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# Microbial, Phytochemical Analysis and Potential of Bignay (*A. Bunius* L.) Leaf as Organic Pesticide

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***Abstract:*** ***A****ll other life depends on the foundation of plants. They are regarded as the foundation of all life on Earth and are crucial to human well-being. They are also vital to the industry and field of medical innovation. Traditional medical practices have employed plant-based repellents for decades as a more effective defense against mosquitoes that are host-seeking (A. aegypti). Bignay (A. bunius) has insecticidal and antibacterial capabilities, which has prompted researchers to launch this investigative effort to reduce the danger of mosquito-transmitted diseases and control, if not completely eradicate, mosquito populations. ethanol as the extracting medium underwent a rotary evaporator for phytochemical screening, the plant's pure extract from the leaves was made for microbiological analysis. The results of the microbiological experiment showed that bignay leaf extract did not prevent S from growing. E. aureus E. coli and C. albicans. The leaves do, however, contain significant chemical substances such as tannins, terpenoids, phenolic compounds, and saponins, according to the phytochemical examination. These substances are known to be effective against mosquitoes. The most effective insect repellent among the five setups (C. 75% extract+ 25% distilled, B. 50% extract+ 50% distilled, and A. 25% extract+ 75% distilled) was also the most effective when compared to commercial insecticides. As a result, Bignay is a more effective alternative source of organic insecticides than commercial pesticides.*

**Keywords:** A.bunius, Bignay, A. aegypti, microbial, phytochemical, leaves, pesticide

## Introduction

Life is everywhere. The planet is made up of millions of different types of species and groups of them, which together form a healthy ecosystem. Plants are one of those numerous types of organisms. According to William (2017), plants serve as the foundation upon which every other life on Earth protects itself. They are also a vital source for human well-being. They are extremely important to our daily lives since they provide us with food, drink, and air.They also play significant factor in the field of medical innovation and industry. Several plants are proven to have valuable role in preparation of vitamins and drugs. Studies show that one-quarter of all prescription drugs come directly from or are derivatives of plants. Additionally, four out of five people around the world today rely on plants for primary health care.

*Antidesma bunius* is a specie of fruit trees in the Phyllanthaceae. It is native to Southeast Asia and Northern Australia. *Antidesma bunius* Spreng., is called *bignai* in the Philippines. This variable plant may be short and shrubby or tall and erect, approaching 30 metres in height. It has large oval-shaped leathery evergreen leaves up to about 20 cm long and seven wide. They form a dense canopy by being joined to the tree's twigs by short petioles. From the lower Himalayas in India, Ceylon, and Southeast Asia (but not Malaya), to the Philippines and northern Australia, the Bignay is indigenous and widespread in the wild. In the Philippines, it is a common and invasive plant; it is also occasionally cultivated in Malaya, and it is grown in every village in Indonesia, where the fruits are sold in clusters. The leaves are used to cure snakebites in Asia and are sudorific in medicine (Morton, 2017). The insecticidal and antimicrobial properties of *Bignay* as well as their abundance in Akle, San Ildefonso have motivated the researcher to conduct this investigatory project, to control if not eradicate the mosquitoes and protect the people from the risks of mosquito-transmitted diseases. Since the school is near the irrigation where there is stagnant water within the area mosquitoes is can breed everywhere. This situation may lead to a serious problem that need to address proper solution.

America is home to an invasive species called Aedes aegypti. It most likely arrived on slave boats from Africa in the seventeenth century, along with the yellow fever it carried. They don't fly far for live long; major traveller would few hundred yards and on average, survive as an adult for ten days. They prefer to bite victims in the ankles or legs and eat during the day. They typically stay low to the ground and attack in silence.

*Aedes aegypti* is the type of mosquitoes that cause Dengue.Dengue has always been considered a tropical illness. Usually, dengue fever resolves on its own. According to the World Health Organization, there is presently no specific antiviral treatment for dengue fever (WHO) (Shepherd, 2015). Several studies have been conducted on plants to explore their possibility as organic source of insecticide. It is in this light that the researcher get interested evaluating the potential of bignay plant extract as a substitute for organic insecticides and a natural antibiotic against *Staphylococcus aureus*, *Escherichia colli* and *Candida albicans.*

**Statement of the problem.** Specifically the researcher aims to; determine the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins and terpenoids; determine the possible uses of *Bignay* plant leaves as insecticides based on the abundance of phytochemicals as revealed by the phytochemical analysis; and determine if *Antidesma bunius* can inhibit the growth of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*.

