South SEED-LPDH College
College of Medical Laboratory Sciences

Anti-Urolithiatic Activity of *Musa acuminata* (Lakatan Banana) Leaves Extract Against Calcium Oxalate Crystals Induced *Rattus Norvegicus*

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The Faculty of College of Medical Laboratory Sciences
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Bachelor of Science in Medical Laboratory Science

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This thesis, entitled, **Anti-Urolithiatic Activity of *Musa acuminata* (Lakatan Banana) Leaves Extract Against Calcium Oxalate Crystals Induced *Rattus norvegicus*.,** prepared and submitted by Bare, Alexander Jr. L., Aguilar, Apolinario Jr. C., Conui, Daniul Jonn P., Javier, Cxyrile Kristof C., Pelaez, Joseph A., Tomas, Adriel Joseph C., in partial fulfillment in the requirements for the degree of Bachelor of Science in Medical Laboratory Science, has been examined and is recommended for acceptance and approval for oral defense.

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CERTIFICATE OF ORIGINALITY

We hereby certify that this THESIS is our own work and that, to the best or our knowledge, beliefs, and understanding, that this paper contains no material previously written or published by another person or organization nor any research material which has been accepted and nominated for awards for any other degree or diploma from any educational institution, except where due acknowledgement is made thereof.

Furthermore, we declare that the intellectual contents of this THESIS is the product of our work although receiving assistance from other people in the manner of organization, presentation, grammar, vocabulary, and style.

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THESIS ABSTRACT

Title:

Anti-Urolithiatic Activity of *Musa acuminata* (Lakatan Banana) LeavesExtract Against Calcium Oxalate Crystals Induced *Rattus Norvegicus*

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Objectives: The study was entitled "Anti-Urolithiatic Activity of *Musa acuminata* (Lakatan Banana) Leaves Extract Against Calcium Oxalate Crystals Induced *RattusNorvegicus".* This study was conducted to determine the anti-urolithiatic activity of lakatan banana leaves. Crystals were induced in the rats using ethylene glycol ad libitum. The anti-urolithiatic activity of the lakatan banana leaves was determined using microscopic examination of the urine excreted by the rats.

Research Method: The active phytochemical components of the *Musa acuminata* leaves were also identified. The lakatan banana leaves were collected from a small plantation in Bacoor, Cavite and was authenticated at the National Museum in Manila. The obtained *Musa acuminata* leaves were tested and have undergone phytochemical testing and it was identified that the leaves contain the following: Triterpenes, Flavonoids, Alkaloids, Saponins, Glycosides, and Tannins. Five kilograms of lakatan banana leaves were oven dried and subjected to 4 liters of ethanol solvent for nine days and was concentrated on the tenth day using a rotary evaporator. This was performed in the Department of Science and Technology in Taguig.

Subjects/ Respondents: This experiment is limited to *Rattus norvegicus* species only. The subjects used are twenty-nine six week old male *Rattus norvegicus* which were purchased from the Food and Drug Administration in Muntinlupa City and was transferred to the Department of Science and Technology in Taguig for housing.

Research Cost: The total expense of the experimentation was P29,941. Housing of the rats in the ITDI-DOST cost P11,571, the rats cost P3000, the extraction cost 2816, the remaining cost amounts cover the disposable materials, transportation, and utilities used throughout the experimentation period.

Data Treatment Method: This study was based on the controlled experimental research design wherein subjects are kept at similar variables to be able to obtain the effects of the test samples in this study. Twenty-nine six-week old male *Rattus norvegicus* weighing approximately 100-200 grams. The test animals were divided into three groups. All groups were induced with 0.75% ethylene glycol ad libitum to promote formation of calcium oxalate crystals and is given constantly throughout the 13 days of experimentation period. The positive and experimental control group was treated with Cystone and *Musa acuminata* leaves ethanolic extract respectively and was given in three doses; 300mg/kg, 400mg/kg, and 500mg/kg after 6 days of crystal induction period. Urine of the rats were collected and examined for microscopic examination for the baseline crystals, 6 days post urolith induction, and the 7 days post treatment.

Findings: Based on the results obtained from experimentation, it shows that there is no significant difference in the effects between the positive control and experimental control group (P is not <0.050). Also, there is no significance difference between the negative control group and the experimental control group (P is not <0.050). This shows that the *Musa acuminata* leaves extract produced the same results as the positive control in the experimentation but was also not significant when compared to the negative control. But based on the successfully induced rats in table 4.1, It is supported that the highest dose of the lakatan banana leaves extract possess a potential anti-urolithiatic activity.

Conclusion: Based on the experimentation data and the analysis of the researchers, we conclude that the *Musa acuminata* leaves extract is not an effective anti-urolithiatic agent against calcium oxalate crystal in the following doses; 300mg/kg, and 400mg/kgbut in the highest dose, 500mg/kg showed probable signs of anti-urolithiatic activity.Also that the phytochemical components of *Musa acuminata* leaves include the following; triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins.

Based on our statistical data the researchers conclude that there is no significant difference between the positive control and experimental control group also, that there is no significant difference between the negative control group and experimental control group but can be supplemented by the raw experimental data to show significance.

Recommendation: Based on this study, the researchers recommend to increase the duration of the experiment in further studies; induction period of calcium oxalate crystals, and the duration of treatment as well as higher doses of *Musa acuminata* leaves extract be used in further studies than what was used in this research (300mg/kg, 400mg/kg, and 500mg/kg). The researchers recommend using other solvents which can be used for active component extraction which can be used for comparison in potency. Other studies are encouraged using *Musa acuminata* leaves which can exhibit other metabolic results due to its abundance in active components.

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Chapter 1

THE PROBLEM AND ITS BACKGROUND

Introduction

Urination is a normal body process wherein the organism excretes a fluid called urine. It is under voluntary control for normal individuals and for some elderly individuals with neurologic injury, it occurs as a reflex. A normal adult urinates about 4 to 6 times a day. The urinary system comprises mainly of the kidneys, ureters, urinary bladder, and the urethra. A major problem faced by individuals today is urolithiasis or formation of stony concretions called renal calculi in the urinary tract. This study focuses on preventing the formation of these stony concretions which can cause painful urination on the individual which can lead to kidney damage or lesions in the urinary tract.

The kidneys are two bean shaped organs found in a normal vertebrate. It is a vital organ of the urinary system that serves several regulatory roles in the body. It eliminates the end products of body metabolism, as well as excess body water and foreign substances such as drugs through urination, maintenance of electrostatic balance as well as hormonal secretion of erythropoietin for red blood cell production. It is the most common site of renal calculi formation.

Renal calculi's or urinary stones are the most common cause of acute urinary system obstruction. It is formed when supersaturated urine containing salt and minerals such as calcium oxalate, ammonium magnesium phosphate, uric acid and cysteine. Among these, the most common is calcium oxalate. It is formed by calcium, a normal urine constituent and oxalic acid, a waste product removed by the kidneys. About 60 to 80 percent of renal stones contain calcium. *Musa acuminata* (lakatan banana) leaves are commonly used for cooking, wrapping and food servings. It is also used for decoration and homebuilding. While most may be unaware of, these banana leaves contain very large amounts of polyphenols and EGCG (epigallocatechin gallate) which are known to dissolve and prevent kidney stones.

This research will provide experimental data on the effects of *Musa acuminata* in preventing calcium oxalate crystal formation. This may also serve as reference for further research about *Musa acuminata* and its potential in preventing kidney stone formation – as the extracts are mixed in drugs or made as food supplements.

Background of the Study

Renal calculi formation is one of the most common types of diseases our society faces today. In severe cases, it would require expensive treatments and surgeries to be removed. Nowadays, people are searching for ways to prevent renal calculi formation including proper diet and supplementation.

Banana leaves have been identified of having high polyphenols and EGCG (epigallocatechin gallate) which are known to dissolve and prevent kidney stones. These are partially transferred to food when wrapped in the leaves and then cooked. The full phytochemical capability of the *Musa acuminata* (lakatan banana) leaves have yet to be tested and fully utilized.

Banana leaves are well known in today's era as eco-friendly disposable plates, as a means of healthier cooking and wraps in treating burns. But what people are neglecting is its potential to treat diseases when the extracts are taken orally.

Rationale

Due to modernization and cultural changes over the years, a lot of people are getting nephrotic-related diseases due to bad lifestyle habits and sedentary mode of living. This is becoming an increasingly serious matter in the health sector. A lot of problem have arisen that lead to researches about the prevention and cure to kidney-related diseases. A lot of measures were made but at a high monetary costs like dialysis and kidney surgeries.

The researchers chose this study as a way to provide an alternative in preventing these certain diseases from happening at a cheap and effective way such that more people may be able to prevent such illnesses without having to pay too much on hospital bills and services.

The researchers want to evaluate the anti-urolithiatic activity of *Musa acuminata* (lakatan banana) leaves extract which can provide information on how lakatan banana leaves extract will prevent calcium oxalate urolithiasis and to provide a potential alternative for preventing calcium oxalate crystal formation in the urinary tract. This study wants to educate the readers about the importance of protecting the urinary tract against renal calculi formation.

The study was also chosen because it may help for further studies that can produce a more potent and viable cure to kidney related diseases.

Statement of the Problem

The purpose of the study is to observe and find out the anti-urolithiatic activity of *Musa acuminata* (lakatan banana) leaves and answer the following problems:

1. Is *Musa acuminata* leaf extract effective as an anti-urolithiatic agent in preventing calcium oxalate crystal formation in the rats?
2. What are the phytochemical contents of *Musa acuminata* leaf extract?
3. Is there a significant difference between the positive and experimental control among the following concentrations?
    a. 300mg/kg
    b. 400mg/kg
    c. 500mg/kg
4. Is there a significant difference in the urine examination of the rats between the negative control group and experimental control group among the following concentrations?
    a. 300mg/kg
    b. 400mg/kg
    c. 500mg/kg

Hypotheses

The following hypotheses were formulated based on the problems stated above:

1. *Musa acuminate* is an effective compound in preventing calcium oxalate formation.

2. *Musa acuminate* leaf extract contains phytochemical constituents such as polyphenols, specifically flavonoids.

3. There is no significant difference in the urine examination of the rats between the positive control group and experimental control group among the following concentrations (300mg/kg, 400mg/kg, and 500mg/kg).

4. There is a significant difference in the urine examination of the rats between the negative control group and experimental control group among the following concentrations (300mg/kg, 400mg/kg, and 500mg/kg).

