TAXONOMIC IDENTIFICATION, PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF COMMON ANGIOSPERMS FOUND IN MT. BAYOSO, PASSI, CENTRAL ILOILO

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APPROVAL SHEET

This research proposal hereto entitle:

"Taxonomic Identification, Phytochemical and Antibacterial Screening of Common Angiosperms found in Mt. Bayoso, Passi, Central Iloilo."

Prepared and presented by Faye Margaret R. Fantilanan, Hazel G. Garcia, and Jane T. Habaradas in partial fulfillment of the requirements in Science Research II has been examined and is recommended for acceptance and approval.

Approved by the Committee in Oral examination with a grade of PASSED on _____. 2006.

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Accepted in partial fulfillment of the requirement in Science Research II.

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Abstract

This study identified the common angiosperms growing in Mt. Bayoso, Passi Central Iloilo, and further subjected the plants for phytochemical screening antibacterial screening. It specifically documented the presence of alkaloids, saponins and tannins. It also checked for antibacterial properties against the bacterium *Staphylococcus aureus*.

The plant specimen that were collected underwent ethanol extraction afterwhich the extracts were used for phytochemical screening and antibacterial testing under standard procedures.

Among the 13 plants collected and identified, results showed that all were positive for saponins and negative for alkaloids. In the tannin testing all were positive except for *Basella rubra Linn.*, commonly known as Alogbate. In the antibacterial testing, ethanol was used as the negative control and Tetracycline was used as the positive control. Results of the antibacterial testing showed that four plants were negative of antibacterial properties against. *S. aureus*. The plants were *Celosia argentea*, *Solanum melongena Linn.*, *Terminalia catappa J.* and *Gmelina arborea J.*

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Chapter I

Introduction

A. Background of the Study

Alkaloids are renowned for their potent pharmacological activities. Tiny amounts have important chemical use such as analgesics, antimalarial, antispasmodics, and treatment for hypertension, mental disorders and tumors (Tebby, 2004). Saponins are glycosides with distinctive foaming characteristics and they consist of a polycyclic aglycone that is either a choline steroid or triterpenoid that is attached via C3 and an ether bond to a sugar side chain (Cornell University, 2004). Tannins are phenolic compounds that precipitate proteins, and they are composed of a very diverse group of oligomers and polymers (Cornell University, 2001).

Alkaloids, saponins, and tannins are just some of the bioactive compounds commonly found in plants. These compounds may possess antibacterial properties (Mojab, 2004). The presence of these bioactive compounds in plants may be an indicator of their medicinal values.

Staphylococcus aureus is a gram-positive pathogenic bacterium that causes diseases ranging from boils and wound infections to more severe ones like Ritter's Disease or Scalded Skin Disease, and TSST-1 or Toxic Shock Syndrome (Paccial et al., 1998).

Mt. Bayoso is located 18.7 km from Passi City. Previous visits from the mountain indicated that this is a forest existing with a diverse group of angiosperms such as mango trees, gmelina, cacao, and the like. There have been tree-planting activities conducted in the previous years, but no taxonomic studies of plants found in Mt. Bayoso have been reported. Furthermore, no phytochemical testing or antibacterial screening of the plants growing in the area has been done. This study therefore is a pioneering effort to document the common species of angiosperms found in Mt. Bayoso.

Figure 1. Location of Passi in the map of Panay.

B. Statement of the Problem

This study aimed to answer these following questions:

1. What species of angiosperms are found in Mt. Bayoso, Passi, Central Iloilo?

2. Which plants in Mt. Bayoso contain bioactive compounds such as alkaloids, saponins, and tannins?

3. Do these plants possess antibacterial properties against *Staphylococcus aureus*?

C. Objective of the Study

The objectives of this study are:

1. To collect, classify, and identify angiosperms growing in Mt. Bayoso, Passi, Central Iloilo.

2. To determine which of these angiosperms growing in Mt. Bayoso contain bioactive compounds in their leaves such as alkaloids, saponins, and tannins.

3. To determine which of the identified angiosperms possess antibacterial properties against *Staphylococcus aureus* as indicated by the zones of inhibition of ethanol leaf extracts of the plants against the test organism.

D. Significance of the Study

Bioactive compounds from plants possess antibacterial properties (Mojab, 2003). More diseases of bacterial origin are arising today, claiming the lives of millions of people. By knowing whether there is a presence of the bioactive compounds in plants, we will be informed on which plants can have potential antibacterial or other medicinal properties.

Also, the knowledge of the phytochemical constituents of plants are needed for the information that could be used in disclosing new sources of material such as tannins, oils, and precursors for the synthesis of complex chemical substances.

This study is a pioneering effort to document the diverse group of angiosperms growing in the forest of Mt. Bayoso, Passi, Central Iloilo. Knowing the diversity of the plants in the area, and the bioactive compounds in them, will provide baseline information which can help the community manage these resources.