**Hypothesis**. The researcher follow the null hypothesis; First there is no significant difference between microbial and phytochemical analysis of *Bignay* plant extract against *S. aureus, E. coli* and *C. albicans*. Second there is no significant difference between the *Bignay* plant leaves extract to commercially based pesticides.

**Conceptual Framework**

| INPUT | PROCESS | OUTPUT |
|---|---|---|
| A. Gathering of Plant Source (Bignay)<br><br>B. Preparation of Extracts<br>□ Cleaning the Leaves using distilled Water<br>□ Cutting of Leaves<br>Preparation of Pure Extract<br><br>C. Preparation of extract with different concentration<br>-Experimental group<br># 25% extract + 75% Distilled water<br># 50% extract +50% distilled water<br># 75% extract +25% distilled water<br>-Positive Control group using commercial pesticides<br>Negative control using distilled water | A. Microbial Test Bioassay<br>*S aureus, E coli,* and *C albicans.*<br><br>B. Phytochemical Profiling of Bignay Plant Extract<br>o Alkaloids<br>o Flavonoids<br>o Glycosides<br>o Phenolic compounds<br>o Saponins<br>o Tannins<br>o Terpenoids<br><br>C. Experimentation<br>Experimental group<br>Control group | A.Antimicrobial Property and Possible Uses of Bignay plant.<br>Findings indicated that Large plants can't impede the development of *Staphylococcus aureus, Escherichia coli and Candida albicans.*<br><br>B. Phytochemical Analysis<br>Botanical Chemical Profiling shown that there was Phenolic substances, Saponins, Tannins, and Terpenoids. However, alkaloids glycosides and flavonoids are absent.<br>The chemical compound present is can be source of organic pesticide.<br><br>*C.* Experimentation Result<br>Based on experiment among the 5 set-up C.75%+25% distilled water is most effective as organic pesticide. |

The study use the Input, process and Output (IPO) model. This is a functional diagram/graph that determines the inputs, outputs, and the required processing tasks needed to transform inputs into outputs. Sometimes, this model is set up to take into account any data that might be generated during the process (Schembri, 2012).The first frame shows the inputs which include the independent variables of the research, beginning the collection of plant sources and creation of various concentrations of plant extracts. The second reflects the process which includes the phytochemical profiling of *Bignay* plant extract, antibacterial assay, antifungal assay and experimentation of the different concentrations of the extracts, this serves as the moderating variables of the research. The third frame is for the outputs, which describes the results of phytochemical analysis, antibacterial assay, antifungal and the results of the experiment, this serves as the dependent variables of the research.

**Significance**

The significant of this research is to discover the plant that will show valuable resources for the potential of new organic products. It will highlight plants that have the potential to yield advantageous medicinal components.Bignay plants will serve as the answer to the very expensive drugs and repellent present in our society nowadays. Studies have shown that having plants around the office and school reduces anxiety in both staff and students and has great effects on health, including lowering blood pressure, alleviating tension in the muscles, and boosting

mood. These advantages can lead to better health and increased worker and student productivity (Getter, 2015).The beneficial goal of this research project to one utilize the cheap, natural abundance of nature, and its advantages as an alternative remedy of dengue causing mosquitoes.

**Scope and Limitation.** The study is limited to the use of *Bignay* plant leaves as a source of pure extract to be tested. Antimicrobial assay was done at MSRI at UP Diliman. This test was carried out to find out how effective the extract was against E. coli, S. aureus, and C. albicans. Only the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, tannins, terpenoids, and saponins were found when the phytochemical features of this plant were examined. The collection of plant leaves is done at San Ildefonso Bulacan. The extraction of *Bignay* leaves was done at Calawitan National High School laboratory. This research started on June 13, 2017 and it will end at September 10, 2017 for the school year 2017-2018.

*Antidesma bunius (L.) spreng* leaves were obtained from Barangay Akle. The extraction process was conducted at Calawitan National High School laboratory San Ildefonso, Bulacan. Ethanol was used as an extracting medium of the plant leaves. An extract were submitted for the phytochemical analysis and appropriate test in the College of Chemistry at University of the Philippines Diliman, Quezon City. The screening shall determine the presence of each phytochemical component as absent, with traced amount, slightly detectable or strongly present.