Scope and Limitations

This study is limited in determining the anti-urolithiatic activity of *Musa acuminata* (lakatan banana) leaves against formation of calcium oxalate crystals only.

The species of the rat used in the experiment are *Rattus norvegicus*. Twenty-ninesix-week old male *Rattus norvegicus* weighing approximately 100-200 grams were acquired from the Food and Drug Administration in Muntinlupa City. These rats were subjected in a controlled environment and diet for the whole period of experimentation (thirteen days).The extract administration was done through oral gavage.

The experimentation took place at the Department of Science and Technology in Taguig. The experiment had one negative control group, three positive control group, and three experimental control group. Each group consisted of four male *Rattus norvegicus* except for the negative control which had five. The negative control group was induced with 0.75% ethylene glycol *ad libitum* without anti-urolithiatic treatment. The positive control group was induced with 0.75% ethylene glycol *ad libitum* and treated with cystone (300mg/kg, 400mg/kg, and 500mg/kg). The experimental control group will be induced with 0.75% ethylene glycol *ad libitum* and treated with 80% ethanolic extracts in the same doses as cystone (300mg/kg, 400mg/kg, and 500mg/kg).

Significance of the Study

This study aims to prove the anti-urolithiatic ability of ability of the *Musa acuminata* (lakatan banana) leaves extract in the urine of *Rattus norvegicus*. If the study yields a positive result by preventing the formation of calcium oxalate crystals, it will be beneficial to the following people:

Community. This research will give knowledge regarding the utilization of banana leaves as a preventive compound in renal calculi formation.

Government (DOH). This research will help promote an alternative method of preventing renal calculi formation.

Medical Industry. This research may be used as a basis for further studies in utilizing *Musa acuminata* (lakatan banana) leaves extract as a supplement for preventing renal calculi formation.

Students. This research may educate them about the anti-urolithiatic activity of *Musa acuminata* in preventing calcium oxalate crystal formation.

Future researchers. This research may serve as a basis for future researches regarding the use of lakatan banana leaves as an anti-urolithiatic agent and preventing calcium oxalate crystal formation.

Definition of Terms

Calcium Oxalate. This refers to the crystals that will be formed in the urinary tract induced by ethylene glycol.

Cystone. This refers to the medication used as positive control to prevent urolithiasis in *Rattus norvegicus*.

Ethanol. This refers to the solvent which was used to obtain the extract of *Musa acuminata* to be used for experimentation.

Ethylene Glycol. This refers to the reagent used to induce the formation of calcium oxalate crystals in the urinary tract of *Rattus norvegicus*.

Experimental control. This refers to the control group in which the *Musa acuminata* leaves extracts are used to prevent calcium oxalate crystal formation in the urinary tract of *Rattus norvegicus.*

Extract. This refers to the compound acquired from the *Musa acuminata* leaves to be used in the experimental control.

Musa acuminata. This refers to the plant used by the researchers to prevent the formation of crystals in the urinary tract.

Negative control. This refers to the group in which no anti-urolithiatic response is expected to occur.

Oral Gavage. This refers to the forceful administration of food and drugs through a tube leading down the throat to the stomach of the *Rattus norvegicus* specimens.

Phytochemical. This refers to the active compounds found in plants

Polyphenol. This refers to the one of the substances found in *Musa acuminata* which are tested for anti-urolithiatic activity.

Positive control. This refers to the group in which a known anti-urolithiatic response is expected to occur.

Rattus norvegicus. This refers to the subject used in the experimentation.

Renal Calculi. This refers to stones which are formed in the urinary tract.

Urolithiasis. This refers to the crystals formed in the urinary tract.

Research Flowchart

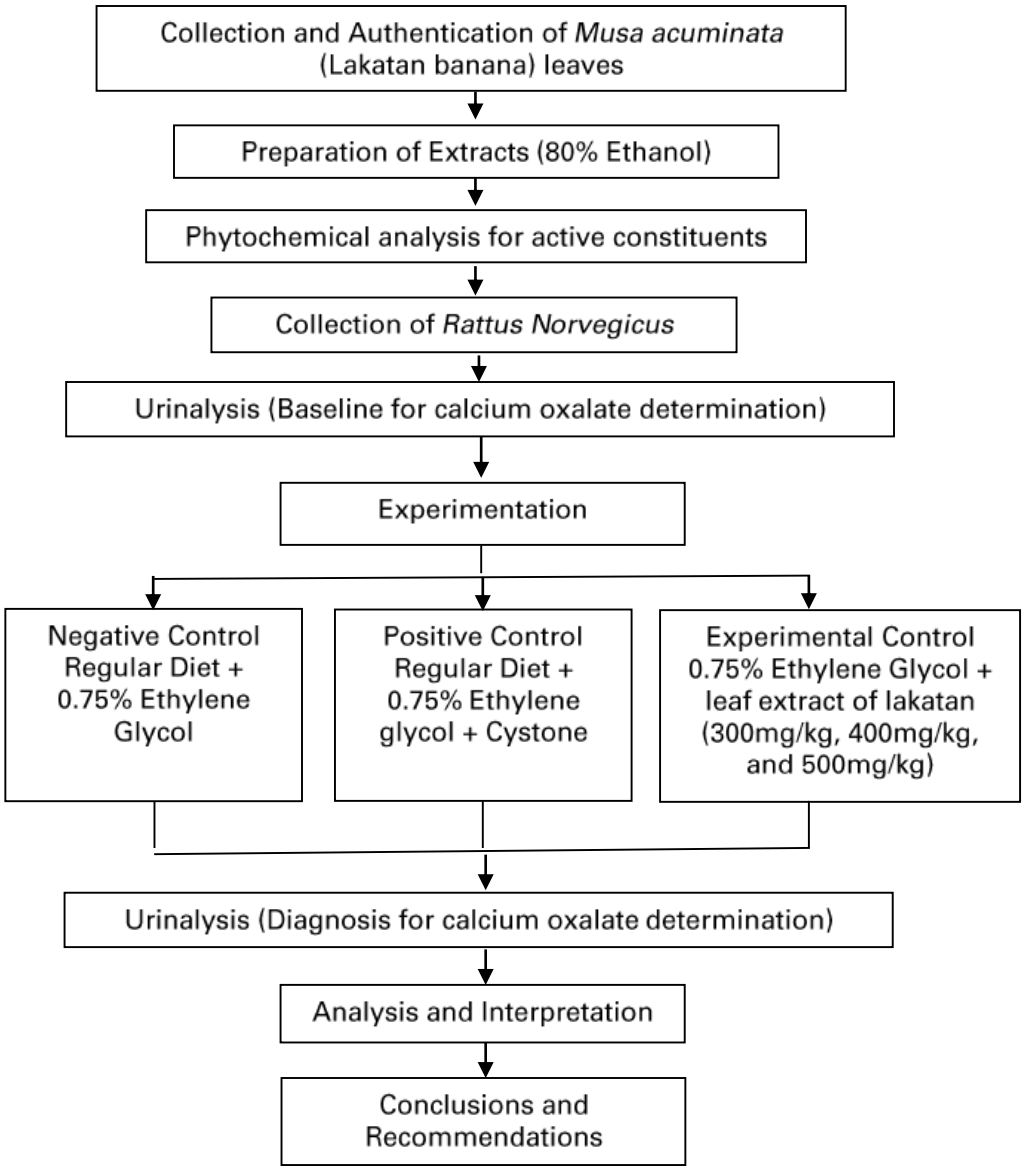

Figure 1.1. A flow chart showing the step-by step procedure in this experimentation

Figure 1.1 above describes the step-by-step procedures in this study. The research begun with the collection and authentication of *Musa acuminata* (Lakatan Banana) leaves. *Musa acuminata* leaves extract were prepared in 80% ethanolsolvents by crude extraction and was then concentrated by a rotary evaporator. Fresh leaf samples have undergone phytochemical analysis in Department of Science and Technology in Taguig.

Rattus norvegicus rats were bought from the Food and Drug Administration in Muntinlupa and was transferred to be housed in the Department of Science and Technology. Before any treatment has started, all experimental subjects have undergone urinalysis as baseline for absolute negative calcium oxalate crystal formation. To do this,the rats were placed in metabolic cages for collection of urine. The experiment started by separating the rats into groups of negative control, positive control and experimental control. Negative control will be induced in 0.75% ethylene glycol. Positive control will be induced in 0.75% ethylene glycol treated with Cystone (300mg/kg, 400mg/kg, and 500mg/kg). The experimental control group will be induced with 0.75% ethylene glycol treated with 80% ethanolic extracts (300mg/kg, 400mg/kg, and 500mg/kg).

The total experimentation period was thirteen days. The data was collected, analyzed, and interpret statistically using Mann-Whitney U test. Conclusion and recommendations were made after the analysis has been finished.

Chapter 2

REVIEW OF RELATED LITERATURE

This chapter presents literatures, studies which are significant in the current study.

RELATED LITERATURE

Banana

The term "banana" is derived from the Arab word meaning "finger". The earliest reference written about bananas is in Sanskrit in 500 BC. Banana plants are not trees but more of a very high herb which can grow with an average of about fifteen meters. The fruits are speculated by horticulturists to be the first fruit on earth. It originates in the Southeast Asian regions in the forests of Malaysia, Indonesia and the Philippines where various species of bananas still grow nowadays. By the end of fourteenth century, bananas started to be traded internationally and is considered nowadays as "The Most Popular Fruit in the World". There are about 1000 varieties of bananas which are subdivided into 50 groups. One-hundred fifty countries grow bananas and produce about 105 million tons of fruit annually. In these, 43 million tons are dessert bananas and 45 million tons are cooked. Bananas are a staple food in most tropical countries which play a major role in food security.

The leaves of banana can reduce serum glucose levels, improved insulin secretion and glycogen storage, and enzyme activity inhibition related to glucose absorption have been experimentally prove to corroborate the beneficial effects on the regulation of glucose

homeostasis. The roots are antibilious and antihelmintic in folk medicines; young leaves are used to cover burns on the skin as cool compress; the astringent ashes of the unripe peel and of the leaves have antidiarrheal and antidysenteric properties. Green bananas are used as a curative for intestinal disorders and for antidiarrheal. Banana stem helps detoxify the body and employed as diuretic. It is commonly cooked and eaten. It is commonly cooked and eaten to prevent or treat kidney stonespossibly by regulating calcium uptake by the presence of potassium. Stem juice of fruited plant is used for treating diarrhea, dysentery, cholera, otalgia, and hemoptysis.The plant is also used in inflammation, pain, and snakebite (Caballero et al., 2016). Banana flower has vitamins, flavanoids and proteins that used as a medicine for bronchitis and eases menstrual cramps. Banana stem helps for those who want to lose weight due to fibers found on it. The inner tender part of the stem can be cooked and can eat to keep their high-blood pressure controlled. It is also a diuretic food that helps detoxify the body. Banana leaves adds flavor to the food by wrapping on it before steaming or grilling. This action will impart some of the polyphenols found on the leaves to the food (Jones et al., 2012).

