inhibition of 36.67 mm while the experimental control 1 (Paragis) is second to amoxicillin which is also very active with Mean zone of inhibition of 22.33 mm while the other two (2) controls do not produce a promising result against Escherichia coli.

Nevertheless, the positive control (Amoxillin) is very active against S. aureus and still the most active among the 4 controls with 50.667 mm mean zone of inhibition as illustrated. Next in line is the Paragis (Eleusine indica) yielded 45.67 mm mean zone of inhibition, and Guava (Psidium guajava) yielded 18.67 mm mean zone of inhibition which means that the Staphylococcus aureus is susceptible to the three (3) controls mentioned above.

Table 3.1. *Test of Significant Difference in the Antibacterial Activity of the different Treatments against E. coli and S. aureus*

	F-value	p-value	Decision
E.coli	714.515	.000	Reject Ho
S aureus	1101.400	.000	Reject Ho

The table shows that there is a significant difference in the antibacterial activity measured through the zone of inhibition of the different treatment groups against *E. coli* and *S. aureus.*

Table 3.2. *Multiple comparisons of the Difference in Antibacterial Activity of the Different Treatments against E. coli*

	Positive control	Negative Control	Treatment 1	Treatment 2
Positive control				
Negative Control	.000*			
Treatment 1	.000*	.000*		
Treatment 2	.000*	1.000	.000*	

The table shows that *E. indica* manifested significantly better antibacterial activity than *P. guajava* and the negative control against *E. coli*. However, it can also be seen that the positive control exhibited significantly better antibacterial activity than *the E. indica.*

Table 3.2. *Multiple comparisons of the Difference in Antibacterial Activity of the Different Treatments against S. aureus*

	Positive control	Negative Control	Treatment 1	Treatment 2
Positive control				
Negative Control	.000*			
Treatment 1	.001*	.000*		
Treatment 2	.000*	.000*	.000*	

The table shows that both *E. indica* and *P. guajava* exhibited significantly better antibacterial activity than the negative control. However, the positive control exhibited signifcanty better antibacterial activity than both *E. indica* and *P. guajava*. Moreover, *E. indica* manifested significantly better antibacterial activity that *P. guajava.*

DISCUSSION

This research study was intended to compare the antimicrobial property of Paragis (Eleusine indica) and Guava (Psidium guajava) against Escherechia coli and Staphylococcus aureus. To attain the objective of the study, researchers performed susceptibility testing, and phytochemical screening (Guevarra et.al, 2005).

Based on the results gathered by the researchers, the two extracts, namely, Paragis (Eleusine indica) and Guava (Psidium guajava) elicited secondary metabolites specifically Flavonoids, Tannins, and Saponins that were responsible for the activities of the plants.

The negative control did not show any inhibiting activity on the two microorganisms, while the positive control amoxicillin showed the maximum zone of inhibition which is 50.667 against Escherechia coli and Staphylococcus aureus.

The positive control (Amoxicillin) yielded the highest zone of inhibition at 50.667 mm and 36.67 mm on the microorganisms Staphylococcus aureus and Escherichia coli, respectively. Thus, these microorganisms are susceptible to positive control (Amoxicillin). This finding is comparable to the results yielded by Paragis (Eleusine indica) and Guava (Psidium guajava) wherein the bacteria tested are susceptible, except Escherichia coli in Guava (Psidium guajava) which is resistant.

The Paragis (Eleucine indica) was found to have an antimicrobial effect against the two microorganisms tested. With its zone of inhibition of 45.67, tested in the Staphylococcus areus, this bacteria is said to be susceptible. To support this, according to Moruh and Otuk (2013), Staphylococcus aureus was moderately susceptible to the methanol extract of Paragis. In addition, the Escherichia coli was susceptible with paragis extract with its zone of inhibition of 22.33 as supported by Moruh and Otuk (2013), and Diva, K. (2018) that this plant extract was very active on inhibiting Escherichia coli infection.

Another finding says that the Guava (Psidium guajava) was found to have antimicrobial effects against Staphylococcus aureus but not with Escherichia coli. The Staphylococcus aureus is susceptible while Escherichia coli is resistant, similar to the finding of Biswas, et.al, 2013, that only Gram-positive bacteria, Bacillus cereus and Staphylococcus aureus, were susceptible to the Guava (Psidium guajava) extracts, while neither of the Gram-negative bacteria, Salmonella enteritidis and Escherichia coli showed any inhibition. This is opposed by Farhana, et.al, (2017) that Guava extracts inhibit both gram-positive and gram-negative bacteria. In this study, there were two gram positive and three-gram negative bacteria.

Staphylococcus aureus & Bacillus cereus are gram-positive bacteria and E. coli, Shigella sonnei & Salmonella typhi are gram-negative bacteria. The resistance of gram-negative bacteria may be due to its cell wall, compromising thin lipopolysaccharides that have the property of permeability barrier which prevent the penetration of plant extract (Biswas, et.al, 2013).

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antimicrobial agents. The results and findings of this study could be a source of potential candidates in the search for active constituents that could lead to the development of drugs.

The results and findings obtained in this study utilizing methanolic leaf extracts of Paragis (Eleusine indica) and Guava (Psidium guajava) have been shown to have some antimicrobial property against the two microorganisms, namely, Staphylococcus aureus and Escherichia coli. The researchers concluded that the most susceptible bacterium in the research study was Staphylococcus aureus according to the results. In addition, between the two experimental controls used in this study, Paragis (Eleusine indica) is more sensitive to the two microorganisms.

RECOMMENDATIONS

Based on the aforementioned findings and conclusions drawn, the following recommendations and suggestions are deemed significant:

- The researchers may conduct further studies regarding various microorganisms using Paragis (Eleusine indica) and Guava (Psidium guajava).
- The researchers may consider the use of the different solvents for extraction on the same plants used in this study.
- The researchers may consider the use of the different concentrations of one or more solvents for extraction on the same plants used in this study.
- The researchers may also consider the different soaking time of the same plants used in this study samples on the solvents.
- Same study should conduct use of animals (rats) and formulate a dosage form for ease of administration.
- The researchers should conduct toxicity studies, determination of maximum effective dose and the minimum toxic dose.
- The researches should conduct histopathology on rats to determine the possible side effects of the extract.

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