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Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endoderm's). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and by preventing the establishment of pathogenic bacteria within the intestine. It causes illnesses like typhoid fever, paratyphoid fever, and food borne.

This strain of bacteria, according to studies and research, is confirmed to be inhibited by organic compounds produced and contained in extracts of kamias leaves and garlic bulbs. These two organic products are commonly used in adding flavor to food and they both exhibit their antibacterial properties on the same strain of bacteria. So, this study aims to compare the observable characteristics of the extracts and the statistical values of the effectiveness and rates of inhibition of the extracts produced from the two organic products against E. coli.

#### Statement of the Problem

##### Main Problem:

This study aims to compare the effectiveness of kamias leaf extract and garlic extract as antibacterial agents against Escherichia coli.

##### Specific Problem:

To prove or disprove that the kamias extract and garlic extract have antibacterial properties against E. coli

To compare and contrast the physical attributes of the two extracts

To compare and contrast the statistical values of the rates of inhibition of the two extracts

Hypotheses

Operational:

The kamias leaf extract has greater effectiveness of its antibacterial property against *E. coli* compared with garlic bulb extract.

The garlic bulb extract has greater effectiveness of its antibacterial property against *E. coli* compared with kamias leaf extract.

Null:

The kamias leaf and garlic bulb extracts have equal statistical values in the effectiveness of its antibacterial property against *E. coli*.

The kamias leaf and garlic bulb extracts are both not effective antibacterial agents against *E. coli*.

Significance of the Study

Kamias is a fruit-bearing tree of the genus *Averrhoa*, family Oxalidaceae. It was originated in Indonesia, but now, are cultivated and found throughout Philippines, Indonesia, Sri Lanka, Bangladesh, Myanmar, and Malaysia. In Philippines, kamias leaves was being used as a alternative paste on itches, swelling, rheumatism, mumps or skin eruptions. Also it is used on bites of poisonous creatures. Malaysians use fermented or fresh bilimbi leaves to  
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treat venereal diseases. In French Guiana, syrup made from the fruit is used to treat inflammatory conditions. To date there is no scientific evidence to confirm effectiveness for such uses. In India, the fruit of this plant in folk medicine to control obesity.

Garlic is a species of onion genus, *Allium*. For health benefits, garlic is known as “cure all” food because it prevents us from most of the diseases or you can say almost all the diseases. Garlic is used to improve immune system of the body. Also it can cure colds and heart diseases. It helps to overcome cholesterol level and blood pressure level.

Kamias and garlic are very common to our environment. They are also usually used in our food. The significance of our study is to know which of the extracts have a more effective antibacterial property and to gain more information about the strain of *E. coli*.

#### Definition of Terms

The following terms are written below for us to know better about the topic:

**Serotypes-** refers to distinct variations within a species of bacteria or viruses or among immune cells of different individuals. These microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level.

**Pathogenic bacteria-** are bacteria that cause bacterial infection.

Paratyphoid fever- Paratyphoid fevers are a group of enteric illnesses caused by serotypic strains of the Salmonella genus of bacteria, S. Paratyphi.

### Scope and Limitation

This study will cover the comparison of kamias leaf and garlic extract as an antibacterial agent. The extract to be used will be concocted from the leaves of the kamias and the bulbs of the garlic. This study will be conducted in a laboratory and further testing will be conducted in the Microbiological Research and Services Laboratory of the Natural Sciences Research Institute in University of the Philippines, Diliman. The study will be performed within an estimated time of fifteen (15) days.

## Chapter 2

### Review of Related Literature

#### Camias (Averrhoa Bilimbi)

Kamias is the Filipino name for a tree scientifically known as Averrhoa bilimbi. In English, it is known as the cucumber or sorrel tree. This tropical tree is found naturally in Malaysia and Indonesia, and its fruit is used both for cooking and traditional medicine. Though kamias is a highly acidic fruit, it can be consumed after certain preparations are taken, and it does provide trace amounts of vitamins and minerals. The height of the kamias tree can reach up to 33 feet, and its leaves, similar to those of the otaheite gooseberry, tend to cluster at the ends of the branches. All year long, it bears small yet fragrant flowers that are either yellow-green or purple. The fruit is yellow-green and belongs to the same family as the starfruit, which is sweeter than kamias. Though sour, the flesh of the kamias fruit is juicy and

soft. (Lynne Sheldon, 2011) Before kamias was introduced to the western world, it was a treatment for rheumatism, mumps and fever in Indonesia, according to Infopedia. nl. sg. It was also used in Malaysia to treat syphilis, high blood pressure and diabetes. Along with its medicinal purposes, kamias is a key ingredient in making drinks and jams and its juice has even been used as a substitute for vinegar.