In some areas, mainly in the provinces, the banana leaves are utilized for medicinal purposes and are able to heal open-skin wounds faster (Capuno, 2012). An article entitled "What Are the Benefits of Banana Leaves?" supports that aside from banana plants being grown for fruits or ornamental reasons, banana leaves which are commonly used for cooking or burn treatments also offer nutritional and medicinal benefits (2011). Banana is commonly named for the herbaceous plants of the genus *Musa*. Dating back as far as early civilizations it has been cultivated for all its parts have medical applications: the flowers in bronchitis and

dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap in cases of hysteria, epilepsy,leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stingsand bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers; the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas. The antibiotic acts against Mycobacteria. A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp. The first two elevate blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines (Kumar et al, 2012).

Bioactive compounds such as amines, dopamine, noradrenaline, octopamine, histamine, 2-phenylethylamine, and tyramine have been reported in banana. Flavonoids also present in the pulp of bananas. Unripe banana has Leucocyanidin that can serve as protective effect against aspirin-induced erosions. Gallocatechin, epicatechin, and condensed tannins present in banana pulp albeit in trace quantities may serve as antioxidants. Flavonoids extracted from unripe fruits have been attributed with significant hypolipidemic activities (Caballero et al., 2016).

Musa acuminata is a specie in the *Musa* family which is *commonly* called "dwarf banana". It grows best in warm, humid tropical or subtropical climates. The soil should be well drained and moist but can grow on a variety of soils. It is the wild ancestor of cultivated banana. Most of dessert bananas which are eaten are derived from *Musa acuminata* and is usually eaten raw. The wild species contain larger seeds than cultivated bananas which are almost seedless. The leaves are arranged spirally and reach about 2.7 meters long and 60 centimeters wide ("*Musa acuminate* (Banana)").

Polyphenols are innately found in the banana. These polyphenols help in slowing the degradation rate in the banana. One of these polyphenols are flavonoids, flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. It has one of the largest nutrient families that are known to scientists. Raw bananas contain flavonoids such as catechin, epicatechin, epigallocatechin, and prodelphinidin dimer b3. Catechins are phytochemical compounds that are found in plants and plant-based food and beverages.Consumptions of these catechins has been associated with a variety of beneficial effects such as increased plasma oxidant activity which is the ability of the plasma to scavenge the free radicals, brachial artery dilation, and resistance of LDL to oxidation. Epicatechin is phytochemical that reduces lipid peroxidation and inhibits platelet aggregation. It is also a strong antioxidant, has insulin mimic reaction that promotes heart health due to its protective role in the osmotic fragility of cells. Epigallocathechin is a flavonoidthat is a chemoprotective agent against cancer that is commonly found in the green tea. It also inhibits tel transferase (Qu et al., 2013).

Calcium Oxalate

Majority of renal calculi contains calcium, usually in calcium oxalate (CaC2O4) and calcium phosphate (CaPO4). No specific causes have been identified, but most cases of patients have idiopathic hypercalcuria without hypercalcemia (Hacking).

The International Kidney Stone Institute stated that nearly eighty percent of renal calculi compose of calcium and oxalate. About half of patients with calcium stones experience abnormally high levels of calcium excreted in the urine also known as hypercalcuria. This condition may occur because of increased absorption of calcium in the intestines, calcium secretion from the bones, or nephrological disorders involving the control of normal calcium levels in the urine. Excess amounts of urinary calcium may be affected by several factors including diet, metabolism, and heredity. An example would be the formation of calcium stones in individuals suffering from parathyroid gland over activity which regulate the calcium (parathyroid hormone release). Excess oxalates released in the urine may also be a cause of calcium oxalate stones formation. Majority of cases are seen in individuals with a history of inflammatory diseases or surgeries in the gastrointestinal tract.

Cystone

Cystone tablets a proven ayuverdic method of treatment for kidney stones. It is a traditionally used for relief of various urological problems including nephrolithiasis. The known effects of this is the prevention of the supersaturation of lithogenic substances, oxamide control (substances which precipitate stone formation) from the intestine and correction of crystalloid-colloid imbalance. Cystone is able to inhibit calculogenesis by by reduction of stone forming substances like oxalic acid, calcium hydroxyproline, etc. It causes the expulsion of these substances by micropulverization. It causes the disintegration of calculi by acting in the mucin that binds the particles together. Cystone also exhibits anti-microbial, antispasmodic and anti-inflammatory properties which prove to be beneficial in the ureteric colic, pain and burning sensation, as well as prevention of urinary tract infections associated with urinary stones and crystalluria (Erickson et al., 2011).

Ethylene Glycol

Ethylene glycol (1, 2 ethane diol) is more known to be used as radiator antifreeze in cars. Its half-life has an average of about 3 hours and is metabolized into three major toxic compounds: glycolaldehyde, glycolic acid, and glyoxylic acid. Glycolaldehyde is formed by the oxidation of ethylene glycol by liver alcohol dehydrogenase catalization. Oxalic acid and formic acid are formed in minute amounts. Oxalic acid is a highly toxic compound on its own and can immediately precipitate as calcium oxalate crystals which are found in various tissues a well as in the urine. The inconsistency of finding these crystals in the urine is an important indicator

of diagnosing ethylene glycol poisoning. The highest concentrations of metabolites which accumulate in the blood is the glycolic acid, and the concentrations in the blood and urine are directly related to symptoms and mortality. These concentrations are the major contributor to high anion gap which are significant in metabolic acidosis. Fatal doses of ethylene glycol are around 100 grams. The principal symptoms of acute poisoning are anuria and necrosis. Other symptoms may include nausea and vomiting, myoclonus, seizures, convulsions, slow reflexes, and coma. Primary diagnosis of ethylene glycol intoxication is done by HPLC measurement of serum ethylene glycol and glycolic acid (Henry, 2011).

Ethylene glycol is rarely known to be found on cosmetics, paint products and detergents. Suicide attempts related to swallowing such products had seen ethylene glycol through blood, urine and other test. Ethylene glycol itself is not toxic but rather their product after it metabolize. The effects on renal system happen at one to three days after ingestion. This includes the pain on the side or back of the abdomen and the formation of calcium oxalate crystals. There are two forms of calcium oxalate during ethylene glycol intoxication. Monohydrate crystal is the most common found in urine but the most specific is the dehydrate crystal. Monohydrate crystal form can also be seen for those who intake a high quantity if Vitamin C diet while dehydrate crystal form needs a high concentration of oxalate present, resulting to a more indication of ethylene glycol poisoning. Primarily, hemodialysis is the most common treatment in ethylene glycol intoxication. Hemodialysis removes the metabolite glycolate and ethylene glycol itself in the blood. Patients who are intoxicated need a

hemodialysis if his serum ethylene glycol reaches more than 50mg/dL with an indication of severe metabolic acidosis and renal failure (Buller et al., 2012).

Polyphenols

Polyphenols are a natural compound class present in all vascular plants and are identified by benzene rings containing one or more hydroxyl functionalities. Some sources of polyphenols include tea, grapes, wine, beer, olive oil, coffee, peanuts, chocolate, as well as some fruits and vegetables. Employment of polyphenols in pharmaceutical and biomedical fields are being suggested in preventing the development of pathologic conditions. Treating the various pathologic effects of food additives by food processing companies, cosmetic formulations in anti-aging products in nutraceutical form. Despite the benefits of polyphenols, it is very sensitive to environmental factors namely, heat and light, as well as exhibit low water solubility in its free form, a high metabolism rate and rapid secretion. Also, the component may undergo degradation in water or oxidation, together with a loss in activity, and most of it contain high molecular weights and cannot be absorbed easily. All the factors mentioned contribute to a deficiency in long-term stability and to poor vascular and oral bioavailability which greatly reduce the effectiveness of the component that depends on preserving stability, bioactivity, and bioavailability. A lot of polyphenolic molecules are identified by an unpleasant or bitter taste that also limits its potential applications, new strategic approaches in developments to overcome the draw-backs included with the stability and bioavailability of polyphenols is truly a great obstacle and requires the attention of many researchers. The administration of the components require the ideas of suitable formulas to be able to preserve

the structural integrity of the phenolic molecules, increase its water solubility, bioavailability, and bioactivity (Watson et al., 2014).

Polyphenols are able to act as reactive oxygen species scavengers, iron chelators, and enzyme modulator. Reactive oxygen species (ROS) play a key role in several pathophysiological processes in a wide variety of renal diseases. These anti-oxidants are expected to be able to decrease vulnerability of the kidneys to oxidative stress. The renoprotectiveactivity of polyphenols is assumed to be mainly because of its wide range of biological actions such as, free radical-scavenging, chelation of metal, and enzyme modulation activities (Rodrigo et al., 2006). Cell disorders and development of diseases such as atherosclerosis, cataracts chronicinflammation and neurodegenerative disease are that ROS can do. In the presence of ROS, the food we take will oxidize. Antioxidant such as polyphenols delays or inhibits the oxidation (Zhan et al., 2011).

The highest yields for extraction of polyphenols are usually achieved with methanol or ethanol and its mixtures with water. Although other solvents such as ethyl acetate or acetone have been used in the extraction of polyphenols in plants. Water or aqueous solutions are the most widely used for extraction due to its low toxicity and high extraction yield as well as the advantage of modulating the polarity of solvents by different concentrations by different ratios. Its main drawback is the low yield for antioxidants with low polarity or liposoluble antioxidants (Sineiro et al., 2008).

Rattus norvigecus

Otherwise known as the brown rat, the *Rattus norvigecus* is a common household lab rat. These rats were used for experimentation ever since the 1800's. Strains were developed to study neuroanatomy, nutrition, endocrinology, genetics, and behavior. Genetic manipulation and testing made these animals susceptible and immune to diseases which can be altered with induced diseases and cures. Diseases of rats are usually handled as a herd (colony) rather than on an individual animal basis. In most cases the goal is to prevent introduction of a disease into a colony than to treat individual animals. Disease prevent is practiced by institution of a health monitoring (sentinel) program based on serological and microscopic diagnosis of problems in a representative sample of animals (John Hopkins University).

