Antimicrobial effect of chinese creeper *(Mikania Micrantia)* leaf extract to E. Coli *(Escherichia Coli)* causing diarrhea

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ABSTRACT

The study utilizes an experimental quantitative method. It was conducted in the science laboratory 5th floor Administration Building of the University of the Visayas. Antimicrobial activity of the leaf extract of Chinese creeper *(Mikania micrantha)* used in traditional folk medicines were screened against E. coli *(Escherichia coli*) compared to Tetracycline, pharmaceutical drug. The highest antimicrobial potentially was exhibited by the Tetracyclic drug, followed by the different concentrations (100 %, 75 %, 50 %, and 25 %). The leaf extract of *M. micantha* can be considered to be equally potent as the most common effect antibiotic, such as Tetracycline. A sensitivity test performed with commonly used sensitivity test resulted in the appearance of drug and plant resistance of the bacteria tested. A comparison of data in the inhibition zones of E. coli (*Escherichia coli*) showed that *M. micantha* leaf extract and Tetracycline were effective against bacteria strain tested. Furthermore, the results suggest that traditional folk medicine could be used as a guide on the continuing search for new natural products with potential medicinal properties.

Keywords: *antimicrobial, diarrhea, Escherichia, Mikania Micrantha*

I. INTRODUCTION

In the advancement of modern medicine, many people do not give importance on plants to their health. Many of us use plants merely for food, flavoring, and also for decorations. Before, people used herbs as medicine to cure different illnesses. At present, many of us depend on synthetic medicines that are manufactured by man using chemicals which may cause side effects in the body. Some plants have medicinal effects on our health from curing common colds to lowering the blood pressure. One of the most common plants that have a medicinal value is the Chinese creeper (*Mikania micrantha*) which the earlier people used to heal their wounds (Facey, Mulder & Porter, 2004; Li, Li, Wang & Cao, 2013). Thus, the study aims to test the antimicrobial effect of the Chinese creeper (*Mikania micrantha*) in *E. coli* which causes diarrhea.

Mikania micrantha, commonly known as Chinese creeper or mile-a-minute weed, is an extremely fast-growing, perennial creeping weed

(Ayensu, 1981). According to Facey and Hassan (2004), *M. micrantha* is the most popularly used for wound dressings and promote the healing of sores. In addition, it has been attracted the attention of natural product chemists because of its antibacterial, antitumor, cytotoxic, analgesic, inflammatory, antiproliferative, and phytotoxic activities. Stem and leaf of the plant sample is used to treat malaria and eczema. Leaf in liquid mixture is used for washing rashes, skin eruptions and smallpox, chicken pox or for measles, by Juice from macerated leaves is applied to persistent sores. Macerated leaves are vigorously rubbed on skin as a treatment for rashes (DeFilipps, Maina, & Crepin, 2004). *Escherichia coli* (E. coli) bacteria normally live in the intestines of people and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, which means they can cause illness especially diarrhea. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons. *E. coli* consists of a diverse group of bacteria.

The antimicrobial activity of *M. micrantha* extracts was evaluated against *Escherichia coli.* However, the antimicrobial constituents were ambiguous though mikanolide and dihydromikanolide as antimicrobial ingredient of *M. micrantha* were reported. There has been no reports which shows that effectiveness of *M. micrantha* as antimicrobial agents agains *E. coli.* which causes diarrhea (Patamona, 2004).

This study provides description about the antimicrobial constituent and antimicrobial activities in an attempt to improve the understanding of the practicality of *M. micrantha.* This will further identify the different phychemical constituents of *M. micrantha* as an antimicrobial agent against the *E.coli* bacteria that causes diarrheal diseases.

II. OBJECTIVES

The study aims to test the anti- microbial effect of Chinese Creeper (*Mikania micrantha*) leaf extract on *E.coli* bacteria causing diarrhea. Furthermore, it specifically aims to determine the effectiveness of Chinese creeper (*Mikania macrantha*) as an anti- microbial drug on *E. coli*; compare the antimicrobial effect of Chinese creeper (*Mikania micrantha*) in other pharmaceutical drug (Tertracycline); assess the level of concentrations of the Chinese creeper (Mikania micrantha) leaf extract on the E. coli; and determine the phytochemical constituents of Chinese creeper *(Mikania micrantha).*

III. METHODOLOGY

The study uses experimental quantitative research design to determine the antimicrobial effect of Chinese creeper (*Mikania micrantha*) leaves extract on *E. coli* causing diarrhea. Samples of *M. micrantha* were collected from Basak, Mandaue City. The extraction and phytochemical testing was done in the chemistry laboratory in the 5th floor, Room 516A of the Administration building in the University of the Visayas (Main Campus), D. Jakosalem Street, Cebu City. The plant sample was freshly picked and washed with tap water to remove unnecessary impurities. Leaves were squeezed and filtered to separate the residue from the pure extract. Extract was subject for phytochemical testing and biological testing. The researchers obtained different levels of concentrations in the pure extracted leaves of *M. micrantha* and undergoes phytochemical and biological testing.