**Review of Related Studies**

**The Potential of Antidesma bunius (Linn) Spreng (Bignay) Leaves**

A. bunius or commonly known as "Bignay" is a specie from the It is indigenous to Northern Australia and Southeast Asia and is a member of the Phyllanthaceace family. It has an ever green alternate oblong leaves, leathery, glossy and short petioles. The tree's leaves and bark were found to contain alkaloid, which is an active component of commercial pesticides. The researchers conducted the study of the potentials of Bignay leaves as pesticide; with the utilization of its leaves as raw materials in preparing pesticides. Based on the research, they found out that the leaves of the plant are rich in alkaloids which is basically one of the important components of pesticides. The study prove that bignay can be a substitute for commercially based pesticides (Bautista, 2015).

**Antidesma bunius (Bignay) fruit extract as an organic pesticide against Epilachna spp**

The Epilachna app., a member of the Coccinellidae family, was the target of this study's investigation into the potential of Antidesma bunius fruit extract as an organic pesticide. The findings indicate that A. Bunius fruit extract can be used as a creative substitute for pesticides to combat ladybirds (Family Coccinellidae), which are considered to be a dangerous pest that cause significant harm to a variety of agricultural crops (Belmi et al, 2014).

**Plant-based repellents: a review on their efficacy, development and testing**

Since ancient times, people have employed plant-based repellent to defend themselves from mosquitoes that are looking for hosts. For the creation of new natural goods, ethnobotanical studies' knowledge of plants used as traditional repulsives is a great resource. The majority of plants have chemicals that help people defend themselves against attacks from phytophagous (plant-eating) insects. These chemicals fall into several categories, including repellents, feeding deterrents, toxins, and growth regulators. The majority of them fall into one of five main chemical categories: nitrogen compounds, terpenoids, phenolics, proteinase inhibitors, and growth regulators. These substances work primarily as a plant-eating insect defense, but they also work well against mosquitoes (Maia and Moore, 2011).

**Antidiabetic Activity test by inhibition of a Glucosidase and phytochemical screening from the most active fraction of Buni (Antidesma bunius L.) Stem Barks and Leaves.**

Antidesma bunius L. stem bark and leaf fractions of 80% ethanol (EtOH) extract were tested for glucosidase inhibitory activity. Sugars, terpenes, and flavonoids were found in the phytochemical analysis of the ethyl acetate fraction of the buni stem barks, whereas sugars, saponins, flavonoids, and tannins were found in the MeOH fraction of the buni leaves. Both the G subfraction and the F subfraction included sugars and flavonoids, according to the phytochemical screening. The kinetic analyses both supported the competitive mode of hydrolysis inhibition (Elya et al., 2012).

**Changes in Physico-Chemical Properties, Polyphenol Compounds and Antiradical of Maoluang (*Antidesma Bunius L. Spreng)* Fruits**

In Maoluang fruits, the effects of fruit development and ripening on changes in physical and chemical characteristics, antiradical activity, and the accumulation of polyphenolic chemicals were studied. According to this study, it also has the greatest gallic and total phenolic levels among other phenolic acids. The primary phenolic components include procacyanidin B2, procyanidin B1, (+)-catechin, (-)-epichatechin, rutin, and trans-veratrol. The most antioxidants are present in Maoluang. As a result, the information that has been released offers helpful daily diet guide for industrial utilization (Butkhup and Samappito, 2011).

**Definition of terms**

The following terms were use in completing this study.

A nitrogen ring is a component of the class of chemical compounds known as alkaloids. Alkaloids are natural substances that are produced by a wide range of species, including bacteria, fungi, plants, and mammals. By using acid-base extraction, several alkaloids can be separated from unpurified extracts. (Casiano, 2017).

**One type of fruit tree in the Phyllanthaceae family is called Antidesma bunius.** It is indigenous to northern Australia and Southeast Asia. Bignay, bugnay, or bignai, Chinese-laurel,

Herbert River-cherry, Queensland-cherry, salamander-tree, wild cherry, and currant tree are some of its alternate names. This plant can range in height from a few meters and be shrubby to about 30 meters and be tall and erect. It has broad, oval, leathery leaves that can grow up to 20 cm long and 7 cm wide. They form a dense canopy by being joined to the tree's twigs.