E. Scope and Delimitation of the Study

The goal of this study is to classify and identify the common angiosperms growing in the forest of Mt. Bayoso, Passi, Central Iloilo. This study also determined whether the bioactive compounds such as alkaloids, saponins, and tannins are present in the leaves of common flowering plants found in Mt. Bayoso. The research was also limited to testing for the presence of only three bioactive compounds which are alkaloids, tannins and saponins. The study will only determine the presence of the said bioactive compounds and not to quantify the amount present in the leaves of the plants. Furthermore, antibacterial screening using ethanol leaf extracts of these angiosperms was done against *S. aureus*.

F. Definition of Terms

The following are the conceptual and operational definitions of some terms used in the study:

Alkaloids

- Nitrogenous compounds met with plants in combination with organic acid (Webster New School and Office Dictionary)
- In this study, it is one of the bioactive compounds whose presence was detected in common plants found in the study site

Angiosperm

- flowering plants that first appeared during the Cretaceous Period
- Belongs to the division Anthophyta (Bio-Web Group, 2000)

Saponins

- Glycosides that have distinctive foaming characteristics
- In this study, it is one of the bioactive compounds whose presence was detected in common plants found in the study site

Staphylococcus aureus

- a spherical bacterium (coccus) which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters (Foodborne Pathogenic Microorganisms and Natural Toxins Handbook)

Tannins

- Phenolic compounds that precipitate proteins
- In this study, it is one of the bioactive compounds whose presence was detected in common plants found in the study site

Chapter II

Review of Related Literature

A. Phytochemicals

Phytochemicals are nonnutritive plant chemicals that contain protective, disease preventing compounds. More that 900 different phytochemicals have been identified as component of food, and many more phytochemicals continued to be discovered today. It is estimated that there may be more than 100 different phytochemicals in just one serving of vegetables (Dresbach et al. 1996).

Phytochemicals are naturally occurring biochemicals that give plants their color, flavor, smell and texture. They can have complementary and overlapping mechanisms of action in the body, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism, and antibacterial and antiviral effect (Tantillo, 2006). These phytochemicals also possess medicinal properties which may help prevent diseases that are responsible for over 60 percent of deaths annually. Currently, the term is being used only for those plant chemicals that may have health-related effects but are not considered essential nutrients (Davidson, 2004).

B. Alkaloids

Alkaloids are compounds known for their potent pharmacological activities. They have important clinical use such as analgesics, antimalarial, antispasmodics, for pupil dilation, and treatment for hypertension, mental disorder and tumor (Tebby, 2004) even

in very small amounts, the alkaloids produce strong physiological effects on the body. All contain nitrogen atoms that are structurally related to those of ammonia. Alkaloids are arguably the most potent therapeutic compounds and have been manufactured as various allopathic drugs. Derived from amino acids, alkaloids represent a varied and complex class of nitrogenous crystalline or oily compound. The therapeutic value of alkaloids differs according to the subcategories, which simply include benzyl, papaveracaea, codeine, protopine, protoberberine, and ipecac isoquinolines. The protoberberine, which include berberine, hydrastine, and canadine, are antibacterial, antiprotozoal, astringent, tonic, bitter tasting, and respiratory, vasomotor, and circulatory stimulants. Some 30 of the known alkaloids are used in medicine. For example, atropine, obtained from belladonna, causes pupil dilation; morpine is a pain killer; quinine is a specific remedy for malaria; nicotine is a potent insecticide; and reserpine is a valuable tranquilizer. The higher the alkaloid content of the extract, the more potent was the antibacterial activity of the extract (Milot, 2005).

C. Saponins

Saponins are poisonous but human generally do not suffer severe poisoning from saponins. Human cholesterine inactivates them so that only the mucus membrane is affected. Because of this saponins have been used in sneezing powders, emetics and cough syrups to facilitate expectoration, most saponins are also diuretic. In the diet, phytochemical saponins have a wide spectrum of activity as antifungal and antibacterial agents, lowering of blood cholesterol, and inhibition of cancer cell growth (Clark, 2005).

In humans, this effect disappears within a week following the neutralizing action of cholesterine. Alfalfa saponins may have potential in human health issues because they reduce serum cholesterol by preventing its reabsorption after it has been excreted in the bile. It is hypothesized that the saponins that bind with bile salts are unavailable to bind with cholesterol (Tebby, 2004). Saponins are used as anticancer. Saponins found in Panax Ginseng are known to enhance the activity of the natural killer (NK) cells, stimulate macrophages and promote production of antibodies (Dharmanda, 2003). The Chinese use saponins in cancer treatment and studies showed that it is more effective than chemotherapy. Saponins can also reduce Urinary Tract Infection (UTI) (Pallarito, 2004).