Kamias provides a minimal amount of daily recommended values of vitamins and minerals. According to FoodRecap. net, 0. 61 g of protein, 0. 6 g of fiber, 3. 4 mg of calcium, 11. 1 mg of phosphorus, 1. 01 mg of iron, 0. 01 mg of thiamine, 0. 26 mg of riboflavin, 0. 302 mg of niacin and 15. 5 mg of vitamin C are contained in every 100 g of kamias fruit consumed. (Tina Pashley, 2010)

#### Medicinal Uses of Kamias

Medicinally, kamias can be made into a paste and applied topically to itchy or swollen skin or skin affected by bug bites. In traditional Malay medicine, they create an infusion of the fruit and leaves to remedy a cough, as well as administer a tonic to women after they give birth. This infusion is also used on pimples, hypertension, dizziness and diabetes. Those in Indonesia use the kamias fruit as a treatment for fevers, inflammation, rectal bleeding, boils and other conditions. The flowers of the kamias are also used as a remedy for toothaches. (Lynne Sheldon, 2011) In the Philippines, the leaves are applied as a paste or poulticed on itches, swellings of mumps and rheumatism, and on skin eruptions. Elsewhere, they are applied on bites of poisonous creatures. Malaysians take the leaves fresh or fermented as a treatment for venereal disease. A leaf infusion is a remedy for coughs and is

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taken after childbirth as a tonic. A leaf decoction is taken to relieve rectal inflammation. A flower infusion is said to be effective against coughs and thrush. (EntrePinoys Atbp., 2009)

#### Antibacterial Properties of Kamias

Study of the aqueous extract of *Averrhoa bilimbi* leaves and fruits showed antibacterial activity against Gram-positive and Gram-negative bacteria. The antibacterial activity could be associated with the presence of bioactive compounds of the flavonoids type, like luteolin and apigenin.

#### Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is a member of the onion family and has been called nature's most versatile medicinal plant. Though it is best known as a culinary herb, garlic has been used all over the world for thousands of years as an herbal remedy for a wide range of conditions. It is mentioned in the Christian Bible and in ancient Egyptian records. In ancient times, garlic was used to treat wounds, infections, tumors, and intestinal parasites. Modern scientists in numerous clinical trials have concluded that eating garlic is a potent antioxidant. This perennial herb is known for its white colored bulb that is composed of small white cloves that have a very peculiar odor and tangy taste.

#### Medicinal Uses of Garlic

Garlic does indeed have scientifically-proven medicinal properties. It contains a substance called Allicin, which has anti-bacterial properties that are equivalent to a weak penicillin. Garlic appears to have anti-bacterial and anti-viral properties. The list is long when it comes to its uses as a remedy.

This list includes wounds, ulcers, skin infections, flu, athlete's foot, some viruses, strep, worms, respiratory ailments, high blood pressure, blood thinning, cancer of the stomach, colic, colds, kidney problems, bladder problems, and ear aches, to name a few. It is believed to cure worms in both people and animals. Externally, garlic is a known anti-bacterial and anti-infection agent.

### Antibacterial Properties of Garlic

Garlic's antibiotic properties have been more extensively studied than some of its other reputed health benefits. Louis Pasteur examined garlic's use as an antibacterial back in the nineteenth century and showed how it killed bacteria under laboratory conditions. Numerous modern studies confirm that garlic has definite antibiotic properties and is effective against many bacteria, fungi and viruses. According to Wright State University, garlic is approximately one per cent as potent an antibiotic as penicillin. Researchers have compared the effectiveness of garlic with that of commercial prescription antibiotics.

The result is often that garlic can be more effective as a broad spectrum antibiotic. However if a particular bacterium or virus is being treated a more specifically targeted antibiotic if available could be a more effective treatment than the more generic effect of garlic. One significant advantage of garlic is that the bacteria do not seem to evolve to build up a resistance to it as they do to many modern antibiotics; " garlic does not seem to produce such resistant strains." This also makes it potentially effective against hospital superbugs - or at least less likely to contribute to their evolution. The antibiotic qualities of garlic appear to be a direct result of the allicin



produced from raw, crushed garlic. This is destroyed by age and cooking – cooked garlic has virtually no antibiotic value although it still retains other benefits.

### Escherichia coli

Escherichia coli, also known as E. coli, is a bacterium that is commonly found in the gut of endotherms (warm blooded organisms). Several types of E. coli exist as part of the normal flora of the human gut and have several beneficial functions, such as the production of vitamin K2. They also prevent harmful bacteria, known as pathogenic bacteria, from establishing themselves in the intestine. Most E. coli strains pose no harm to human health, except for serotype O157: H7, which can cause food poisoning in humans and can become life-threatening. Other less common serotypes, such as O104: H4, O121, O26, O103, O111, O145, and O104: H21 can also cause serious infection.