Candida albicans. Is a dimorphic fungus. This implies that C. A branching hyphal form and an oval-shaped yeast form are the two distinct phenotypic forms of albicans. C. The mucosal membranes of people and other mammals, such as the mouth, gut, vagina, and even the skin, are albicans' natural home. Typically, C. Albicans coexists symbiotically with its human or animal host, causing no harm and even assisting in the breakdown of trace amounts of dietary fiber. The regular bacterial flora of the mouth, vaginal mucosa, and digestive tract serve as a check against the development of fungal diseases like C. albicans. One of the key causes that makes someone more likely to contract C is the loss of this natural flora (Strickland, 2010).

Escherichia coli, or simply E. coli) is a rod-shaped, gram-negative Escherichia bacterium that is typically found in the lower intestine of warm-blooded species (endotherms). Most E. While most E. coli strains are safe, some serotypes can give their hosts significant food illness and occasionally trigger product recalls owing to food contamination.

**Flavonoids** are a group of secondary metabolites found in plants. Chemically, they are composed of two phenyl rings (A and B) and a heterocyclic ring with a typical 15-carbon skeleton (C). The abbreviation for this carbon skeleton is C6-C3-C6.

**Glycosides-** The term "glycoside" refers to substances in which a sugar molecule is joined to a non-carbohydrate modification, often a tiny chemical molecule. It has a variety of significant functions in living things. Inactive glycosides are the primary form of chemical storage in many plants. These can be made active through enzyme hydrolysis, which separates the chemical from the sugar component and makes it usable. Plant glycosides are widely employed in medicine.

**Phytochemicals** are substances made by plants. The word is now only applied to plant compounds that have potential health-related effects but are not regarded as necessary nutrients.

**Plant** ingredients are tracked via a procedure called phytochemical screening. There is a set technique, and color changes as indicators are typically used.

**Saponins** -A group of chemical compounds known as saponins is present in remarkable abundance in a variety of plant species. More specifically, they are amphipathic glycosides that are categorized structurally by having one or more hydrophilic glycoside moieties paired with a lipophilic triterpene derivative and phenomenologically by the soap-like foaming they create when shaken in aqueous solutions (Kannal, 2017).

***Staphylococcus aureus***- is a gram-positive, Firmicutes-class coccus bacteria that is frequently found on the skin and in the human respiratory tract.

**Tannin**- also known as vegetables tannin, natural organic tannins or sometimes tannoid, b ( ie type of biomolecule as opposed to modern synthetic tannin) is an astringent bitter plant polyphenolic compound that binds to and precipitates protein and various other organic compounds including amino acid and alkaloids. These substances are extensively dispersed in a variety of plant species where they serve as a form of defense against predators and may also act as insecticides to control plant development (Chung et al., 2017).

**Terpenoids** represent the largest and most diverse class of beneficial plant chemicals; more than 40,000 individual terpenoids exist, and new ones are discovered every year Accumulating research suggests terpenoids may help prevent metabolic disorders, fight cancer, exert anti-aging benefits Terpenoids are a valuable resource for the flavor and fragrances industries because they are responsible for a diversity of plant scents and flavors (Mercola, 2017).

## Methods

### A. Materials

The following materials were utilized in the study:
Bignay leaves Beaker Empty container Ethyl alcohol
Erlenmeyer flask Distilled water
Water (distilled,Tap) Dropper
Sprayer Blender
Strainer

### B. Procedures

1. Plant Components

In Akle, San Ildefonso, Bulacan, the leaves of the Antidesma bunius (Bignay) tree were collected. In order to properly authenticate the Bignay plant leaves, they were cleaned, washed, and dried before being given to a plant taxonomist at the College of Biology at the University of the Philippines, Quezon City, who confirmed the species as Antidesma bunius (Linn) Spreng.Another set of *Bignay* leaves were cleaned and washed for extraction of the plant for microbial assay. From 1 kg of plant leaves 250 ml of pure extract was obtained by squeezing out In order to collect the plant material for microbiological analysis, another set of Bignay leaves were cleaned and washed. 250 ml of pure extract were produced from 1 kg of plant leaves by pressing the juice with a cheese cloth. A bottle of wine was also prepared, along with another pair of fresh Bignay leaves.The leaves were chopped and weighed 1 kg of plant leaves were mixed with 1L 95% of ethyl alcohol then soaked it for 72 hours. After soaking, it was squeezed using cheesecloth or a strainer before being put in an empty, clean bottle to hold the extract. The College of Chemistry Analytical Analysis Division received the extract for rotary evaporation. After 4 days the extract was given to the University of the Philippines, Diliman, Quezon City's Phytochemical Analysis Division for phytochemical analysis.