D. Tannins

Tannins are naturally occurring plant polyphenols. Tannins are common in fruits in tea, in chocolate, in legume forages, in legume trees, in grasses. Tannins occur in many trees and the best sources include oak galls and the bark of sumac. Tannins have a yellow-white to brown color and a faint, characteristic odor. Tannins are widely distributed in the plant kingdom. They are common in both gymnosperms and angiosperms. Within angiosperms, tannins are more common with dicotyledons than monocotyledons.

Tannic acid is valuable as an external medicine because it is an astringent and styptic. Chinese also use tannin-containing herbs for medicine. The most common uses of tannin are curing intestinal disorders, such as diarrhea and dysentery, intestinal parasites, rectal prolepses and hemorrhoids; bleeding, including functional bleeding, hematochezia (blood

in the stool), bleeding hemorrhoids, and topically for bleeding wounds and ulcerations; and excessive discharge, such as enuresis and frequent urination; leucorrhoea; hyperhidrosis (excessive sweating) night sweating and involuntary seminal emission. Tannins are also used as anti-aging remedies (Tebby, 2004).

E. Common Plants in the Philippines

Filipino Name	Scientific Name	English Name
Abukado	*Persea americana Mill.*	Avocado
Adelfa	*Nerium indicim Mill.*	Adelfa
Acapulco	*Cassia alata L.*	Ringworm bush
Andiyo	*Premna odorata Blanco*	Fragrant premna
Alubati	*Basella rubra L.*	Malabar night shade
Kulasiman	*Purtulaca olearacea L.*	Pueslane
Amarillo	*Targetes erecta L.*	Marigold
Ampalaya	*Momordica charantia L.*	Bitter gourd
Anonas	*Anona reticula L.*	Custard apple
Atis	*Anona squamosa L.*	Sugar apple
Atsuete	*Biva orillana Linon*	Annatto
Balanoy	*Osimin basilicum L.*	Sweet basil
Balete	*Ficus stipulosa Miq. L.*	
Bawang	*Allium sativa L.*	Garlic
Bayabas	*Psidium guajava L.*	Guava

Bunga de China	*Arecta catechu L.*	Betel nut palm
Buyo anis	*Piper beetle L.*	Betel leaf pepper
Kakaw	*Theobroma cacao L.*	Cocoa plant
Duhat	*Syszygium jambolanum*	Black plum
Gusol	*Kaempferia galanga L.*	
Gumamela	*Hibiscus rosa-sinensis L.s*	China rose

F. Plants reported to contain alkaloid, saponins, and tannin

Plants recorded to contain saponins:

European soapwort *Saponaria officinalis,* Dioscoreaceae, Liliaceae, Amaryllidaceaefoxglove *Digitalis spp.*, fenugreek *Trigonella foenum-graecum*, wild yam *Dioscorea villosa*, sarsaparilla, beth root *Trillium erectum*, false unicorn root *Chamaelirium luteum,* ginseng *Panax ginseng,* licorice *Glycyrrhiza glabra,* lobelia *Lobelia inflate,* ipecac *Cephaelis ipecacuanha,* Squills *Urginea maritime,* cowslip *Primula vera*common, daisy *Bellis perennis*, mullein flowers *Verbascum thapsus*, violet family *Viola spp.*, snakeroot *Polygala senega,* figwort *Scrophularia nodosa, Akebia trifoliata,Bupleurum chinense,* silver birch *Betula pendula*, corn silk *Zea mays,* horse chestnut *Aesculus hippocastanum,* yarrow *Achillea millefolium*, lime flowers *Tilia spp.*, Chai Hu *Bupleurum chinense,* Dang Gui *Angelica sinensis,* ginseng *Panax ginseng,* Hu Mu *Aralia chinensis,* Jujube *Zizyphus jujube,* Siberian ginseng *Eleutherococcus senticosus,* Yuan Zhi *Polygala tenuifolia,* Wu Wei Zi *Schizandra chinensis,*

Plants recorded to contain tannins:

Tea, coffee, cocoa, sorghums, Bayberry, Bilberry, Black cohosh, Borage, Burdock, Cedar, Chamomile, Cinnamon, Comfrey, Crampbark, Echinacea, Elder, Eucalyptus, Evening rimrose, Eyebright, Feverfew, Flax, Ginkgo biloba, Horehound, Hyssop, Juniper, Marshmallow, Nettle, Pennyroyal, Peppermint, Plantain, Red raspberry, Rhubarb, Rose Hips,, Rosemary, Sage, St. John's Wort, Skullcap, Slippery elm, Squawvine, Suma, Thyme, Uva ursi, Valerian, Wild yams, Wintergreen, Witch hazel, Wood betony, Yarrow

Plants recorded to contain alkaloids:

Opium poppy *Papaver somniferum*, San Pedro cactus *Trichocereus pachanoi*, rattleweed *Astragalus pomonensis*, morning glory *Ipomoea tricolor*, Mexican morning glory *Turbina corymbosa*, "fly agaric" mushroom *Amanita muscaria*, psilocybin mushroom *Psilocybe cyanescens*, peyote *Lophophora williamsii*, jimsonweed *Datura*, tree datura *Brugmansia sanguinea*,

G. *Staphylococcus aureus*

Staphylococcus aureus is a spherical bacterium (coccus), which on microscopic examination appears in pairs, short chains or bunched, grapelike clusters. These organisms are gram-positive. Some strains are capable of producing a highly heat stable protein toxin that causes illness. In humans it can cause severe food poisoning. It has been identified as the causative agent in many food poisoning outbreaks and is probably responsible for more cases of individuals in family groups than the records show. Foods are examined for the presence of *S. aureus* and/or are entertoxins to confirm that *S.*

aureus is a causative agent of food borne illness, to determine whether a food is potential source of "staph" food poisoning and to demonstrate post processing contamination, which is generally due to human contact or contaminated food contact surfaces (CFSAN, 1992). *S. aureus* has become resistant to many commonly used antibiotics. Up to 90% of all *Staphylococcus* isolates are resistant to penicillin (Wikipedia). Some plants that contain phytochemicals may possess antibacterial properties against *S. aureus*, some of these plants are *Parkia biglobosa, Parkia bicolor, Sophora exigua* (M. Iinuma et al., *2004*), (E. O. Ajaiyeoba, 2002).

Chapter III

Materials and Methods

A. Materials:

Mortar and pestle

Test tubes

Funnels

Dropper and Pasteur pipette

Filter paper

Calibrated test tubes or 10 ml graduated cylinder

Reagent bottles

Laboratory masks

Laboratory goggles

Spatula

Test tube rack

250 ml beaker

Plant leaves

Ammoniacal chloroform

1M sulfuric acid

Platform balance

Mayer's reagent

Autoclave

Clean sheets of paper

Erlenmeyer flask

Ethyl alcohol

Water bath

Buckner funnel

Tightly stoppered container

Hexane

Stirring rod

Chloroform

Acetic anhydride

Hot plate

Distilled water

Salt

Gelatin salt reagents

Ferric chloride

Inoculating loop

Forceps

Autoclaved filter paper discs

Transparent ruler

Bacterial culture (*S. aureus*)

Mueller-Hinton agar

Nutrient agar plates

Polyethylene bags

Knives or scissors

Coarse black sand

Basin

B. Description of the Plant Collection Site

Taxonomic classification as well as phytochemical and antibacterial screening of plants has been done on angiosperms collected from Mt. Bayoso, Passi, Central Iloilo. Mt. Bayoso is located 18.7 kilometers from Passi City.

Around Mt. Bayoso were several crop plantations but there weren't any field plantation on the mountain itself. There were a few households (2-3) around the mountain and there is one residential house right at the foot of the mountain at the entrance gate that serves as shelter for the people that were obliged to protect and guard Mt. Bayoso. However, at the study site itself, there is yet another native hut that was small in size yet was enough to provide rest and shelter. This is another shelter for the people that were protecting the mountain whenever they were inspecting the mountain itself. The study site showed that the mountain holds a diverse group of flowering trees (angiosperms). The site from which the angiosperms were collected was located approximately halfway up from the foot of the mountain itself since it is the highest site accessible by foot. No taxonomic study has been conducted in the area. Furthermore, no phytochemical screening on the plants growing in the area has been reported.

C. Collection of Plant Specimen for Identification, Phytochemical and Antibacterial Screening

In the field, a branch containing the leaves, flowers and fruits from each angiosperm was cut using shearing scissors. The branches were placed inside polyethylene bags. These are for the identification of the plant species.

About 500 grams of healthy leaves from each plant were also collected for the extraction process. The plants that were collected were the most common in the area. The leaves were placed inside separate plastic bags and were brought to PSHS-WV campus.

D. Processing of Collected Plants for Identification

In the PSHS-WV laboratory, plants collected were analyzed to verify the identification performed on the site. Leaf arrangements ware noted for the proper identification of the plants. Plants were identified from the family, genus, and species level with the aid of the reference book "Flora of Manila" (Merill, 1982) and "A Pictorial Cyclopedia of Philippine Ornamental Plants" (Domingo M. Reyes, 1999).

E. Preparation of Materials

E. 1 Sand treatment

The coarse black sand was washed with water. The washed sand had been let stand for 24 hours and was air-dried. When dried, the sand was placed in the container and was left for storage.

E. 2 Ammoniacal chloroform

Materials: anhydrous sulfate ammonia

Chloroform filter paper

200 ml Erlenmeyer flask (5)

Procedure:

One liter of chloroform was mixed with 3.66 ml of concentrated ammonia. Sufficient anhydrous sodium sulfate was added to the mixture, and the solution was filtered.