E. coli was procured in the University of the Visayas-College of Medicine, Micro-Parasitology Laboratory in Banilad campus. *E. coli* broth samples were placed properly in a tightly sealed box for the safety of the researcher and people around the place. In preparing the bacterial culture, the researchers prepared nutritive agar plate to be used as a media for bacterium to be cultured. In culturing bacteria, the researchers took a sample from the broth culture of one bacteria colony. Different levels of concentrations of *M. micranta* leaf extract and antibacterial agent were then added.

Kirby-Bauer Disk Diffusion Susceptibility Test was used to determine the sensitivity and resistance of pathogenic facultative anaerobic bacteria to various antimicrobial compounds in order to assist the researchers in selecting a treatment option. It is a simple method where the culturing surface inoculated with microbe is exposed to small disks containing known amounts of chemical agents. This resulted in a zone of inhibition showing the growth of the microbe corresponding to the susceptibility of the strain to the agent. In the application of different level of concentrations to the bacterial contaminated petri dishes. The researchers used filter paper. The filter papers were soaked with the different concentrations (25 %, 50 %, 75 %, 100 % and Tetracyclin) and then applied to the petri dishes.

Statistical Treatment (Math is Fun, 2014):

- To get the total area of aura + disk diffusion area $= \frac{\pi\, x\, diameter^{\,2}}{4} = \frac{3.1416\, x 10^2}{4}$

Note: Same formula to get the area of disk diffusion

- To get the ring:
  = area of aura – area of disk diffusion area
- To get the percentages: $\frac{area\ of\ ring}{total\ area\ of\ aura+dot}$ x 100
- To get the average *: T1 + T2 +T3*

IV. RESULTS AND DISCUSSION

The following data were tabulated and statistically treated for easier discussion of the results.

Table 1. Total diameter of the Aura (zone of inhibition)

Total Diameter of the Aura				
Dosage	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
100 %	10 mm	23 mm	25 mm	19.33
75 %	7 mm	16 mm	17 mm	13.33
50 %	6 mm	14 mm	14 mm	11.33
25 %	0 mm	11 mm	13 mm	7.66
Tetracyclin	29 mm	30 mm	29 mm	29.33
Controlled	0 mm	0 mm	0 mm	0

The table shows the total diameter of the aura (zone of inhibition) in each trial. Each dosage, the experimental and the positive control, exhibited changes in the diameter of the aura compared to the controlled which shows no changes. Tetracycline was observed to be the most effective with the average of 29.33 followed by the experimental controls. This further shows that the greater the diameter of the aura the greater the chance that the bacteria increases.

In getting the total diameter, the researchers used a ruler in measuring each zone with the unaided eyes while viewing the back portion of the petri dish.

Table 2. Total Area of the aura + Dot

Total number of the aura + dot				
Dosage	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
100 %	78.5	415.5	490.9	328.33
75 %	38.5	201.1	227.0	155.53
50 %	28.3	153.9	153.9	112.03
25 %	0.0	95.0	132.7	227.03
Tetracyclin	660.5	706.9	660.5	675.96
Controlled	0.0	0.0	0.0	0.0

The table shows the total area of the aura and the dot in every concentration. This further shows that the concentration the least number of bacteria present. This means that 100% solution contains lesser number of bacteria compared to the other solutions. While Tetracyclin, which is antimicrobial drug has the least number of bacteria compared to the 100% solution of *M. micrantha.* The researchers used the formula written below in determining the total area of the aura, added the disk and the dot.

$$= \frac{\pi\, x\, diameter^{\,2}}{4} = \frac{3.1416\, x 10^2}{4}$$

Table 3. Total area of the Ring

Total area of the ring				
Dosage	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
100 %	58.9	395.8	471.2	308.63
75 %	18.8	181.4	207.3	135.83
50 %	8.6	134.3	134.3	92.4
25 %	0.0	75.4	113.1	62.83
Tetracyclin	640.9	687.3	640.9	656.33
Controlled	0.0	0.0	0.0	0

Note: To determine the total area of the ring, the researcher used the formula: = Area of the aura - area of the aura of the disk diffusion

The table shows that the higher the percentage of the extract the lesser the contamination of the ring. This means that 100 % solution has the greater possibility of inhibiting the bacteria.

Table 4. Percentage of *E.coli* killed

Percentages of Bacteria Killed				
Dosage	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
100 %	75.0 %	95.3 %	96.0 %	88.76
75 %	49.0 %	90.2 %	91.3 %	76.83
50 %	30.6 %	87.2 %	87.2 %	68.33
25 %	0.0 %	79.3 %	85.2 %	54.83
Tetracyclin	97.0 %	97.0 %	97.0 %	97
Controlled	0.0 %	0.0 %	0.0 %	0

The table shows the effectiveness of leaf extract concentration in killing *E. coli*. One hundred percent solution is more effective than the 25 % solution. Thus, this shows that the higher the concentration the higher the amount of the bacteria killed. Furthermore, the *M. micrantha* leaf extract is comparable to the pharmaceutical drug which is Tetracycline.