Urolithiasis

Urolithiasis refers to any calculi which can be identified anywhere along the pathway of the urinary tract. There may be several terms used which can be interchangeable but have slightly varying meanings. The interchangeable terms include urolithiasis, nephrolithiasis, and renal or kidney stones (Hacking). It is currently identified that cases of recurring renal calculi in susceptible people is a result of many factors but mostly affected by a reduced urine flow rate (influenced by decreased fluid intake), and urine saturation including large amounts of naturally insoluble substances. Chemical analysis of these stones are very significant in determining the causes of the condition. Specialized x-ray diffraction and infrared spectroscopy techniques are the methods mainly utilized for the purpose (Bishop, 2010). The calculi may vary in sizes, commonly identified as sand, gravel, or stone. Physical characteristics

of the various calculi are rarely of help to its identification, though a few characteristics are worth noting. Uric acid and urate stones are characterized by yellow to brownish red color and of moderate hardness. Phosphate stones are pale and friable. Calcium oxalate stones are dark in color and are very hard with a rough surface. Cystine stones are yellow brown in color and may feel greasy to touch (Henry, 2011).

A major effect of this condition is pain. Urinalysis may give information on hematuria, urine pH, and urine crystals that can contribute to stone formation (Mundt et al., 2011). The pain may suddenly manifest as a sharp, stabbing below the ribcage in the back. The pain is often termed as "colic" and usually happens when a stone blocks the urine flow out of the kidneys. The ureter is a long narrow tube which transport the urine from the kidney to the bladder. As a stone moves from the kidneys to the ureter, the chances of experiencing symptoms are higher. As the stones start to move towards the bladder, the pain may also move towards the lower abdomen or groin. A patient have chances to experience nausea and vomiting if there is pain and the urge to frequently urinate or have a burning sensation when urinating. The severity of symptoms is not directly related to the size of the stone. Small stones are more common in producing significant pain. Though some stones may be asymptomatic, particularly the stones which are located in the kidneys. These kidney stones may be present if there is a trace of blood in the urine which may not be seen by the naked eye. Even if the stones may not cause discomfort, it is important to be diagnosed as it may grow too large to be excreted out of the urinary tract (International Kidney Stone Institute).

RELATED STUDIES

Renal disorders are now being one of the major concerns of health institutions around the world nowadays. Many hospitals and institutions are working together to search for new approaches to treat and prevent these diseases. Urolithiasis is one of these disorders and has affected many individuals worldwide. Treatments for urolithiasis were made to help combat and treat these disorders however these treatments produced many side effects to the human body which has done so little to help the individual to improve his overall health. Being a slowly increasing problem in our society, medicines were also made to combat these disorders but most of the medicine that were made came at a high cost and therefore researchers and scientists come up with alternatives such as herbal medicine and natural treatment. Banana was also used as one of natural antioxidants that were studied by the previous researchers which are believed to be effective for prevention of calcium oxalate formation and has been used since ancient times as a way to treat urinary tract infections and kidney stones (Kalpana et al, 2013).

A research was conducted by St. John College of Pharmacy in India on the anti-urolithiatic activity of *Solanium virginianum* on Ethylene Glycol induced urolithiasis in *Sprague dawley* rats. The goal was to study the anti-urolithiatic activity of the plant extract of *Solanium virginianum* on Ethylene Glycol induced urolithiasis in *Sprague dawley* rats. The whole plants of *Solanum virginianum* were cleaned and chopped into small pieces and dried under shade. The coarse powder was obtained by mechanical grinding. The powdered material (100 g) was subjected to continue hot extraction in soxhlet apparatus at a temperature of (60- 700 ^{0}C) by

using ethanol (95% v/v) as solvent. After complete extraction, the extract was dried. The yield was about 5% w/w and it was stored at 4°C in desiccator. The extract was suspended in distilled water using 1% acasia as suspending agent for oral administration to animals..The whole experimentation period was 28 days, urine was collected and analyzed for calcium and phosphate. The urine was also subjected to microscopic examination in identification of presence or absence of crystal formation.The urolithiasis was induced by orally feeding water infused with Ethylene glycol (0.75%v/v) for 28 days. The ethanolic extract of *Solanium virginianum* (200 mg/kg, 400 mg/kg) was orally administered on the 1st day for preventive measures and from the 15th day for curative measures. It was observed that the inducing agent, Ethylene Glycol, elevated the calcium and phosphate levels in urine, whilst also increasing blood urea nitrogen (BUN), serum creatinine and serum uric acid levels. The treatment with the methanolic extract of *Solanium virginianum* significantly reduced calcium and phosphate levels in urine, serum creatinine, blood urea nitrogen (BUN) and uric acid levels of urolithiasis were also reduced by the preventive and curative measures of treatment. Based on the information given above, the researchers were able to conclude that *Solanium virginianum* has anti-urolithiatic activity against the urolithiatic effects of Ethylene Glycol.*Solanum Virginianum* is endowed with various chemical components such as alkaloids, flavonoids, phytosterols, mucilage and fixed oil etc., which possibly contribute to its vast uses in folklore medicine.

A study recorded in the International journal of Pharmacy and Pharmaceutical Sciences focused on the inhibition of calcium oxalate crystallization in vitro by extract of banana cultivar monthan. Banana cultivar monthan corm extracts are used with different solvents of varying polarity. Each were tested for antilithiatic potential. Kidney stone formation was simulated in vitro by three different assays such as crystal nucleation, aggregation, and growth. The effects of the extracts are observed spectrophotometrically. The results showed that the ethanol extract of banana cultivar monthan prevented crystal nucleation, growth and aggregation (Kalpana et al., 2013).

According to a study conducted in the Department of Science and Technology, "Banana stalk may be the next alternative herbal remedy against kidney stones". In this study, the anti-urolithiatic effects of *Musa paradisiaca*pseudo-stem capsules against ethylene glycol induced *Rattus norvegicus*. Laboratory examinations showed that mice fed with banana capsules did not show signs of developing kidney stones. Blood and urine examination conducted on the mice revealed healthy functioning kidneys (2015).

Banana stem juice is a well-known remedy for urinary disorders. It improves the functional efficiency of kidney and liver such as alleviating the discomforts and diseased condition in them. It protects the excretory organs in the abdominal region from toxins and helps to eliminate them by excretion in the urine. It has been found to be of great help in the treatment for the removal of calculi in the kidney, gall bladder, and prostate. It is recommended to mix this juice whenever possible with the juice of ash pumpkin (Aravindh Herbal Lab, 2013).

An article by the Journal of Agriculture and Food Chemistry focuses on the study of phenolics and and antioxidant properties of fruit pulp and cell wall fractions of postharvest *Musa acuminata* cultivars. This study aims to support studies regarding the phenolics associated in the cell walls of the plant. In the study, catechin, gallocatechin, epicatechin, as well as condensed tannins were detected in the soluble fruit pulp extract. In the soluble cell wall fraction, two hydroxycinnamic acid derivatives were predominant. On the other hand, in the insoluble cell wall fraction, the anthocyanidindelphinidin, which was reported in banana cell walls for the first time, was predominant. Cell wall fractions exhibit remarkable antioxidant capacity, specifically after acid and enzymatic hydrolysis, which was related with the total phenolic content released after the hydrolysis of the water-insoluble polymer, but is not seen in the posthydrolysis water-soluble polymer. The acid hydrolysis released various mono-saccharides, enzymatic hydrolysis released one peak of oligosaccharides. These resultsindicate that banana cell walls are a potential source of natural antioxidants and that they could be bioaccessible in the human gut.

Synthesis of the Study

Bananas are very large herbs which yield "finger-like" fruits. It is assumed by horticulturists as the first fruit on earth. It originates from Southeast Asian countries such as Malaysia, Indonesia, and the Philippines and many species still grow in these countries nowadays. Bananas are known to be the most popular fruit in the world.

The peel and pulp of the banana fruit are proven to exhibit antifungal and antibiotic properties. In India and most Asian regions, the banana leaves are commonly used for wrapping food before cooking. Aside from industrial uses of banana leaves, bananas are used for medicine. It is used for treatment of bronchitis, dysentery, hysteria, epilepsy, leprosy, fevers, hemorrhages, diarrhea, hemorrhoids, bites, burns, and many more. The stem is proven to help in detoxification of the body and is an effective diuretic. It is commonly cooked and eaten in prevention or treatment of kidney stones as it regulates calcium uptake by the presence of potassium.

Musa acuminata (Lakatan banana or dwarf banana) is a specie of banana that grows best in warm, humid tropical or subtropical climates like the Philippines. It is a well-known dessert banana. Its leaves reach about 2.7 meters long and 60 cm wide. These banana leaves contain very high amounts of polyphenols.

Polyphenols are natural compounds present in all vascular plants. One of these plants is the banana plant. One polyphenols which are found in the banana plant are flavonoids. Polyphenols are employed in pharmaceutical and biomedical field for the prevention of pathologic conditions. Polyphenols are best extracted with solvents such as ethanol or

methanol. Water or aqueous solutions are the most widely used solvent for extraction but has a very low yield for antioxidants. A lot of compounds containing polyphenols are identified of having unpleasant and bitter taste. Polyphenols are able to react a reactive oxygen specie scavengers, iron chelators, and enzyme modulators thus being able to exhibit renoprotective activity. Reactive oxygen species play a key role in pathophysiological processes in a wide variety of renal diseases. Polyphenols are antioxidants which are expected to be able to decrease kidney vulnerability to oxidative stress. High yields of polyphenols can be obtained from methanol or ethanol extracts with different water mixtures. Although some studies use ethyl acetate or acetone are used in polyphenol extraction in plants. Aqueous solutions are widely used for its low toxicity and high extraction yields but low yields for anti-oxidants of low polarity or liposoluble antioxidants.

Urolithiasis may also be called in other terms such as, nephrolithiasis, renal or kidney stones. It is the presence of stones in the urinary tract. A major manifestation of presence of stone is colic pain. This happens when the stone blocks the urinary flow out of the kidneys. Renal disorders are now one of the major health concerns worldwide. Treatments for urolithiasis became too expensive that researchers are now conducting studies for an alternative means of prevention or treatment for these diseases.