### Methodology

#### Materials/ Equipments

| Quantity | Materials | | 3 | Beaker | | 200 g | Camias leaves | | 200 g | Garlic bulbs | | 500 mL | Solvent (Diethyl ether) | | 1 | Gas stove | | 1 | Separatory funnel | | 1 | Strainer | | 3 | Funnel | | 2 | Florence flask | | 3 | Filter paper | | 1 | Weighing scale | | 2 | Reagent bottle |

#### Procedure:

##### 1. Preparation of Materials

The materials needed for the preparation of the extract were gathered and

the researchers measured the required amount of materials to be used for a more accurate result.

## 2. Preparation of Extracts

The kamias leaves were boiled in distilled water under constant temperature. After 15 minutes of boiling, the kamias leaves were strained from the liquid. The liquid contained the extract from the kamias leaves. Then, the garlic bulbs underwent the same procedure. Both materials produced reasonable suffice amounts of liquids for extraction. Then, the liquids were thoroughly filtered in the filtration process using filter paper. In the process of extraction, the extraction setup was assembled composed of iron stand, iron ring and the separatory funnel. The kamias extract and the garlic extract separately underwent the process of extraction by transferring the liquid into the separatory funnel and pouring 300 mL of diethyl ether as solvent of extraction. After vigorously shaking the mixture, the mixture was separated into layers consisting of the extract and the water. Then, the extracts were produced and contained in two sterilized reagent bottles for laboratory testing.

## 3. Laboratory Testing

The kamias extract and the garlic extract were tested based on their antibacterial assays at the Microbiological Research and Services Laboratory. The methodology the researchers from the laboratory performed was as follows:

Microbial suspension was prepared from 24-hour old culture of the test organism. The suspending medium used was 0.1% peptone water.

Pre-poured Nutrient Agar (NA) plates, about 3 mm thick, were inoculated with the microbial suspension by swabbing the agar surface. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove the excess inoculum from the swab. The swab was streaked over the entire agar surface. This procedure was repeated two more times, rotating the plate 60° each time to ensure even distribution of the inoculum. Three equidistant wells were made on the agar plate using a cork borer (10 mm diameter) and 200 µl of the sample was placed in each hole. A single well or disc was placed on the control plates.

The NA plates were incubated at 35°C and observed after 24 hours. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated. The antimicrobial index (AI) was computed using the following the formula:

## Results and Discussion

After performing the procedure, the researchers noted observations regarding the physical attributes of both extracts produced. | Extract | Color | Appearance | Smell | Shelf Life | | Kamias | Deep green | Cloudy | Odorless to leafy smell | 1-2 weeks | | Garlic | Clear to dirty white | Cloudy with minimal settling | Strong garlic smell | 1-2 weeks | | | particles | | |

The researchers also observed during the filtration process that the garlic extract took longer time before being completely filtered of larger particles compared to the kamias extract. In terms of viscosity, the kamias extract was observed to be more viscous than the garlic extract.

After receiving the results from the Microbiological Research and Services Laboratory, the table below shows the outcome of the experiment: | Test

organism | Sample | Clearing zone, mm | AI | | | 1 | 2 | 3 | | | E. coli

| Kamias Extract | -a | - | - | 0 | | | Garlic Extract | 20 | 18 | 18 | 0.9 | | |

Chloramphenicolb | 27 | 3.5 |

aNo inhibition of growth of the test organism

b6-mm disc contains 30 µg chloramphenicol

From these results, we can interpret that the garlic extract exhibited a more effective antibacterial assay against *Escherichia coli* compared with kamias extract with an antimicrobial index of 0.9. Kamias extract did not exhibit any inhibition to the growth of the test bacteria. Also, chloramphenicol, the control setup of the experiment, exhibited greater effectiveness in terms of antibacterial assay with an antibacterial index of 3.5.

The kamias extract did not exhibit antibacterial properties against *E. coli*, despite previous researches performed by scientists who concluded that kamias extract did exhibit antibacterial assay. The researchers analyzed that the sample kamias extract did not inhibit the growth of *E. coli* because of possible contamination by foreign materials and inaccurate execution of the procedure which resulted to an ineffective sample.

Chapter 5

Conclusion and Recommendation

Conclusion:

The researchers therefore conclude that the garlic bulb extract has greater effectiveness of its antibacterial property against E. coli compared with kamias leaf extract. Also, the kamias leaf did not exhibit its antibacterial assay and did not inhibit the growth of E. coli, as expected based on previous researches about its antibacterial assay against E. coli.

#### Recommendation:

After interpreting and analyzing the results, the researchers highly recommend: 1. Further research on the antibacterial assays of the kamias leaf and garlic bulb extracts 2. Careful execution of the procedure to avoid inaccuracy in the results 3. More thorough and carefully planned procedure for this type of study 4. Further study on E. coli and the compounds that inhibit its growth

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