Table 5. Phytochemical testing of *Mikania micrantha*

Active Constituets	**Reagents**	**Results**	**Actual results**	**Remarks**
Alkaloid	Mayer's reagent	Creamy	Creamy color	+
Saponins	Water	Foam	Foamy	+
Glycosides	Fehling solutions A and B	Red	Red	+
Tannins	Ferric Chloride	Dark green or deep blue	Dark green ppt.	+
Phenols	Ferric Chloride	Dark green or deep blue	Dark green ppt.	+

Legend: (-) Negative; (+) Heavy precipitate

Table 5 shows the results of phytochemical testing of *M. micrantha* in the different reagents used. It further shows that *M. micrantha* has an active chemical contituents. The result further shows that the leaf extract of *M. micrantha* has an effective antimicrobial agent's against *E. coli* causing diarrhea. The extract holds active constituents that are responsible for the death of *E. coli.*

V. CONCLUSION

Based on the gathered data, the leaf extract of *M. micrantha* (Chinese creeper) is an effective antimicrobial agent against E. coli causing diarrhea. The leaf extract contains different active constituents that are responsible for the death of *E. coli.* The effectiveness of the antimicrobial activity of *M. micrantha* depends on the level of concentration. This means that higher the level of concentration the higher the amount of the bacteria killed. Furthermore, the antimicrobial effect of the leaf extract of *M. micrantha* is comparable to the pharmaceutical drug which is the Tetracycline. The leaves extract of *M. micrantha* may now be a promising source in the quest for new antimicrobial drugs due to its efficacy.

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Inhibitory activity of *Plumeria rubra* (Kalachuchi), *Ipomea quatica* (Kangkong), *Mimosa pudica* (Makahiya), *Euphorbia hirta* (Gatas-Gatas) and *Coleus aromaticus* (Oregano) plant extracts against *Staphylococcus aureus* coagulase production

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ABSTRACT

The leaf extracts of *P. rubra* (Kalachuchi), I. aquatica (Kangkong), *M. pudica* (Makahiya), *E. hirta* (Gatas-Gatas), and *C. aromaticus* (Oregano) were screened for inhibitory activity against coagulase production of *S. aureus*. The Tube Coagulase Test and Colony Count were used for the inhibitory assay. The *E. hirta* (Gatas-Gatas), and C. aromaticus (Oregano) were found to have inhibitory activity against *S. aureus* coagulase production both having a mean grade level of 1 and a mean colony count of 193.33 and 229.67, respectively. However, the in vitro tests conducted do not, in any way, stimulate the complexity of the human body. Instead, these results warrant the *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) plant extracts to further anti-coagulase investigation.

Keywords: *inhibitory activity, Staphylococcus aureus, coagulase production, antibacterial, herbal plants*

I. INTRODUCTION

Herbal plant is a vast wealth of nature not only from the global environmental perspective but also from the medicinal point of view. It plays a significant role in improving the disease resistant ability and combating against various unfavorable metabolic activities within the living system. Numerous infectious diseases have been known to be controlled by herbal remedies that have been proven variously since primitive to present history of mankind. Since time immemorial, man has used various parts of plants in treatment and prevention of various ailments. Unimaginably, unrevealed and unmatched varieties of compounds are present in the diversified herbs on earth. From these points of view, it is obvious that natural products, either in a form of pure compounds or as standardized plant extracts, provide unlimited opportunities to develop a variety of new drugs.

The increase in drug-resistant bacteria has pressed on the search for alternative and natural sources of antibiotics (Saeed et al., 2005). One potential source of antibiotics is plants (Joshi et al., 2009). Plants such as Kalachuchi, Kangkong, Makahiya, Gatas-Gatas, and Oregano are widespread species in the Philippines and are used in traditional medicine in the country. Moreover, plants are not only very accessible and effective against disease-causing microbes but also safer to use than commercial antibiotics (Chaudhry et al., 2006). A study was shown

that extraction of the crude plant *P. rubra* that contains iridoids that have been reported to have antibacterial, algicidal, cytotoxic, and/or plant growth inhibitory activity (Kardono et al., 1990). It was found that *I. aquatica* exerted a high magnitude of antimicrobial activity against the tested four types of bacterial species: namely, *Escherichia coli, Pseudomonas aeruginosa, S. aureus* and *Micrococcus luteus* (Majumdar et al., 2009). *M. pudica* was found to exhibit in vitro bacteriostatic activity (Genest et al., 2008). Leaves of *E. hirta* which were extracted by maceration in ethanol were used in traditional medicine for the treatment of boils, wounds and control of diarrhea and dysentery (Ogueke et al., 2007). A study was previously conducted on the efficacy of Oregano oil which contains carvacrol and thymol against planktonic *S. aureus* and *S. epidermidis* (Nostro et al., 2004).