Ethylene glycol is commonly known as anti-freeze in car radiators. Fatal doses of ethylene glycol is around 100 grams. Ethylene glycol is not poisonous in itself, but rather the products it produces after it is metabolized in the body. Inside the body, it metabolizes into three major toxic compounds; Glycolaldehyde, glycolic acid, and glyoxylic acid. Glycolaldehyde

is formed by oxidation by liver alcohol dehydrogenase. The others are found in minute amounts. It can rarely be found in cosmetics, paints, and detergents. The effects start to show as pain in the abdominal area and the beginning of calcium oxalate crystals and will later progress to calculi formation if left untreated.

Calcium oxalate is the most common type of renal stones which are diagnosed. Eighty percent of renal stones are composed of calcium and oxalate. Various conditions which can lead to calcium oxalate crystal formation may include over reactivity of the parathyroid hormone which regulates the amount of calcium to be secreted or reabsorbed. This manifests as hypercalcuria and may be affected by increased calcium absorption in the intestines, excessive calcium secretion from the bones, nephrological disorders or a clinical significance of ethylene glycol poisoning. There are two forms of calcium oxalate which can be encountered in ethylene glycol poisoning. Monohydrate and dehydrate crystals. The most common treatment of this is hemodialysis to clear out the ethylene glycol in the serum but it would cost a great amount of money.

Banana parts are being studied as herbal remedy against induced kidney stones of calcium oxalate and its crystals specifically the stem. The traditional medicine in some Asian countries is to cook the banana stem and eat it as treatment and prevention against kidney stone formation.

Cystone tablets are proven ayuverdic method of treatment against kidney stones. It is traditionally used for relief of urological diseases. It's known mechanism of action is the prevention of supersaturation of lithogenic substances, as well as controlling the metabolism

of crystal formation. This will serve as the basis in proving the anti-urolithiatic activity of the experimental compounds.

Similar researches have been done in preventing calcium oxalate induced kidney injuries by ethylene glycol poisoning. One of these researches used *Solanium virginianum* in *Sprague dawley* rats. The whole plants of this are cleansed and chopped and dried before mechanical grinding. The fine powder is then subjected to warm soxhlet extraction. In the analysis of the biochemical components of *Solanium virginianum*, it was found that it contains alkaloids, flavonoids, etc. which show some similarities to the contents present in bananas.

Another study using banana cultivar monthan corm and its antilithiatic activity simulated in vivo. The research proved that ethanolic extracts are proven to be effective in expressing antilithiatic properties. The assays simulate crystal nucleation, aggregation, and growth. The results have been obtained by using spectrophotometric tests.

A local research which has been conducted on the efficacy *Musa paradisiaca* in *Rattus norvegicus* against calcium oxalate crystals induced by ethylene glycol poisoning. The means of prevention involves the oral feeding of specimens with *Musa paradisiaca* pseudo-stem capsules. The results of the study are favorable as the specimens which are induced with the pseudo-stem capsules did not show signs of crystal formation.

Rattus norvegicus is the most common household lab rat. Ever since the 1800's, these rats have been used for experimentation in various studies such as neuroanatomy, nutrition, endocrinology, genetics and behavior. In most studies using rats, the aim is to prevent the

whole herd of rats from developing the disease under study by the use of experimental substances which can possibly yield positive effects against the disease under study.

By comparing past reasearches based on plants that consist of compounds and characteristics similar to that of *Musa acuminata*leaves, the researchers are able to obtain a solid theoretical background which supports the possible anti-urolithiatic activity of *Musa acuminata* leaves against calcium oxalate crystal formation in ethylene glycol induced Rattus norvegicus.

Chapter 3

RESEARCH METHODOLOGY

This chapter will deal with the methods and procedures in determining the anti-urolithiatic activity of *Musa acuminata* (Lakatan Banana) leaves extract in preventing calcium oxalate crystal induced kidney injury in *Rattus norvegicus*.

Research Design

The researchers used a controlled experimental type of research design. It is a scientific investigation wherein both the control and experimental groups are kept under similar variables apart from the factor under study which would help in identifying the effects or influence of that factor. The experiment will be used to identify and compare the anti-urolithiatic activity of *Musa acuminata* leaf extract to cystone, a proven preventive compound on *Rattus norvegicus* specimens. The experimentation period will last for 13 days. Before beginning introduction of any chemical or compounds, the animal specimens have undergone routine urinalysis, and microscopic examination for formed elements (Sanders, 2008).

Data Gathering Procedures

1. **Collection of Materials**

   A. **The Test Organism.** The test organisms used in the experiment are Twenty-nine six-week old male *Rattus norvegicus* weighing approximately 100-200 grams which were purchased from the Food and Drug Administration (FDA), Muntinlupa City. These rats were subjected to 13 days of experimentation.

   B. **The Plant Sample.** The plant material used in this experiment were five kilograms of *Musa acuminata* (Lakatan Banana) leaves which were collected in a small plantation in Bacoor, Cavite and was authenticated in the National Museum. 200 grams of fresh plant extract were subjected to phytochemical testing for identification of active components. The leaves were oven dried and have undergone crude extraction method using 80% by ethanol which will be used in the prevention of calcium oxalate crystal formation in ethylene glycol induced *Rattus norvegicus* specimens. The extracts were used in the span of 13 days of experimentation period.

2. **Preparation and Extraction of Plant Material**

After authentication, the leaves were washed with distilled water, oven dried and subjected to crude extraction of 80% ethanolic solvent. The extraction time lasted for a total of ten days.

3. Preparation of Concentrations

The researchers used 80% ethanolic extract. The doses given were based on the weight of the rats. Three doses were used; 300mg/kg, 400mg/kg, and 500mg/kg. The extracts were given to test specimens by oral gavage during the experimentation of 13 days.

Research Instrument

The following research instruments and methods were utilized by the researchers during the period of experimentation:

Determination of the presence of calcium oxalate. Urinalysis was utilized for the detection. Formed calcium oxalate crystals will be examined by microscopic examination of the urine samples from the *Rattus norvegicus* specimens.

Crude Extraction Method. This method of extraction was utilized by the researchers for the reason that it can retrieve active compounds in efficiently and are less likely to deteriorate the active components by heat. The solvent used for extraction of the active compounds is 80% ethanol. The extraction period lasted for 10 days. The extract was then concentrated by a rotary evaporator.

Oral Gavage Induction Method. This method was utilized by the researchers to induce the specimens with the definite amounts of solutions to assure proper administration of controlled factors in the experimentation. This method used a stomach tube which will be inserted in the mouth of the specimens to reach the stomach. The stomach tube will be connected to a syringe containing the compounds to be induced.

Phytochemical Analysis of the Plant Sample. The samples of the obtained extracts have undergone phytochemical testing in the Department of Science and Technology (DOST) in Taguig. The phytochemical test will focus on the identification of polyphenols, an anti-oxidant compound which is claimed to show anti-urolithiatic activity.

Urinalysis. This test was used to determine the values which may increase or decrease from normal values in the urine of the specimens before the experimentation period, on the 6th day of the experimentation period and after the experimentation period after the 13th day. The urine was subjected to microscopic examination for observation of formed crystals.

Data Collection

The rats were placed in metabolic cages to collect the urine that they will excrete on designated days of urinalysis throughout the whole experimentation period of 13 days. The researchers examined the urine constituents of the test organisms on the designated days of urinalysis (baseline crystals, 6 days post induction, and 7 days post treatment). This test was utilized for the identification and grading of calcium oxalate crystals which are the key variable in this research.

Statistical Treatment of Data

The values tabulated are collected from the urinalysis. The significant differences among control groups are determined with the Mann-Whitney U test using Asymptomatic Significance (2-Tailed) to acquire the P value. Results are interpreted as significant if the values are $P < 0.050$.

Chapter 4

PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA

This chapter includes the complete presentation, analysis and interpretation of the data gathered from the experimentation proper.

This study utilized a total of twenty-nine (29) male *Rattus norvegicus* rats that were divided into seven (7) groups. Urine collection by metabolic cages were done three times and subjected to crystal examination for calcium oxalate. All the gathered data were tabulated and computed for significance (P-value).

1. Is *Musa acuminata* leaf extract effective as an anti-urolithiatic agent in preventing calcium oxalate crystal formation in the rats?

Based on our results, the study shows that there is a potential activity in preventing the formation of calcium oxalate crystals but may not be significant enough, given the doses used in this study.

Table 4.1 Tabulated data of examination for calcium oxalate crystals

Experimentation Data for the Examination of Anti-Urolithiatic Activity					
Group/ Sample	**Dose in mg/kg**	**Animal No.**	**Urine Crystals Microscopic Examination**		
			Baseline Crystals	**6d Post Urolith Induction**	**7d Post Treatment**
Group 1 Negative Control: *Distilled water*	0[a]	1	Negative	Negative	Negative
		2	Negative	Calcium Oxalate (+3)	Calcium Oxalate (+3)
		3	Negative	Negative	Negative
		4	Negative	Negative	Negative
		5	Negative	Calcium Oxalate (+2)	Negative
Group 2 Positive control: *Cystone*	300	1	Negative	Negative	Negative
		2	Traces	Negative	Negative
		3	Negative	Negative	Calcium Oxalate (+2)
		4	Negative	Calcium Oxalate (+2)	Calcium Oxalate (+3)
Group 3 Positive control: *Cystone*	400	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Negative	Negative	Negative
Group 4 Positive control: *Cystone*	500	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Calcium Oxalate (+4)	Calcium Oxalate (+2)
		4	Negative	Negative	Negative
Group 5 Treatment: Test Sample *Lakatan Banana Leaves*	300	1	Traces	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Traces	Negative	Negative
Group 6 Treatment: Test Sample *Lakatan Banana Leaves*	400	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Traces	Negative	Negative

Group 7 Treatment: Test Sample *Lakatan Banana Leaves*	500	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Calcium oxalate (+4)	Negative
		4	Negative	Negative	Negative

Legend: [a] -control, the same as in the highest dosed – Days

The table above shows the tabulated format of the calcium oxalate crystal examination in this study. The table shows that the rats which have been treated with the highest doses from Cystone and *Musa acuminata* have shown potential anti-urolithiatic activity. Most of the rats which have shown negative crystals post induction are one of the unexpected variables in this research and therefore hindered in showing further evidences of potential anti-urolithiatic activity against calcium oxalate crystal formation. The negative control group rats which have been successfully induced showed no signs of improving throughout the whole thirteen days of experimentation which is the expected outcome. Though one rat from the group showed that the calcium oxalate crystals disappeared, this is one of the unexpected variables which is why there are several rats per group in this experiment.