E. 3 Mayer's reagent

Materials: mercuric iodide 100 ml graduated cylinder

Distilled water

Procedure:

1.4 grams of mercuric iodide was dissolved in 60.0 ml of distilled water and the mixture was poured into a solution of 50.0 grams of potassium iodide in 10.0 ml of distilled water. Then, enough distilled water was added to make 100.0 ml solution.

E. 4 Sterilization of Materials Needed

Materials that can be autoclaved such as Petri dishes, flasks, etc. were singly wrapped in clean sheets of paper. After then, they were placed inside the autoclave at 121 degrees Celsius at 15 psi for thirty minutes.

F. Preparation of Plant Extracts (Ethanol Extraction) for Phytochemical and Antibacterial Screening

One hundred grams of air-dried leaves were placed inside a reflux condenser. Then 300 ml of 70% ethyl alcohol was added. The condenser was placed over a hot water bath and refluxed for an hour. The water bath was maintained at the boiling point of water.

G. Phytochemical Screening

G.1 Testing for Alkaloids

A. Two to four grams of fresh finely cut leaves were triturated with sufficient sulfuric acid and clean sand using a mortar and pestle. Then 10.0 ml of ammoniacal chloroform was added to the triturated leaves and the mixture was stirred. Triturated leaves were filtered into a test tube; the leaves were pressed to recover the extract. Then 0.5 ml of 1M sulfuric acid was added to the extracts and the test tube was shaken. The clear upper layer of the extract was pipetted off into test tubes and were tested with Mayer's reagent.

A positive test with Mayer's reagent is indicated by a white precipitate. Results were then recorded.

G. 2 Testing for Saponins

A. Liebermann-Burchard test

The equivalent of 10 grams of the plant extract was evaporate into dryness using a water bath. It was the cooled to room temperature. Ten ml of hexane was added, it was then stirred for a few minutes, it was allowed to settle and the supernatant was decanted off. The step was then repeated until the hexane extract has removed most of the color of the extract. The hexane extract was then discarded safely. To the residue, 10 ml of chloroform was added and was stirred for five minutes. It was then decanted into a test tube containing about 100 mg of anhydrous sodium sulfate. It was shaken and passed through a dry filter paper. The filtrate was divided into two clean test tubes. Test tube number one was used as reference. To one portion, three drops of acetic anhydride was added. The mixture was mixed gently. One drop of concentrated sulfuric acid was added and the mixture was mixed gently.

Color changes were observed immediately over a period of one hour. Then the color change was compared to the reference test tube. Results were then recorded.

G. 3 Testing for Tannins

Materials: analytic balance
Water bath
Distilled water
Test tubes
Gelatin salt reagents
Erlenmeyer flask
Autoclave
salt
dropper
filter paper
test tube rack
ferric chloride
hot plate
clean papers

A. Procedure:

Ten grams of equivalent plant materials were ethanol extracted. It was evaporated to incipient dryness over a water bath and was then cooled. Five drops of 10% NaCl solution was then added to salt out undesirable constituents. The mixture was then filtered. The filtrate was divided into three test tubes labeled A, B and C. the contents of test tube A was reserved as blank.

B. Gelatin test

To test tube B, three drops of gelatin salt reagent was added. Any formation of a precipitate was observed and recorded. Precipitation will indicate the presence of tannins.

B.2 ferric chloride test

To test tube C, three drops of ferric chloride solution was added. Any color change was then observed. A blue-black color indicates the presence of hydrolysable tannins While a brownish green color indicates condensed tannins, if the gelatin test is positive, there are no polyphenolic compounds present. Results were recorded.

H. Antibacterial screening

H. 1 Preparation of Nutrient Agar Plates

The Petri dishes were cleaned using liquid antibacterial soap and running water. It has been let stand until the dishes were dry. When dried, the dishes were wrapped

securely with clean sheets of paper and placed inside the autoclave at 121 degrees Celsius for 15 minutes. After 15 minutes it was taken out and was prepared for agar to be placed into it.

H. 2 Preparation of Agar

Using an analytical balance, 5.6 grams of Mueller-Hinton agar was weighed and mixed to 200 ml of distilled water. The mixture was done in a 200 ml Erlenmeyer flask. It was placed on a hot plate and stirred at the same time. The heating and stirring of the agar was maintained until there were no visible solid particles and the solution was clear. The mixture was first cooled before it was poured into the Petri dishes. When more agar was needed the same steps were repeated.

H. 3 Soaking of the Filter Paper Discs

By the aid of a puncher, filter paper discs were punched from filter paper sheets. Per plant extract there were ten filter paper discs made available. Then all produced filter paper discs were wrapped in a clean sheet of bond paper. The wrapped filter paper discs were placed in an autoclave at 121 degrees Celsius at 15 psi for 15 minutes. Sterile paper discs were soaked in the plant extracts and in the positive and negative control for 24 hours.