Antibiotic resistance becomes a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. There is a continuous and urgent need to discover new antimicrobial compounds with diversed chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new discoveries that lead to develop better drugs against microbial infections. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Recent studies have suggested that several plants species exhibit promising antimicrobial effects. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection of various tissues of the body. *S. aureus* is a gram-positive cocci, non-motile, non-spore forming, catalase-positive and facultatively anaerobic organism which belongs to the family Micrococceae. They may be found singly, in pairs, and in irregular clusters that have been described as "bunches of grapes". The cell wall contains peptidoglycan and teichoic acid. The organisms are resistant to temperatures as high as 50°C, to high salt concentrations, and to drying. Colonies are usually large (6-8 mm in diameter), smooth, and translucent. Colonies appear creamy, white, or light gold and "buttery looking" after 18 to 24 hours of incubation (Stoppler, 2009). *S. aureus* colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales, including the skin, oral cavity and gastrointestinal tract. They causes range of illnesses from minor skin infections such as pimples, boils (furuncles), cellulitis folliculitis, carbuncles, impetigo, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Staph-related illness can range from mild and requiring no treatment to severe and potentially fatal. *S. aureus* is identified primarily by the tube coagulase test (Larsen et al., 1995). One important classification of *S. aureus* is its ability to produce coagulase. The role of *S. aureus* coagulase is captivating. It causes coagulation that allows the bacteria to coat itself with a layer of fibrin under which it hides from the immune system making it more virulent. Coagulase is part of the *S. aureus* defense system (Todar, 2011).

The World Health Organization (WHO) estimates that the mortality rate of *S. aureus* invasive infection was about 90 % by the year 2011. *S. aureus* is an opportunistic bacterial pathogen associated with asymptomatic colonization of the skin and mucosal surfaces of normal humans. However, it also is the cause of wound infections and has the potential to induce certain diseases, leading to infections in any of the major organs of the body. It also is responsible for many serious community- and

nosocomially-acquired infections, being the most frequently isolated bacterial pathogen from patients with hospital-acquired infections. Although antibiotic agents are now available in the market, it cannot be denied that antibiotic resistance of *S. aureus* is great due to its ability to form fibrin clot and thus protecting itself from phagocytosis making it more virulent. The use of antimicrobial substances with inhibitory mode of action may have fewer side effects than those with bactericidal mode of action. The latter ones tend to kill all of the bacteria in the body including normal flora whereas the former ones just retard the growth of the bacteria which are further killed by the immune response of the body (Doss et al., 2011).

The researcher then aim to inhibit that coagulase production of *S. aureus* using five herbal plants. These are (1) *P. rubra* (Kalachuchi), (2) *I. aquatica* (Kangkong), (3) *M. pudica* (Makahiya), (4) *E. hirta* (Gatas-Gatas*),* and (5) *C. aromaticus* (Oregano).

II. OBJECTIVES

The study generally aimed to investigate the inhibitory activity of *P. rubra* (Kalachuchi), *I. aquatica* (Kangkong), *M. pudica* (makahiya), *E. hirta* (Gatas-Gatas*),* and *C. aromaticus* (Oregano) plant extracts against *S. aureus* coagulase production. Specifically the researcher aimed to achieve the following:

1. Quantify the inhibitory effect of herbal plants against coagulase production in *S. aureus;*
2. Compare the inhibitory activity of herbal plants in *S. aureus* coagulase production; and
3. Identify herbal plants that have inhibitory effect against *S. aureus* coagulase production

Null hypothesis. There is no significant difference on the inhibitory activity of *P. rubra* (Kalachuchi), *I. aquatica* (Kangkong), *M. pudica* (Makahiya), *E. hirta* (Gatas-Gatas*),* and *C. aromaticus* (Oregano) plant extracts against *S. aureus* coagulase production.

III. MATERIALS AND METHODS

The study utilizes experimental research design, specifically parallel group design where five groups were used at the same time with only one single variable (control group) is manipulated (Calmorin & Calmorin, 2002). In this experiment, five different herbal plant extracts were compared. All of these extracts were obtained through decoction method of extraction with uniform amount of grams of herbal plants and volume of solvent. The experiment was conducted at Pharmacy Laboratory and Medical Technology Laboratory of St. Scholastica's College Tacloban. For easy access of reagent and laboratory apparatuses, all relevant tests and measurements, such as weighing and the extraction of the plants were done at Pharmacy Laboratory. However, the inoculation, culturing of *S. aureus* and performing Tube Coagulase Test were done at Medical Technology Laboratory for safe, regulated, and conducive environment.