The positive control group had three doses; 300mg/kg, 400mg/kg, and 500mg/kg. The lowest dose, 300mg/kg was not able to exhibit anti-urolithiatic activities. Unfortunately, the 400mg/kg group was not able to have any positive crystal induction and is therefore considered no longer significant to show the anti-urolithiatic activity of Cystone given in this dose. The highest dose of Cystone given; 500mg/kg had one rat which was successfully induced with calcium oxalate crystal and showed positive anti-urolithiatic activity. Therefore,

making the highest dose significant for comparison of anti-urolithiatic activity of the experimental plant material. The experimental control group was also given the three same doses as Cystone; 300mg/kg, 400mg/kg, and 500mg/kg. This was done to compare the anti-urolithiatic capabilities of the positive control group to the experimental control group and interpret if there may be doses of the plant extract which may show better anti-urolithiatic capabilities than Cystone. The experimental control groups with the doses 300mg/kg, and 400mg/kg were not successfully induced with calcium oxalate crystals on the induction period and still showed no signs of crystal formation after the treatment period. The highest dose of the plant material had one rat which was successfully induced with calcium oxalate crystals and successfully prevented and removed the calcium oxalate crystals which was present after the induction period. The researchers consider the highest dose (500mg/kg) of the positive control and experimental control significant in showing the anti-urolithiatic activities against calcium oxalate crystal formation but was not significant enough due to unexpected events.

2. What are the phytochemical contents of *Musa acuminata* leaf extract?

Phytochemical findings are obtained from 200 grams of fresh *Musa acuminata* leaves include the following:

Table 4.2 The Phytochemical Findings in 200 grams fresh *Musa acuminata* leaves

Constituent	Presence
Sterols	(-)
Triterpenes	(+++)
Flavonoids	(+++)
Alkaloids	(+)
Saponins	(++)
Glycosides	(+)
Tannins	(+++)

Note: (+) Traces, (++) moderate, (+++) abundant, (-) Absence of constituents

Phytochemical findings on Musa acuminata leaves include abundance in triterpenes, flavonoids, and tannins. There are moderate amounts of saponins and traces of alkaloids and glycosides. The abundance of active phytochemical components shows that the *Musa acuminata* leaves are well and capable of exhibiting biological metabolic processes as well as normalizing body functions.

3. Is there a significant difference between the positive and experimental control among the following concentrations?

Table 4.3 A table showing the significant difference of the Positive Control Compared to the Experimental Control group.

Dose	Positive Control	Experimental Control	P-Value	Interpretation	Decision
300mg/kg	Cystone	*Musa acuminata* leaves extract	0.131	Not Significant	Accept Hypothesis
400mg/kg			0.317	Not Significant	
500mg/kg			0.127	Not Significant	

After the experimentation of thirteen days, the table shows that there is no significant difference between the positive control; Cystone and experimental control group; 80% ethanolic extract of *Musa acuminata* leaves among the following concentrations; 300mg/kg, 400mg/kg, and 500mg/kg. The researchers decided to accept the hypothesis for all three doses used in this study. Having a P-value of <0.050 shows that there is a significant difference between the values compared. The data shows that the doses 300mg/kg, 400mg/kg, and 500mg/kg of the positive control group vs the experimental control group produced the following P-values; 0.131, 0.317, and 0.127 respectively. This supports that the experimental control group and the positive control group have expressed the same anti-urolithiatic activity against the calcium oxalate crystal protocol which was used in this study.

4. Is there a significant difference in the urine examination of the rats between the negative control group and experimental control group among the following concentrations?

Table 4.4 A table showing the significant difference of the Negative Control Compared to the Experimental Control group after the thirteenth day of experimentation.

Dose	Negative Control	Experimental Control	P-Value	Interpretation	Decision
300mg/kg	Distilled Water	Musa acuminata leaves extract	0.286	Not Significant	Do Not Accept Hypothesis
400mg/kg			1.000	Not Significant	
500mg/kg			0.286	Not Significant	

After the experimentation of thirteen days, the table shows the tabulized P values between the Negative control and experimental control group; 80% ethanolic extract of *Musa acuminata* leaves extract which was given in three doses; 300mg/kg, 400mg/kg, and 500mg/kg having the P-values of 0.286, 1.000, and 0.286 respectively. The researchers decided to reject the hypothesis. This shows that the 80% ethanol extract of *Musa acuminata* leaves extract did not exhibit significant anti-urolithiatic activity. Thus, indicating in this study that *Musa acuminata* leaves extract is not a potent anti-urolithiatic treatment in the doses used in this study (300mg/kg, 400mg/kg, and 500mg/kg). Although this should have shown significance, the unexpected values in the experimentation data hindered statistical evidence of this significance. Table 4.1 shows that the rat which have been induced successfully showed evidences of anti-urolithiatic activity compared to the negative control which was successfully induced which showed no anti-urolithiatic activity after the experimentation period.

Chapter 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter discusses the summary of findings, generated conclusion, and recommendations based on the analysis of the result on the anti-urolithitic activity of *Musa acuminata* (lakatan banana) leaves extract against calcium oxalate crystal induced *Rattus norvegicus*.

SUMMARY OF FINDINGS

Based on the results obtained from experimentation, it shows that there is no significant difference in the effects between the positive control and experimental control group (P is not <0.050). Also, there is no significance difference between the negative control group and the experimental control group (P is not <0.050). This shows that the *Musa acuminata* leaves extract produced the same results as the positive control in the experimentation but was also not significant when compared to the negative control. But based on the successfully induced rats shown in table 4.1, It is supported that the highest dose of the lakatan banana leaves extract possess a potential anti-urolithiatic activity.

Conclusions

Based on the data presented, the following conclusions were made:

1. The *Musa acuminata* leaves extract is not an effective anti-urolithiatic agent against calcium oxalate crystal in the following doses; 300mg/kg, and 400mg/kg. The highest dose, 500mg/kg showed probable signs of anti-urolithiatic activity.
2. The phytochemical components of *Musa acuminata* leaves include the following; triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins.
3. There is no significant difference between the positive control and experimental control group. Therefore, the researchers decided to accept the hypothesis.
4. There is no significant difference between the negative control group and experimental control group. Therefore, the researchers decided to reject the hypothesis.

Recommendations

Based on this study, the following recommendations were made:

1. The researchers suggest to increase the duration of the experiment in further studies; induction period of calcium oxalate crystals, and the duration of treatment.
2. The researchers suggest higher doses of *Musa acuminata* leaves extract be used in further studies.
3. Consider using other solvents which can be used for active component extraction which can be used for comparison in potency.
4. Other studies be done using *Musa acuminata* leaves which can exhibit other metabolic results due to its abundance in active components.

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APPENDICES

APPENDIX A

COMPUTATION AND PREPARATION OF REAGENTS

A. PREPARATION OF ETHYLENE GLYCOL SOLUTION

Equipment:

- Ethylene Glycol
- Distilled water

Technique:

1. The ethylene glycol concentration should be 0.75%.
2. For every 75 ml of ethylene glycol, 925 ml of distilled water is added to prepare the desired concentration.

B. PREPARATION OF CYSTONE FOR ORAL ADMINISTRATION

Equipment:

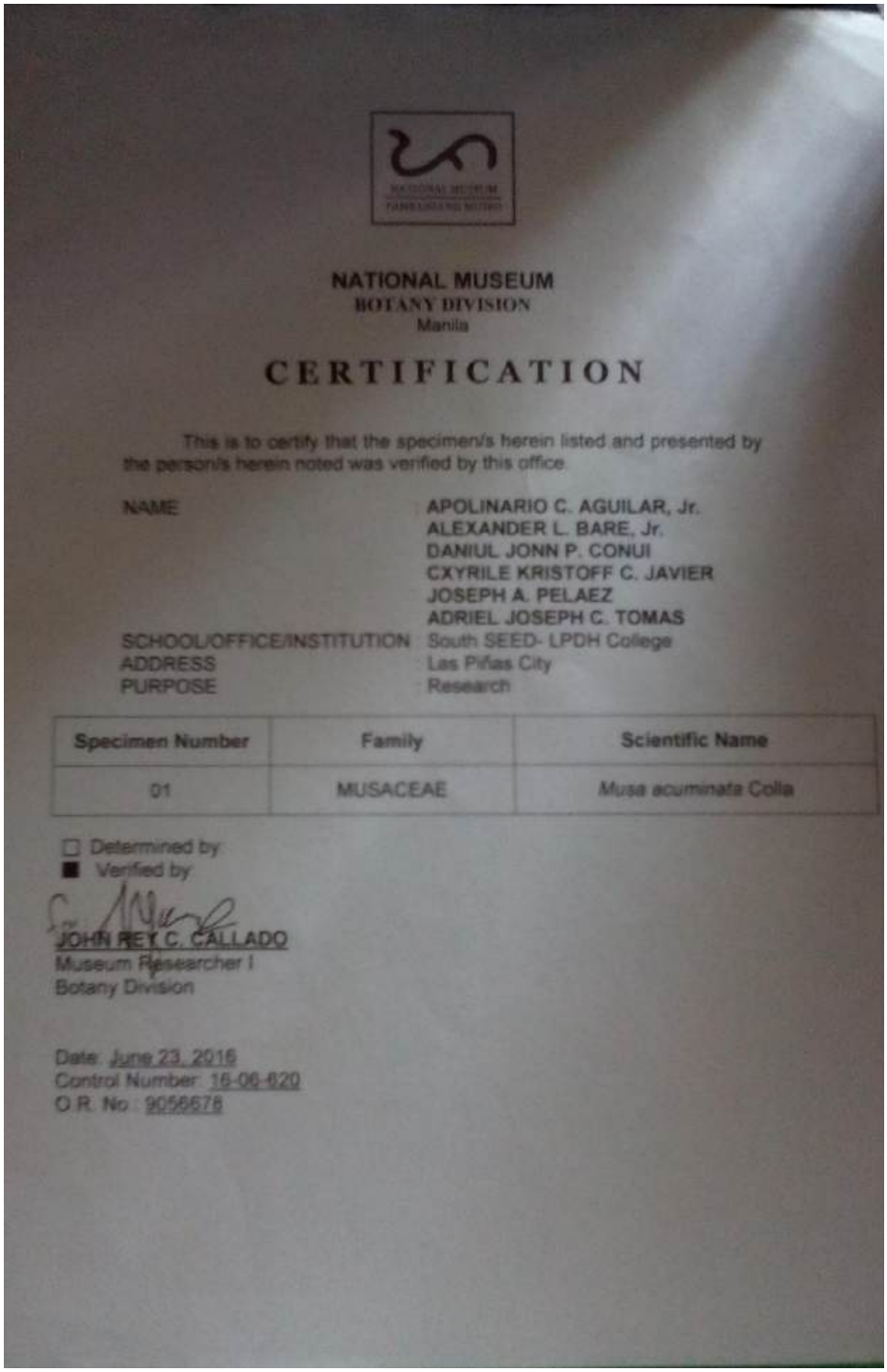
- Mortal and Pestle
- Cystonetablets
- Distilled water

Technique:

1. Cystone tablets are pulverized using the mortar and pestle.
2. For every 1000mg of pulverized cystone, 1ml of distilled water is added.