1. Collection of Insects

Using mosquito nets, adult mosquitoes were gathered from Calawitan National High School's various classrooms. No matter their size or age, roughly 450 mosquitoes were caught and

brought to the testing location. For 30 minutes, mosquitoes were moved from clean plastic petri dishes into the jars to help them relax and become acclimated. Then, 150 mosquitoes were divided among five jars, each containing 50 mosquitoes. Cling wrap that had been pierced was placed over every plate to keep
3. Mortality Test

On the test plates, sprays of various quantities of plant extract and the controls were applied. In each treatment, 2ml of solutions were used. Every 15 minutes, insect mortality was measured and the initial spraying time was recorded. Based on the results of the initial experiment, which used distilled water as the negative control group and a commercial pesticide as the positive control group, this time interval (15 min) was established. Each treatment's mortality was noted for up to five hours. With three experiments at three distinct concentrations, three trials were undertaken. 4. Data Analysis

Each treatment's average mortality was calculated. The data were subjected to one-way analysis of variance (ANOVA) at both the 0.05 and 0.01 threshold of significance (Statistics Solution, 2013). Then, the F-test was used to compare the differences between the treatments.

## Results

I. Antibacterial property of *Antidesma bunius* leaves extract

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

Pre-poured Nutrient Agar (NA) plates, about 3 mm thick, were inoculated with the respective bacterial suspension and Glucose Yeast Peptone Agar (GYP) with the yeast suspension. Inoculation of agar plate surfaces was done by swabbing technique. To remove extra inoculums from the cotton swab, it was dipped into the microbial solution, twisted several times, and then pressed firmly against the interior wall of the tube above fluid level. Over the entire agar surface, the swab was streaked. In order to guarantee that the inoculum was distributed evenly, this technique was repeated twice more, each time rotating plate 600. On the agar plate, three (3) evenly spaced wells were created using a cork borer (10 mm diameter). Each well received a part of the sample equal to 200 µl.

After 24 hours of incubation at 350C, the NA and GYP plates were examined. The clearance zone was measured in millimeters, and its typical diameter was determined. The following formula was used to determine the antibacterial index (Al).

$$AI = \frac{\text{Diameter of clearing zone} - \text{diameter of the well}}{\text{Diameter of the well}}$$

As a result, Staphylococcus aureus, Escherichia coli, and Candida albicans cannot be inhibited by Antidesma bunius. The computed AI was 0. The positive control was chloramphenicol.

the insects from escaping while yet enabling regular breathing. Just before the mortality test, mosquitoes were given an additional 30 minutes to acclimate in the testing plates. In three trials for each concentration, the procedure was repeated.

*Table 1 Summary of the Bioassay analysis*

| Test Organism | Sample | Clearing zone mm | AL |
|---|---|---|---|
| *E. coli* | Bignay ( *A. bunius* | | 0 |
| | Choramphenicol disc[b] | -27 | 3.5 |
| *S. aureus* | Bignay ( *Antidesma bunius* | - - - | 0 |
| | Choramphenicol disc[b] | 33 | 4.5 |
| *C. albicans* | Bignay ( *Antidesma uunius* | - - - | 0 |
| | Choramphenicol disc[b] | 35 | 2.5 |

a. A phytochemical analysis of the ethanol-based extract of Bignay leaves.

b. Alkaloids

The Wagner's reagent was employed to check for the presence of alkaloids. Three (3) cc of distilled water were used to dissolve the plant extract. Wagner's reagent used dropwise causes a blue-black precipitate, which denotes the presence of alkaloids. When Wagner's reagent was added to the beaker, the Bignay extract's dark brown original color changed, indicating that there had been no reaction.

c. Glycosides (Killiani-Keller test)

The extract was dissolved with 2 ml distilled water. To the solution, 1% $FeCl_3$ was added dropwise. One (1) mL concentrated H2 SO4, was added without disturbing the solution. The observance of a brown ring is indication of cardiac glycosides.