IV. PREPARATION OF HERBAL PLANT EXTRACTS

Plant Collection. *P. rubra* (Kalachuchi), *I. aquatica* (Kangkong), *M. pudica* (Makahiya), *E. hirta* (Gatas-Gatas*),* and *C. aromaticus* (Oregano) samples were collected from Happy Homes Diit, Tacloban City, Philippines. The individual plant was randomly collected between 8:00 am to 10:00 am by uprooting method. Collected samples were wrapped in clean plastic bags and transported directly to the Pharmacy Laboratory for the preliminary procedures. The samples were thoroughly washed with running water to remove debris. The plant materials were rinsed with distilled water. Each sample was weighed 10 g. Only the healthy looking matured leaves in every herbal plant were picked and randomly selected to be used for decoction.

Extraction and Purification of Plant Extract. Decoction of each herbal plant was prepared by boiling 10 g of the leaves in 50 ml distilled water in a flask for 20 minutes. The flask containing the leaves and decoction was removed

from the heat and allowed to cool. The content of flask was filtered through filter paper to obtain clear decoction.

Syringe filter with a general size of 0.45 micron was used to purify the plant extracts. Then, each plant extract was stored in separate autoclaved reagent bottles and was placed inside the refrigerator.

Test for Contaminants in Plant Extract. Trypticase Soy Agar (TSA) was used to check the presence of organisms in every plant extract and was prepared according to the manufacturer's instructions. A small sample of extract was inoculated into TSA and was incubated at 37° C overnight.

V. PREPARATION OF BACTERIAL CULTURES

Isolation of *S. aureus* Strains. Stock cultures of *S. aureus* were obtained from the Eastern Visayas Regional Medical Center (EVRMC). The stock cultures were maintained by sub-culturing in nutrient agar media and were incubated at 37° C overnight.

Identification of Bacteria. Gram's staining technique was performed to check the morphological characteristics of the *S. aureus*.

Tube Coagulase Test was done for the further confirmation for the presence of *S. aureus*. One tube was filled with 0.5 ml of human plasma with EDTA and 0.1 ml of *S. aureus.* Then, it was incubated at 37° C and was observed up to four hours and 24 hours after incubation. The grade level was measured as (4 +) if the fibrin clot filled the complete volume occupied by the broth; (3 +) if the clot fills more than half but less than the total volume occupied by the broth; (2 +) if the clot fills less than half the total volume occupied by the broth; (1 +) if there is a little disorganized clot formation; (negative) if no clot observed but a little amorphous deposit might be seen (Spencer & Tatini, 1974).

Standardization of the Bacteria in this study. 0.5 McFarland Standard was used to standardize the approximate number of bacteria in a liquid suspension with a chemical solution of 1 % barium chloride (0.05ml) and 9 % sulfuric acid (9.95ml). McFarland Standard was stored in standing position at 4°C to 8°C and protected from light during 12 weeks. The suspension was whirled again for at least one minute to obtain a homogenous suspension and to break the clumps**.** A full loop of bacterial growth was obtained with an inoculating loop and placed in a test tube with 3 ml of Nutrient Broth. The test tube was whirled for at least one minute to break the clumps until a fairly turbid suspension was obtained. The bacterial suspension's turbidity was adjusted to be the same as the turbidity of the 0.5 McFarland standards. More broth was added to the bacterial suspension to reduce turbidity, while more colonies were added to increase turbidity. The bacterial concentration of the bacterial suspension was 1.5×10^8 CFU/ml (colony forming unit/milliliter).

Inhibitory Assay of Herbal Plant Extract. The inhibitory activity of the *P. rubra* (Kalachuchi), *I. aquatica* (Kangkong), *M. pudica* (Makahiya), *E. hirta* (Gatas-Gatas*),* and *C. aromaticus* (Oregano) plant extracts were determined by measuring the grade level of the inhibition of the clot in the Tube Coagulase Test. Six test tubes were prepared, each tube contains 0.5 ml human plasma and 0.1 ml of standardized *S. aureus*. Five tubes were treated with 0.1 ml of plant extract, with tube 1 containing *P. rubra* (Kalachuchi), tube 2 *I. aquatic* (Kangkong), tube 3 *M. pudica* (Makahiya), tube 4 *E. hirta* (Gatas- Gatas) and tube 5 *C. aromaticus* (Oregano). All tubes were incubated at 37° C and observed up to four hours and 24 hours of incubation. Tube six was left untreated and served as basis for comparison on the grade level for coagulase production.

In every plate of Mueller Hilton Agar (MHA), 0.1 µl of the mixture in each tube were obtained and immediately streaked with the use of bacterial cell spreader. Growth of colonies in each MHA plate was counted to check if the treated bacteria have the same number of colony growth as to the untreated bacteria.

VI. STATISTICAL ANALYSIS

The Tube Coagulase Test and MHA colony counting were done in triplicates and the mean values and standard error of the mean were calculated. Tabular form representation was the main tool in the study for easy comparison of results obtained.