APPENDIX B

CERTIFICATION OF PLANT SAMPLE



NATIONAL MUSEUM
BOTANY DIVISION
Manila

CERTIFICATION

This is to certify that the specimen/s herein listed and presented by the person/s herein noted was verified by this office.

NAME	APOLINARIO C. AGUILAR, Jr. ALEXANDER L. BARE, Jr. DANIUL JONN P. CONUI CXYRILE KRISTOFF C. JAVIER JOSEPH A. PELAEZ ADRIEL JOSEPH C. TOMAS
SCHOOL/OFFICE/INSTITUTION	South SEED- LPDH College
ADDRESS	Las Piñas City
PURPOSE	Research

Specimen Number	Family	Scientific Name
01	MUSACEAE	*Musa acuminata* Colla

☐ Determined by
■ Verified by

JOHN REY C. CALLADO
Museum Researcher I
Botany Division

Date: June 23, 2016
Control Number: 16-06-620
O.R. No.: 9056576

APPENDIX C

PHYTOCHEMICAL ANALYSIS

Republic of the Philippines
Department of Science and Technology

INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
(Formerly National Institute of Science and Technology)
STANDARDS AND TESTING DIVISION
Gen. Santos Ave., Bicutan, Taguig, Metro Manila 1631

Fax No.: (632) 837-31-67 / 837-00-32

Tel. Nos. 837-20-71 to 82
local 2188, 2189

REPORT OF ANALYSIS
No. ITDI-072016-OCS-0551

Customer's Name : Alexander L. Bare Jr.
Address : South SEED-LPDH College, Las Pinas City

Sample Code : OCS-2016-0880
Sample : ***Banana Leaves without the Middle Stem (Musa acuminata)***
Description & Identification : About 200 g fresh, green leaves cut into pieces in an unmarked resealable plastic pouch
Date received : July 25, 2016
Date (s)Tested : August 02-04 2016

Phytochemical test for plant constituents :

Sterols	-	(-)
Triterpenes	-	(+++)
Flavonoids	-	(+++)
Alkaloids	-	(+)
Saponins	-	(++)
Glycosides	-	(+)
Tannins	-	(+++)

Note: (+) Traces, (++) moderate, (+++) abundant
(-) Absence of constituents

Reference: Pharmacognosy, 15th edition, 2002, Trease & Evans

VALIDITY OF THE REPORT: The test results are those obtained at the time of the test and pertain only to the sample(s) received by this Laboratory. *Codes and words in italics* are quoted solely for the customer's reference; significance of these codes and words are not verified by this Laboratory. This report is not to be used for advertising purposes or sales promotion.

ROSARIO T. FUERTES
Head, Organic Chemistry Section

NATIVIDAD R. MAMPLATA
Head, Chemistry Laboratory

Issued under the Authority of:

DR. CYNTHIA M. NALO-OCHONA
Authorized Officer

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APPENDIX D

ORIGINAL DATA OF EXPERIMENTATION

Republic of the Philippines
Department of Science and Technology
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
(Formerly National Institute of Science and Technology)
STANDARDS AND TESTING DIVISION
Gen. Santos Ave., Bicutan, Taguig, Metro Manila 1631

Fax No.: (632) 837-31-67 / 837-00-32 Tel. Nos. 837-20-71 to 82 local 2188, 2189

Results:

Table 1. The sample administered orally to Ethylene glycol induced urolithiasis in SD Rats produced the following Urine Crystals compared to the positive control Cystone.

Group/ Sample	Dose	Animal No.	Weight, kg		Urine Crystals Microscopic Examination		
			Initial	Final	Baseline	6d Post Urolith Induction	7d Post Treatment
Group 1 Negative Control *Distilled water*	0*	1	0.1312	0.1574	negative	Triple phosphate (+3)	Triple phosphate (+3)
		2	0.1286	0.1442	negative	Calcium oxalate (+3)	Calcium oxalate (+3)
		3	0.1426	0.1640	negative	Triple phosphate (+3)	Triple phosphate (+3)
		4	0.1257	0.1508	negative	Triple phosphate (+4)	Triple phosphate (+4)
		5	0.1591	0.1480	negative	Ca oxalate (+2) Triple phosphate	Triple phosphate (+3)
Group 2 Positive Control *Cystone*	300	1	0.1322	0.1588	negative	Triple phosphate (+4)	Triple phosphate (+4)
		2	0.1432	0.1647	traces	Triple phosphate (+4)	Triple phosphate (+4)
		3	0.1544	0.1777	negative	Triple phosphate (+2)	Triple phosphate (+3) - Calcium oxalate (+2)
		4	0.1088	0.1270	negative	Calcium oxalate (+2)	Calcium oxalate (+3)
Group 3 Positive Control *Cystone*	400	1	0.1221	0.1495	negative	Triple phosphate (+3)	Triple phosphate (+2)
		2	0.1546	0.1615	negative	Triple phosphate (+4)	Triple phosphate (+3)
		3	0.1253	0.1504	negative	Triple phosphate (+2)	Triple phosphate (+3)
		4	0.1427	0.1712	negative	Triple phosphate (+3)	Triple phosphate (+2) -smaller in size
Group 4 Positive Control *Cystone*	500	1	0.1311	0.1573	negative	Triple phosphate (+3)	Triple phosphate (+2)
		2	0.1099	0.1319	negative	Triple phosphate (+4)	Triple phosphate (+3)
		3	0.1291	0.1549	negative	Calcium oxalate (+4)	Calcium oxalate (+2)
		4	0.1507	0.1808	negative	Triple phosphate (+3)	Triple phosphate (+3)

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Republic of the Philippines
Department of Science and Technology
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
(Formerly National Institute of Science and Technology)
STANDARDS AND TESTING DIVISION
Gen. Santos Ave., Bicutan, Taguig, Metro Manila 1631

Fax No.: (632) 837-31-67 / 837-00-32 Tel. Nos. 837-20-71 to 82 local 2188, 2189

Group/ Sample	Dose	Animal No.	Weight, kg		Urine Crystals Microscopic Examination		
			Initial	Final	Baseline	6d Post Urolith Induction	7d Post Treatment
Group 5 Treatment Test Sample *Lakatan banana*	300	1	0.1242	0.1490	traces	Triple phosphate (+4)	Triple phosphate (+2)
		2	0.1318	0.1574	negative	Triple phosphate (+4)	Triple phosphate (+4)
		3	0.1320	0.1584	negative	Triple phosphate (+3)	Triple phosphate (+3)
		4	0.1304	0.1565	traces	Triple phosphate (+4)	Triple phosphate (+3)
Group 6 Treatment Test Sample *Lakatan banana*	400	1	0.1166	0.1399	negative	Triple phosphate (+4)	Triple phosphate (+4)
		2	0.1311	0.1573	negative	Triple phosphate (+3)	Triple phosphate (+3)
		3	0.1389	0.1667	negative	Triple phosphate (+4)	Triple phosphate (+4)
		4	0.1422	0.1612	traces	Triple phosphate (+4)	Triple phosphate (+4)
Group 7 Treatment Test Sample *Lakatan banana*	500	1	0.1007	0.1208	negative	Triple phosphate (+3)	Triple phosphate (+1) smaller in size
		2	0.1113	0.1338	negative	Triple phosphate (+3)	Triple phosphate (+3)
		3	0.1395	0.1604	negative	Calcium oxalate (+4)	Triple phosphate (+4)
		4	0.1330	0.1530	negative	Triple phosphate (+4)	Triple phosphate (+2)

legend: * - control, the same as in the highest dose
d - days

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TABLES

Table 4.1

Tabulated data of examination for calcium oxalate crystals

Group/ Sample	Dose in mg/kg	Animal No.	Urine Crystals Microscopic Examination		
			Baseline	6d Post Urolith Induction	7d Post Treatment
Group 1 Negative Control: *Distilled water*	0[a]	1	Negative	Negative	Negative
		2	Negative	Calcium Oxalate (+3)	Calcium Oxalate (+3)
		3	Negative	Negative	Negative
		4	Negative	Negative	Negative
		5	Negative	Calcium Oxalate (+2)	Negative
Group 2 Positive control: *Cystone*	300	1	Negative	Negative	Negative
		2	Traces	Negative	Negative
		3	Negative	Negative	Calcium Oxalate (+2)
		4	Negative	Calcium Oxalate (+2)	Calcium Oxalate (+3)
Group 3 Positive control: *Cystone*	400	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Negative	Negative	Negative
Group 4 Positive control: *Cystone*	500	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Calcium Oxalate (+4)	Calcium Oxalate (+2)
		4	Negative	Negative	Negative
Group 5 Treatment: Test Sample *Lakatan Banana Leaves*	300	1	Traces	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Traces	Negative	Negative
Group 6 Treatment: Test Sample	400	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Negative	Negative
		4	Traces	Negative	Negative

Lakatan Banana Leaves					
Group 7 Treatment: Test Sample *Lakatan Banana Leaves*	500	1	Negative	Negative	Negative
		2	Negative	Negative	Negative
		3	Negative	Calcium oxalate (+4)	Negative
		4	Negative	Negative	Negative

Legend: [a] -control, the same as in the highest dosed - Days

Table 4.2

The Phytochemical Findings in 200 grams fresh *Musa acuminata* leaves

Constituent	Presence
Sterols	(-)
Triterpenes	(+++)
Flavonoids	(+++)
Alkaloids	(+)
Saponins	(++)
Glycosides	(+)
Tannins	(+++)

Note: (+) Traces, (++) moderate, (+++) abundant, (-) Absence of constituents

Table 4.3

A table showing the significant difference of the Positive Control Compared to the Experimental Control group.