H. 4 Antibacterial Screening

In our antibacterial test we regarded tetracycline as our positive control and ethanol as our negative control.

The nutrient agar plate was equally divided into five sections. Each section labeled into each rightful plant extract. *S. aureus* was streaked aseptically all over the nutrient agar plate, by first, heating the inoculation loop over the alcohol lamp until the loop turned red. The inoculating loop was then cooled by letting it stand for a few seconds before actually dipping it into the bacterial culture. After cooling, it was dipped into the bacterial culture and streaked nutrient agar plate (note: before opening the nutrient agar plate, its sides were heated by passing them over the flame a couple rounds of its circumference). The streaked plate was allowed to stand for three minutes. The first disc was aseptically (forceps were heated and let cooled before being used) picked from a Petri dish containing the plant extracts and placed it over its respective section. The same steps were done to all other discs from all other plant extracts. The plate was incubated at 37 degrees Celsius for 24 hours. After the incubation period, the growth of the bacteria around each disc was observed. The diameter of the zones of inhibition of the plant extracts against *S. aureus* regarded as positive or negative. Presence of zones of inhibition in each treatment was noted.

I. Handling Disposal of Materials, Equipments and Microorganism

Persons involved were required to wash their hands after they handled viable materials, after removing gloves and before leaving the laboratory. Eating, drinking, handling of contact lenses and applying if cosmetics was not permitted in the work areas. Mouth pipetting was prohibited; 22mechanical pipetting devices were used. Then, all procedures were performed carefully to minimize the creation of splashes or aerosols. Work surfaces were decontaminated on completion of work and at the end

of the day, after any spill or splash, with viable materials with disinfectants that are effective against the agents of concern. All cultures stock and other regulated wastes were decontaminated before disposal by an approved decontamination methods such as: autoclaving. Dishes were autoclaved at 121 degrees Celsius at 15 psi for 30 minutes before being discarded. However materials decontaminated outside the immediate laboratory were placed in a durable leak-proof container and closed for transport from the laboratory. Materials decontaminated offsite from the facility were packaged in accordance with applicable state and federal regulation, before removal from the facility. Laboratory equipments and work surfaces were decontaminated with an effective disinfectant (Lysol) on a routine basis after work.

J. Tabulation of Recorded Data

Data gathered from all types of screening were recorded in a tabular form with respect to the plant species; the data given was qualitative for phytochemical screening (presence, absence). For antibacterial screening the zones of inhibition were also reported as positive if there is any zone of inhibition and negative if there is none.

Chapter IV

RESULTS AND DISCUSSIONS

A. Results

This study aimed to identify taxonomically angiosperms that have been collected from Mt. Bayoso, Passi, Central Iloilo. This study also aimed to determine the presence or absence of certain phytochemicals such as alkaloid, saponin and tannin. It also specifically determined the antibacterial properties of the identified angiosperms against *Staphylococcus aureus*.

Thirteen angiosperms were collected from the study site. All of these angiosperms have been taxonomically identified to the species level with the aid of "Flora of Manila" (Merill, 1982) and "A Pictorial Cyclopedia of Philippine Ornamental Plants" (Domingo M. Reyes, 1999) with their scientific names and family aligned to their common names/ english names (Table 2).

Table 2. Taxonomic identification of common angiosperms found in Mt. Bayoso, Passi, Central Iloilo.

Common name/English name	Scientific name	Family
Agdao,Alagaw/ Fragrant premna	*Premna odorata blanco*	Lamiaceae
Alibhon, Sambong/ False oxtongue	*Blumea balsimifera*	Asteraceae
Alogbate/ Malabar night shade, Malabar spinach	*Basella rubra Linn.*	Basellaceae
Alum-alum/ Silver cock's comb	*Celosia argentea*	Amaranthaceae
Talong/ Eggplant, Aubergine	*Solanum melongena L.*	Solanaceae
Kakaw/Cacao, Chocolate Tree	*Theobroma cacao L.*	Sterculiceae
Talisay/ False kamani	*Terminalia catappa J.*	Combretaceae
Kapayas/ Papaya	*Carica Papaya*	Caricaceae
Mahogany/ Honduras Mahogany, Caoba	*Swietenia macrophylla*	Meliaceae
Acacia/ Sweet acacia	*Acacia farnesiana*	Leguminosae
Okra/ Lady's finger	*Belmochus esculentus L.*	Malvaceae
Gmelina, Kumbil/ Gamhar	*Gmelina arborea J.*	Verbenaceae
Madre de Cacao/ Forest lilac	*Glirida sepium*	Fabaceae

For the phytochemical screening, results showed that all angiosperms identified did not contain alkaloids (Table 3).