The researcher used one-way analysis of variance (one-way ANOVA) to analyze the mean of clot inhibition in the Tube Coagulase Test and Colony Count results of the tubes with plant extract as the experimental group with tube 1 containing *P. rubra* (Kalachuchi), tube 2 *I. aquatic* (Kangkong), tube 3 *M. pudica* (Makahiya), tube 4 *E. hirta* (Gatas-Gatas) and tube 5 *C. aromaticus* (Oregano) to the control group with no plant extract.

Nevertheless, Duncan's Multiple Range Test (DMRT) was also used. It involves the computation of numerical boundaries that allow for the classification of the difference between the mean of clot inhibition in tubes with plant extract (experimental group) to the mean of tube without plant extract (control group) as significant or nonsignificant.

VII. RESULTS AND DISCUSSIONS

The results in experiment 1 using Plasma X and experiment 2 using Plasma Y in grade level after 4 hours, no clot were formed. The grade level and turbidity were measured after 24 hours. 10 µl of the mixture in each tube was obtained and immediately streaked in MHA with the use of bacterial cell spreader. After 24 hours of incubation, numerous growths of colonies were seen. Thus, the amount of mixture streaked in MHA was reduced to 0.1 µl in experiment 3. The number of colony growth obtained shown on Table 1.2 was less than 300 cfu/ml. Also, the plasma used in experiment 3 was pooled plasma from A, B and C.

Table 1.2. Summary of data gathered on Grade Level of Clot Formation, Turbidity and Colony Count on the Inhibitory Assay against *S. aureus* Coagulase Production Using Plasma X (Experiment 1) and Plasma Y (Experiment 2)

	Experiment No. 1				Experiment No. 2			
	Grade Level Clot Formation		Turbidity	Colony Count	Grade Level Clot Formation		Turbidity	Colony Count
Herbal Plant Extract	4 hrs	24 hrs			4 hrs	24 hrs		
P. rubra (Kalachuchi)	No clot	+1	Less Turbid	TNTC	No clot	+2	Less Turbid	TNTC
I. aquatica (Kangkong)	No clot	+1	Less Turbid	TNTC	No clot	+3	Less Turbid	TNTC
M. pudica (Makahiya)	No clot	+2	Less Turbid	TNTC	No clot	+2	Less Turbid	TNTC
E. hirta (Gatas-Gatas)	No clot	+1	Turbid	TNTC	No clot	+3	Turbid	TNTC
C. aromaticus (Oregano)	No clot	+1	Turbid	TNTC	No clot	+3	Turbid	TNTC
Conrol group	No clot	+3	Less Turbid	TNTC	No clot	+3	Less Turbid	TNTC

Table 1.2. Summary of data gathered on Experiment No. 3 Grade Level of Clot Formation, Turbidity and Colony Count on the Inhibitory Assay against *S. aureus* Coagulase Production Using Pooled Plasma (A,B,C)

Herbal Plant Extract	Experiment No. 3											
	Trial 1				Trial 2				Trial 3			
	Grade Level Clot Formation		Turbidity	Colony Count	Grade Level Clot Formation		Turbidity	Colony Count	Grade Level Clot Formation		Turbidity	Colony Count
	4 hrs	24 hrs			4 hrs	24 hrs			4 hrs	24 hrs		
P. rubra (Kalachuchi)	No clot	+1	Less Turbid	12	No clot	+2	Less Turbid	7	No clot	+1	Less Turbid	24
I. aquatica (Kangkong)	No clot	+1	Less Turbid	79	No clot	+2	Less Turbid	110	No clot	+1	Less Turbid	132
M. pudica (Makahiya)	No clot	+2	Less Turbid	129	No clot	+2	Less Turbid	26	No clot	+2	Less Turbid	94
E. hirta (Gatas-Gatas)	No clot	+1	Turbid	153	No clot	+1	Turbid	246	No clot	+1	Turbid	181
C. aromaticus (Oregano)	No clot	+1	Turbid	228	No clot	+1	Turbid	298	No clot	+1	Turbid	163
Conrol group	No clot	+2	Less Turbid	112	No clot	+2	Less Turbid	123	No clot	+2	Less Turbid	149

The first part deals with measuring the grade level of clot formation of experimental group and control group 24 hours of observation and counting the number of colonies formed in MHA for in each test solution including the control group and also observing its turbidity.