Dose	Positive Control	Experimental Control	P-Value	Interpretation
300mg/kg	Cystone	*Musa acuminata* leaves extract	0.131	Not Significant
400mg/kg			0.317	Not Significant
500mg/kg			0.127	Not Significant

Table 4.4

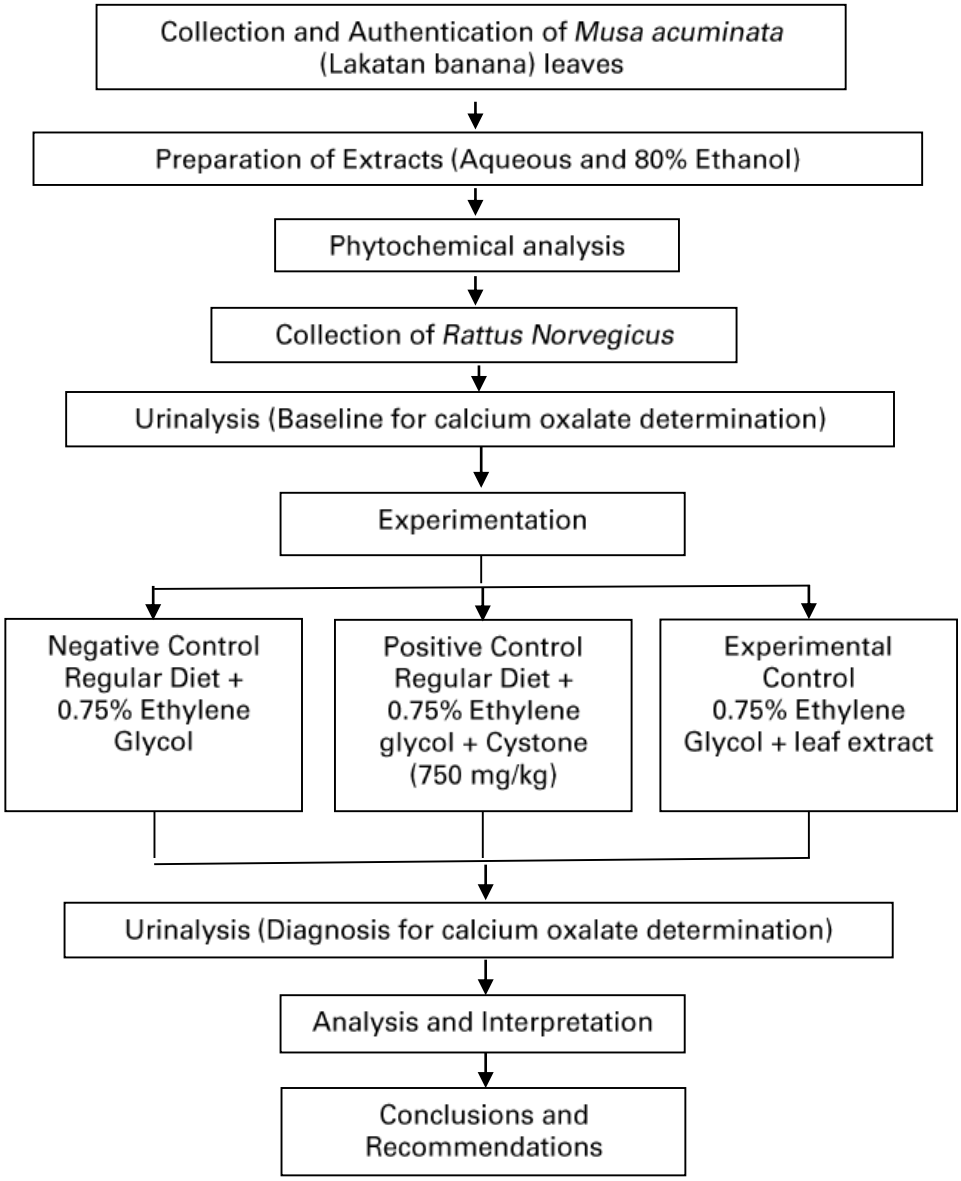
A table showing the significant difference of the Negative Control Compared to the Experimental Control group

Dose	Negative Control	Experimental Control	P-Value	Interpretation
300mg/kg	Distilled Water	*Musa acuminata* leaves extract	0.286	Not Significant
400mg/kg			1.000	Not Significant
500mg/kg			0.286	Not Significant

FIGURE

Figure 1.1.

A flow chart showing the step-by step procedure in this experimentation



PLATES

Plate 1. Collection of *Musa acuminata* (Lakatan Banana) leaves

Plate 2. Drying and Pulverizing of Leaves

Plate 3. Phytochemical Analysis of Leaves

Plate 4. Crude Extraction of Plant Materials

Plate 5. Operation of Rotary Evaporator

Plate 6. Collection of male *Rattus norvegicus*

Plate 7. Adaptation Period of Rats

Plate 8. Cystone and Concentrated Crude Extract

Plate 9. Collection of Urine

Plate 10. Oral gavage of rats

CURRICULUM VITAE

I. Personal Data

Aguilar	Apolinario Jr.	Callorina	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: B-3 L-13 Soldier's Home, Batasan Hills, Quezon City
Contact Number: 09154359129 Email: sslcapolaguilar@gmail.com
Date of Birth: 05/12/1989 Place of Birth: Quezon City Age: 27
Religion: Roman Catholic Nationality: Filipino
Language/ Dialects Spoken: English, Tagalog
Civil Status: Single Occupation: Student

II. Home and Community

Father's Name: Apolinario M. Aguilar Mother's Name: Celia C. Aguilar
Occupation: Deceased Occupation: Histopath Technician

III. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Batasan Hills National High School
Address: IBP Road, Batasan Hills, Quezon City
Inclusive Years: 4

IV. Membership in Any Organization

PHISMETS

V. Awards and Distinction

Dean's Lister 2nd Semester, AY 2013-2016

I. Personal Data

Bare	Alexander Jr.	Laconico	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: Blk 3 Lot 6, Queenstriangle St. Queensrow Central Area A, Bacoor, Cavite
Contact Number: 09163900324 Email: alexanderbarejr@gmail.com
Date of Birth: 05/29/1996 Place of Birth: Taguig City Age: 20
Religion: Born Again Christian Nationality: Filipino
Language/ Dialects Spoken: English, Japanese, Tagalog
Civil Status: Single Occupation: Student

II. Home and Community

Father's Name: Alexander C. Bare Sr. Mother's Name: Susan L. Bare
Occupation: General Foreman Occupation: Housewife

III. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Academy of Jesus
Address: Mother Earth Subdivision, Las Piñas City
Inclusive Years: 4

IV. Membership in Any Organization

Association of Christian Schools International (ACSI)
PHISMETS
Rotary Club Youth

V. Awards and Distinction

High School Valedictorian
ACSI Leader
Jude Garcia Award for Excellence in Science and Technology

I. Personal Data

Conui	Daniul Jonn	Pasno	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: : Blk 2 Lot 7 Narra St., Mutual Homes Subdivision, Soldiers Hills Village, Brgy. Putatan, Muntinlupa City
Contact Number: 09266010092 Email: daniul.conui@gmail.com
Date of Birth: 10/21/1991 Place of Birth: Quezon City Age: 24
Religion: Baptist Nationality: Filipino
Language/ Dialects Spoken: English, Tagalog
Civil Status: Single Occupation: Student

VI. Home and Community

Father's Name: Jose V. Conui III
Occupation: Pastor/ Administrator

Mother's Name: Celedonia P. Conui
Occupation: School Principal

VII. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Christ Baptist Academy
Address: Blk 24, Soldiers Hills Village, Baranggay, Putatan, Muntinlupa City
Inclusive Years: 4

VIII. Membership in Any Organization

N/A

IX. Awards and Distinction

N/A

I. Personal Data

Javier	Cxyrile Kristof	C	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: 25 A C. Gawaran St. Digman, Bacoor, Cavite
Contact Number: 09358061534 Email: cxyjav@gmail.com
Date of Birth: 09/06/1996 Place of Birth: Paranaque City Age: 20
Religion: Roman Catholic Nationality: Filipino
Language/ Dialects Spoken: English, Japanese, Tagalog
Civil Status: Single Occupation: Student

II. Home and Community

Father's Name: Christopher P. Javier Mother's Name: Kate C. Javier
Occupation: Self-Employed Occupation: Operations Staff

III. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Cavite School of Life
Address: EVY Compound, Panapaan III, Bacoor, Cavite
Inclusive Years: 4

IV. Membership in Any Organization

N/A

V. Awards and Distinction

N/A

I. Personal Data

Pelaez	Joseph	Anipan	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: 68 Espiritu Compound, UPS-V, Sucat Paranaque City
Contact Number: 0908939834 Email: japets_pelaez027@yahoo.com
Date of Birth: 05/27/1996 Place of Birth: Manila City Age: 20
Religion: Roman Catholic Nationality: Filipino
Language/ Dialects Spoken: English, Tagalog
Civil Status: Single Occupation: Student

II. Home and Community

Father's Name: Jose S. Pelaez
Occupation: Vendor

Mother's Name: Helen A. Pelaez
Occupation: Tailor

III. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Paranaque National High School
Address: Dr A. Santos Avenue, Paranaque City
Inclusive Years: 4

IV. Membership in Any Organization

PHISMETS

V. Awards and Distinction

5C's Competence Award

I. Personal Data

Tomas	Adriel Joseph	Canlas	Sex: Male
Last Name	First Name	Middle Name	

Permanent Address: No. 39, Nehemiah St, Camella Homes Classic, Pillar Village, Las Piñas City
Contact Number: 800-0309 Email: kolt.pyton@gmail.com
Date of Birth: 03/16/1996 Place of Birth: Manila City Age: 20
Religion: Protestant Nationality: Filipino
Language/ Dialects Spoken: English, Tagalog
Civil Status: Single Occupation: Student

II. Home and Community

Father's Name: Ariel P. Tomas
Occupation: Real Estate

Mother's Name: Grace Fe C. Tomas
Occupation: Instructor/ Professor

III. Educational Background

College Attended: South SEED-LPDH College
Address: Yokohama St. BF Homes International, Las Piñas City
Inclusive Years: 4 yrs. Course: BS Medical Technology
High School Graduated: Las Piñas Baptist Academy
Address: Marcos Alvarez
Inclusive Years: 4

IV. Membership in Any Organization

PHISMETS

V. Awards and Distinction

5C's Competence Award