The extract shows that there are no glycosides present.

d. Phenolic substances

Two (2) mL of distilled water were used to dissolve the plant extract. the emergence of blue-black precipitate or a brownish- green precipitate with the dropwise addition of FeCl3 solution at 1% is a sign that phenolic chemicals are present.

d. Saponins

(5) mL of distilled water were used to dissolve the plant extract. The solution was heated to boiling, cooled, and then shook ferociously. The presence of saponins is indicated by the development of foaming.

The outcome shows that the extract contains saponins.

e. Tannins

The plant extract was dissolved in (1) mL distilled water. The formation of blue-black precipitate or a brownish- green precipitate with the dropwise addition of 15 % $FeCl_3$ solution, is indicative of the presence of tannins.

The result indicates the presence of tannins in the extract.

f. Terpenoids

The dried extract was treated with two (2) mL of ChCl3 and then a layer of saturated H2SO4. The presence of terpenoids is indicated by the presence of a reddish brown interface.

The outcome shows that the extract contains terpenoids.

*Table 2 Results of Bignay Leaf Extract Phytochemical Screening.*

| Test for the presence of the following | A. bunius (bignay) |
|---|---|
| Tannins | + |
| Saponins | + |
| Terpenoids | + |
| Flavonoids | - |
| Cardiac glycosides | - |
| Phenolic substances | + |
| Alkaloids | - |

C. Experimental Design and Treatments

*Table 3 Aedes aegypti mortality under various treatments*

| Treatments | Trial 1 | Trial 2 | Trial 3 | Average |
|---|---|---|---|---|
| *A.bunius* extract | | | | |
| 25% | 25 | 23 | 26 | 25 |
| 50% | 31 | 29 | 32 | 31 |
| 75% | 37 | 38 | 36 | 37 |
| Positive control (Commercial pesticide) | 48 | 50 | 50 | 49 |
| Negative Control (Distilled water) | | | | |

*Table 4. Summary of Result showing the number of mosquitoes died in each treatment*

| Group | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 25% | 3 | 74 | 24.66667 | 1.555556 |
| 50% | 3 | 92 | 30.66667 | 1.555556 |
| 75% | 3 | 111 | 37 | 0.666667 |
| Positive control | 3 | 148 | 49.33333 | 0.888889 |

*Table 5. Computation showing the significant difference in the Bignay leaf extract at varied concentration using ANOVA*

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1002.917 | 3 | 334.3056 | 0.55353 | 0.66004 | 4.06618 |
| Within Groups | 4831.62 | 8 | 603.9525 | | | |
| Total | 5834.537 | 11 | | | | |

**Discussion**

Bignay (Antidesma bunius) leaf phytochemical analysis and bioassay were performed to identify the phytochemical components that were anti-Escherichia coli, anti-Staphylococcus aureus, and anti-Candida albican. Results indicate that Antidesma bunius is unable to stop E. from growing. coli, S. C. aureus and albicans.

In this work, a pythochemical screening test was conducted on the extract Antidesma bunius. In Akle, San Ildefonso, Bulacan, the fresh leaves were harvested. The harvested leaves were divided into little pieces and squeezed through cheesecloth after being cleaned with distilled water. On the other hand, a different batch of leaves was prepared for the bioassay procedure. It was then soaked into Ethanol for 72 hours. After soaking, it underwent rotary evaporation in Analytical Research at the College of Chemistry Research Building and bioassay in Natural Science Research Institute at Miranda Hall while the phytochemical test was done in the College of Chemistry Research Building at UP Diliman, Quezon City. In the pythochemical screening test, alkaloids, cardiac glycosides, flavonoids, tannins, terpenoids, polyphenolic chemicals, and saponins were all tested for. The findings of the phytochemical screening revealed the presence of tannins, terpenoids, polyphenolic chemicals, and saponins. However, flavonoids and glycoside alkaloids were not present.

Saponins may boost the immune system's ability to fight off viruses, parasite, and fungal cells such as *Candida albicans*, according to the study of "ACS Chemical Biology" in March 2010 (Kannal, 2017). The various components that make up plant antioxidants include terpenoids, polyphenolic chemicals, ascorbic acid, and tocopherols. In-depth discussions of these mechanisms and the in vitro analysis of antioxidant activity were conducted. It's possible that the monoterpenes limonene and perillyl alcohol will be effective treatments for cancer (Grassman, 2014).Tannins are used as effective insect repellent. The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by tannins (Chung et al., 2017).