Table 2.1. Grade Level of Clot Formation of five different plant extracts and control group in Tube Coagulase Test

Herbal Plant Extract	Replication			Treatment Total (T)	Treatment Mean ($\bar{x}$)
	Trial 1	Trial 2	Trial 3		
P. rubra (Kalachuchi)	+1	+2	+1	4	1.33
I. aquatica (Kangkong)	+1	+2	+1	4	1.33
M. pudica (Makahiya)	+2	+2	+2	6	2
E. hirta (Gatas-Gatas)	+1	+1	+1	3	1
C. aromaticus (Oregano)	+1	+1	+1	3	1
Control group	+2	+2	+2	6	2
Grand Total				**26**	
Grand Mean					**1.44**

The observed clot formation of the five herbal plant extracts against coagulase production of *S. aureus* may attribute to the compound it possesses. The *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) were found to have greater effect in inhibiting clot formation with the mean of 1. The *P. rubra* (Kalachuchi) and *I. aquatica* (Kangkong) showed the same effect in terms of inhibiting the clot formation with the mean of 1.33. While *M. pudica* (Makahiya) with the mean of 2 did not show any inhibitory activity against clot formation since it was the same as the mean of the control group based on Table 2.1 data. The results for clot formation do not prove that the plant extracts inhibit only the *S. aureus* coagulase production thus observation on turbidity and colony count were performed to further investigate inhibitory activity of the five herbal plant extracts.

Table 2.2. Turbidity of five different plant extracts and control group in Tube Coagulase Test

Herbal Plant Extract	Replication		
	Trial 1	**Trial 2**	**Trial 3**
P. rubra (Kalachuchi)	Less Turbid	Less Turbid	Less Turbid
I. aquatica (Kangkong)	Less Turbid	Less Turbid	Less Turbid
M. pudica (Makahiya)	Less Turbid	Less Turbid	Less Turbid
E. hirta (Gatas-Gatas)	Turbid	Turbid	Turbid
C. aromaticus (Oregano)	Turbid	Turbid	Turbid
Conrol group	Less Turbid	Less Turbid	Less Turbid

The *P. rubra* (Kalachuchi), *I. aquatica* (Kangkong) *M. pudica* (Makahiya) and Control group showed the same characteristic in terms of turbidity which is less turbid compared to *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) showed in Table 2.2. Less turbid either indicates that the coagulase production of *S. aureus* was inhibited or the bacteria were killed by the presence of the herbal plant extracts. Turbid designates the presence of more bacteria in the solution and that the bacteria did not form a clot thus making the solution turbid.

Table 2.3. Colony Count (CFU/ml) of five different plant extracts and control group in Tube Coagulase Test

Herbal Plant Extract	Replication			Treatment Total (T)	Treatment Mean ($\bar{x}$)
	Trial 1	**Trial 2**	**Trial 3**		
P. rubra (Kalachuchi)	12	7	24	43	14.33
I. aquatica (Kangkong)	79	110	132	321	107
M. pudica (Makahiya)	129	26	94	249	83
E. hirta (Gatas-Gatas)	153	246	181	580	193.33
C. aromaticus (Oregano)	228	298	163	689	229.67
Conrol group	112	123	149	384	128
Grand Total				**2,266**	
Grand Mean					**125.89**

However, the inhibitory activity against *S. aureus* coagulase production was further determined by the number of colony growth in MHA. The variation of results in the colony count based on Table 2.3, may due to the different components that plant extracts possesses against *S. aureus*.

The second part deals with determining the significant mean difference of grade level and colony count of the control group and experimental group using one-way ANOVA and DMRT.

Table 3.1. Analysis of Variance (ANOVA) of Grade Level of Clot Formation of five different plant extracts and control group in Tube Coagulase Test

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Computed *F*	Tabular F	
					5%	1%
Treatment	5	3.11	0.62	5.64**	3.11	5.06
Experimental Error	12	1.33	0.11			
Total	17	4.44				

The computed *F* value is larger than the tabular *F* value at the 5% and 1% level of significance. This implies that the treatment difference in grade level of clot formation of five different plant extracts and control group is said to be highly significant indicated by two asterisks (**) on the computed *F* value in the analysis of variance.

Table 3.2. Analysis of Variance (ANOVA) of Colony Count of five different plant extracts and control group in Tube Coagulase Test

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Computed *F*	Tabular F	
					5%	1%
Treatment	5	89,891.78	17,978	10.06**	3.11	5.06
Experimental Error	12	21,448	1,787.33			
Total	17	**111,339.78**				

The computed *F* value is larger than the tabular *F* value at the 5% and 1% level of significance, the treatment difference in colony count of five different plant extracts and control group is said to be highly significant indicated by two asterisks (**) on the computed *F* value in the analysis of variance.

Table 3.3. Duncan's Multiple Range Test (DMRT) for comparing Grade Level of Clot Formation of five different plant extracts and control group in Tube Coagulase Test Using the Alphabet Notation

Herbal Plant Extract	Mean ($\bar{x}$)	DMRT
M. pudica (Makahiya)	2	a b
Control group	2	| |
P. rubra (Kalachuchi)	1.33	c d
I. aquatica (Kangkong)	1.33	| |
E. hirta (Gatas-Gatas)	1	e f
C. aromaticus (Oregano)	1	| |

Any two means having a common letter are not significantly different at the 5% level of significance.