Results also showed that all angiosperms were positive for saponins (Table 3).

As for the tannin testing, all angiosperms were positive except for *Basella rubra Linn.* (Alogbate) (Table 3).

The results of the antibacterial screening showed the nine angiosperms having antibacterial properties against S. aureus and those were: *Premna odorata blanco* (Agdao), *Blumea balsamifera* (Alibhon), *Bassella rubra Linn.* (Alogbate), *Theobrama cacao L.* (Kakao), *Carica papaya* (Papaya), *Swietena macrophylla* (Mahogany), *Acacia farnesiana* (Acacia), *Belmochus esculentus L.* (Okra), and *Glirida sepium* (Madre de Cacao) (Table 3).

Table 3. Results of the phytochemical testing and antibacterial screening against *S. aureus* of the leaf ethanol extracts.

Scientific name	Alkaloid	Saponin	Tannin	Antibacterial testing
Premna odorata blanco	-	+	+	+
Blumea balsimifera	-	+	+	+
Basella rubra Linn.	-	+	-	+
Celosia argentea	-	+	+	-
Solanum melongena L.	-	+	+	-
Theobroma cacao L.	-	+	+	+
Terminalia catappa J.	-	+	+	-
Carica Papaya	-	+	+	+
Swietenia macrophylla	-	+	+	+
Acacia farnesiana	-	+	+	+
Belmochus esculentus L.	-	+	+	+
Gmelina arborea J.	-	+	+	-
Glirida sepium	-	+	+	+

B. Discussion

Despite the diversity of the forest of Mt. Bayoso, only a few species of common angiosperms were collected because the plant collection site was limited to the lowest level of the mountain, which is the only accessible area. The area is also very close to the locals, so the plants available were limited.

The plants collected have been found not to contain alkaloids. Alkaloids are often found in the families: *Convolvulaceae, Cactaceae, Strophariaceae,*and *Solanacea* (Wikepedia, 2006). The plants we have gathered are not included in these families therefore it is possible that the plants gathered does not contain the said phytochemical. A studied performed by the Iranian Journal of Pharmaceutical Research most common phytochemical in plants is the saponin, followed by tannins and flavonoids and least is the alkaloids. Of the 200 plants gathered, 84 percent of plant species had saponin, 42 percent of plant species had tannin, 45 percent of plant species had flavonoid, 13 percent of plant species had alkaloid (Amin Gh. et al., 2004).

All of the collected angiosperms were positive of saponins. This is because the Liebermann-Burchard test used for saponins testing identifies saponins presence regardless of the amount or abundance. Also, saponin is one of the phytochemicals that is common in most plants (Cornell University, 2004).

As for the tannin testing, the results for the plants were not uniform despite the fact that they all grow on the same area and level. There are many different types of tannin, and different types of tannin tests that identify them. Ferric chloride testing for one may determine whether a plant contains hydrolysable or condensed tannins (Cornell University, 2001). This test was used for identifying tannin presence in the plants collected. Some have been found to contain only hydrolysable tannins or only condensed tannins, and for some, none at all. Also, most tannin-containing plants have been found to have woody barks to which most of their tannin content is stored (Wikipedia, 2006).

Since only the leaves were subjected to ethanol leaf extraction and later, to the tannin test, there is a possibility that the plant *Basella rubra Linn.* (alogbati) will show a positive test with its other parts tested, such as the stem.

There have been varying results in the antibacterial testing. This is because the presence of one bioactive compound does not necessarily mean that it has antibacterial potential against S. aureus. This methicillin-resistant bacterium has been found to be resistant of most types of penicillin (Wikipedia, 2006). Though presence of phytochemicals may suggest antibacterial properties (Mojab, 2004), there are still many different types of phytochemicals that can affect the antibacterial potential of a plant. Some of these phytochemicals are phytosterols, flavonoids, carotenes, and anthocyanins. Since only three phytochemicals were tested in this study, the presence or absence of those phytochemicals does not necessarily contribute to the antibacterial potential of the plants.

Chapter V

Summary, Conclusion, and Recommendations

This study aimed to determine the common angiosperms in Mt. Bayoso, Passi, Central Iloilo, and second, their phytochemical properties as well as antibacterial properties against *S. aureus.*

Specifically it aimed to:

1. Collect, classify and identify common angiosperms growing in Mt. Bayoso, Passi, Central Iloilo.
2. Determine which of these angiosperms growing in Mt. Bayoso contained bioactive compounds such as alkaloids, saponins and tannins; and
3. Determine which of the identified angiosperms possess antibacterial properties against *S. aureus* as observed by the zones of inhibition of the ethanol leaf extracts against the test organism.