Natural phenolic chemicals are crucial in the treatment and prevention of cancer. Medicinal and nutritional plants include a variety of phenolic chemicals, such as phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, and quinones. The most recent research is covered in this review, which lists the structural categories and molecular anticancer mechanisms of phenolic compounds from dietary and medicinal plants. (Huang et al., 2010).

For the antimicrobial test, another set of leaves were prepared for bioassay. The *Bignay* pure leaves extract was been submitted in Natural Science Research Institute at Miranda Hall, UP Diliman, Quezon City. The outcome demonstrated that Bignay plants do not impede E. coli, S. C. aureus and albicans.

Other researchers may attempt to generate an Antidesma bunius solution using a different extraction solvent for a potentially superior outcome. Since the extract the researcher used in the microbiological test was pure and there was no solvent combined in the leaves, there was a flaw in the study. The plant is able to stop the E. coli, S. C. aureus and Since the findings of the phytochemical research showed that the plants contained the chemical components responsible for those microorganisms, albicans will grow if the extract reported in NSRI is subjected to rotational evaporation.

Results on Table 4 on the treatment of Bignay leaf extract on mosquitoes showed that in 24 hours, 25% concentration, 25 mosquitoes died in Trial 1, 23 mosquitoes died in Trial 2 and 26 mosquitoes died in Trial 3 with the Mean of 24.67. This indicated the Bignay leaf extract is effective against mosquitoes. As regards, 50% concentration, 31 mosquitoes died in Trial 1, 29 in Trial 2 and 32 died in Trial 3 with of Mean of 30.67. The results indicated that at this concentration Bignay leaf extract is considered as very effective treatment for killing mosquitoes.

When 75% concentration were used, all the 37 mosquitoes died in Trial 1, 38 mosquitoes died in Trial 2 and 36 mosquitoes died in Trial 3 with a Mean of 37. This indicates that at this concentration of Bignay becomes very effective in killing mosquitoes. In the control group, 48 mosquitoes perished in Trial 1, 50 in Trial 2, and 50 in Trial 3, for a mean of 49.33. According to the results, a concentration of 75% suggests a treatment that kills mosquitoes effectively and can be compared to a positive control.

The One Way Analysis of Variance (ANOVA) was employed and assessed at the 0.05 level of significance to see whether there is a significant difference in the ability of Bignay leaf extract to kill mosquitoes at different concentrations based on Table 5.

Table 5's findings of the ANOVA calculation, on the other hand, revealed that there were significant variations in the level of effectiveness of Bignay (Antidesma bunius) leaf extract between 75% concentration and the positive control because they obtained lower than 0.05. As a result, the null hypothesis was disproved.

## Conclusion

The findings of this study demonstrate that the Bignay extract contained a variety of phytochemicals, including phenolic compounds, tannins, terpenoids, and saponins. Natural phenolic chemicals are crucial in the treatment and prevention of cancer. The majority of a pesticide's ingredients are phenolic compounds. Effective insect repellents include tannins. Tannins' antibacterial properties are widely known. Tannins prevented the development of numerous fungi, yeasts, bacteria, and viruses (Chung et al., 2017). According to a study published in "ACS Chemical Biology" in March 2010, the saponins found in many plants may increase the immune system's capacity to combat viruses, parasites, and fungi like Candida albicans (Kannal, 2017). The largest class of natural substances are terpenoids, often known as terpenes. Many terpenes are used to treat human ailments because they have biological effects. The Bignay microbiological and phytochemical screenings differ significantly from one another.

The outcome of the bioassay procedure, which was carried out in Antidesma bunius and involved the use of pure extracts of the Bignay against Escherichia coli, Staphylococcus aureus, and Candida albicans, revealed that the extract of the Bignay cannot suppress the growth of the test organisms.

The researcher rejected the null hypothesis, which claims that there is no significant difference between the commercial pesticide on the market and the extract from Bignay plant leaves in terms of effectiveness, after analyzing the data with the F test (one way ANOVA).

Due to the primary chemicals present, which are nearly as potent and effective as the commercial pesticide, the research of Bignay extract has been recognized as noteworthy by the scientific community.

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