The above table (Table 3.3) showed that there is no significant difference between Makahiya and Control group; Kalachuchi and Kangkong; Gatas-Gatas and Oregano in terms of inhibiting the clot formation of *S. aureus* in Tube Coagulase Test.

Table 3.4. Duncan's Multiple Range Test (DMRT) for Colony Count of five different plant extracts and control group in Tube Coagulase Test using the Alphabet Notation

Herbal Plant Extract	**Mean ($\bar{x}$)**	**DMRT**
C. aromaticus (Oregano)	299.67	a
E. hirta (Gatas-Gatas)	193.33	b
Control group	128	c
I. aquatica (Kangkong)	107	d
M. pudica (Makahiya)	83	e
P. rubra (Kalachuchi)	14.33	

Any two means having a common alphabet are not significantly different at the 5% level of significance.

The difference between the largest R_p value (The R_p value at p = 6) of 83.34 and the largest treatment mean *C. aromaticus* (Oregano) of 299.67 is 299.67 - 83.34 = 146.33. From the array of means obtained, all treatments means, except that of *E. hirta (GATASGATAS),* are less than computed difference of 146.33. Hence, they are declared significantly different from *C. aromaticus* (Oregano). The difference between the second largest treatment mean *E. hirta* (Gatas-Gatas) of 193.33 and the second largest R_p value (the R_p value at p = 5) of 82.35 is 193.33 - 82.35 = 110.97. From the array of means obtained, all treatments means, except that of control group are less than computed difference of 110.97. Thus they are declared significantly different from *E. hirta* (Gatas-Gatas). The difference between the third largest treatment mean which is the control group of 128 and the third largest R_p value (the R_p value at p = 4) of 81.53 is 128 - 81.53 = 46.47. Because the mean of *P. rubra* (Kalachuchi) is less than 46.47, it is declared significantly different from the mean of the control group. The difference between the fourth largest treatment mean which is the *I. aquatica* (Kangkong) of 107 and the fourth largest R_p value (the R_p value at p = 3) of 79.08 is 107-79.08 = 27.92 Because the mean of *P. rubra* (Kalachuchi) is less than 79.08, it is declared significantly different from the mean of the *I. aquatica* (Kangkong). However because the mean of *P. rubra* (Kalachuchi) is the only outside the groupings already made, *P. rubra* (Kalachuchi) mean was compared using the appropriate R_p values with the rest of the means namely control group, *I. aquatica* (Kangkong) and *M. pudica* (Makahiya). Of the three comparisons, the only one whose difference is less than the corresponding R_p value is that between *M. pudica* (Makahiya) and *P. rubra* (Kalachuchi) 83 - 14.33 = 68.67 < R_p (at p = 2) of 75.41. Thus *M. pudica* (Makahiya) and *P. rubra* (Kalachuchi) are declared not significantly different from each other.

The above table (Table 3.4) showed that there is no significant difference between Oregano and Gatas-Gatas; Gatas-Gatas and control group; control group, Kangkong and Makahiya; Kangkong and Makahiya; Makahiya and Kalachuchi in terms of Colony Count.

The last part deals with the identification of herbal plants that have inhibitory effect against *S. aureus* coagulase production.

Table 4. Identification of herbal plants that have Inhibitory Activity against Coagulase Production of *S. aureus*

Herbal Plant Extract	**Anti-bacterial**	**Anti-coagulase**
P. rubra (Kalachuchi)	+	
I. aquatica (Kangkong)	+	
M. pudica (Makahiya)	+	
E. hirta (Gatas-Gatas)	-	+
C. aromaticus (Oregano)	-	+

In the study, the inhibitory activities of the herbal plant extracts against coagulase production in *S. aureus* were assessed through Tube Coagulase Test and MHA colony count. The resulting clot in each tube was measured and the growth of colonies in MHA was counted. The results for the Tube Coagulase Test and colony count revealed that only the plant extract of *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) showed inhibitory activity against the production of coagulase in *S. aureus*. Furthermore, the *P. rubra* (Kalachuchi) and *I. aquatica* (Kangkong) plant extracts were effective in inhibiting the coagulase production due to its antibacterial activity against the bacteria *S. aureus*. Meanwhile, the plat extract of *M. pudica* (Makahiya) did not show any inhibitory activity against *S. aureus* coagulase production.

VIII. CONCLUSION

The results obtained from the measurement of grade level, turbidity and colony count showed that *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) plant extracts have inhibitory activity against the production of coagulase in *S. aureus*. The inhibitory activity might be due to the synergistic effects of its constituents or the presence of bioactive compounds of the two herbal plant extracts. The results of the study do not prove that plant extracts of *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) already has therapeutic value. The in vitro tests conducted do not, in any way, stimulate the complexity of the human body. Instead, these results warrant the *E. hirta* (Gatas-Gatas) and *C. aromaticus* (Oregano) plant extracts to further anti-coagulase investigation.

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