A. Summary

The findings of the study are summarized as:

1. The researchers were able to collect 13 angiosperms found in Mt. Bayoso, Passi, Central Iloilo. They identified the plants while gathering them on the study site. The plants were further verified with the aid of "A Picture Cyclopedia of Philippine Ornamental Plants" by Domingo M. Reyes (1999).

2. The collected and identified angiosperms were extracted and checked for the presence of bioactive compounds such as alkaloids. All the plants were found negative of the said phytochemical.
3. The angiosperms were checked for the presence of saponins and all of the plants were found positive of the said phytochemical.
4. The angiosperms were also checked for the presence of tannins and all of the plants were found positive of the said phytochemical with the exception of *Baselle Rubra Linn.* (Alogbate).
5. Antibacterial testing showed that nine plants namely: *Premna odorata blanco* (Agdao), *Blumea balsamifera* (Alibhon), *Bassella rubra Linn.* (Alogbate), *Theobrama cacao L.* (Kakao), *Carica papaya* (Papaya), *Swietena macrophylla* (Mahogany), *Acacia farnesiana* (Acacia), *Belmochus esculentus L.* (Okra), and *Glirida sepium* (Madre de Cacao) (Table 3), were found positive against *S. aureus* where in the other hand the four were found negative (Table 3).

B. Conclusions

Thirteen common angiosperms found in Mt. Bayoso, Passi, Central Iloilo and it was found that all of the plants were found positive of saponins, and negative of alkaloids. All of them were positive of tannin with the exception of *Basella rubra Linn.* (Alogbate). This would suggest that plants have potent medicinal purposes. It was also found that all of them were positive of antibacterial properties with the exception of

Celosia argentea L (Alum-alum)., *Solanum melongena L.* (Talong) , *Terminalia catappa J.* (Talisay) , and *Gmelina arborea J.* (Gmelina).

C. Recommendation

For further studies, it is recommended that those plants found positive of the said phytochemicals be quantified. The plants with the presence of bioactive compounds should be tested for their effectivity on certain microorganisms such as *Bacillus cereus, Esherichia coli, Pseudomonas aeruginosa, Aspergillus niger* and *Candida utilis.* For future studies, the zones of inhibition of the plant extracts against *S. aureus* are also recommended to be studied and to be measured to determine the effectivity of the extracts.

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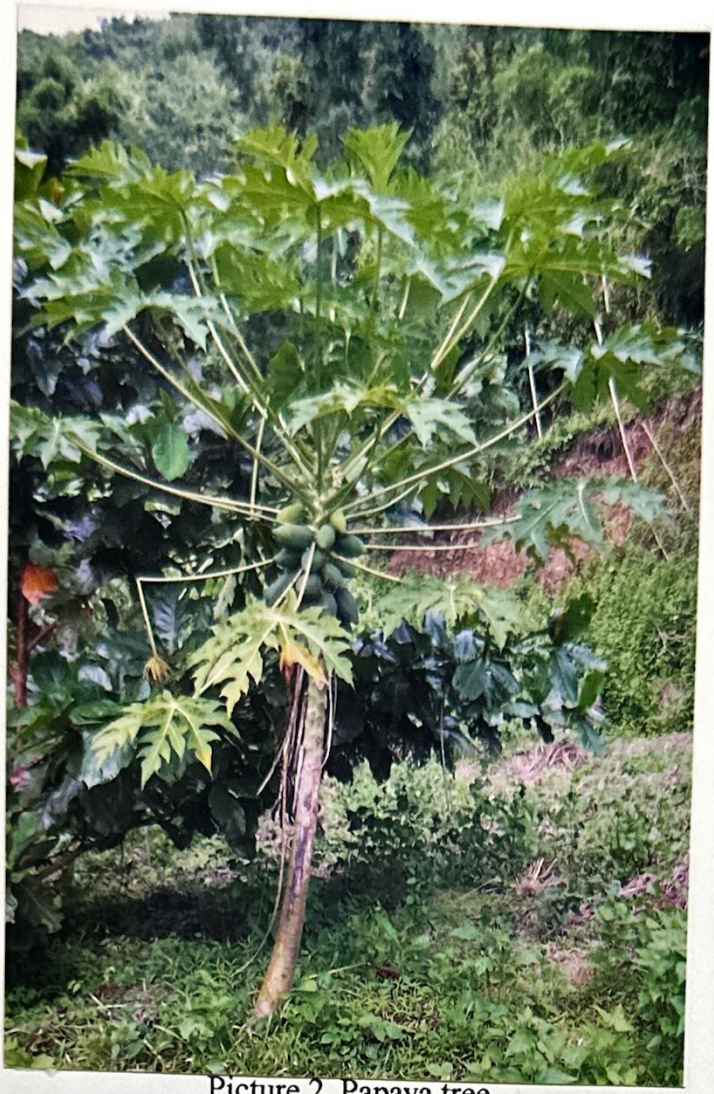
Appendix

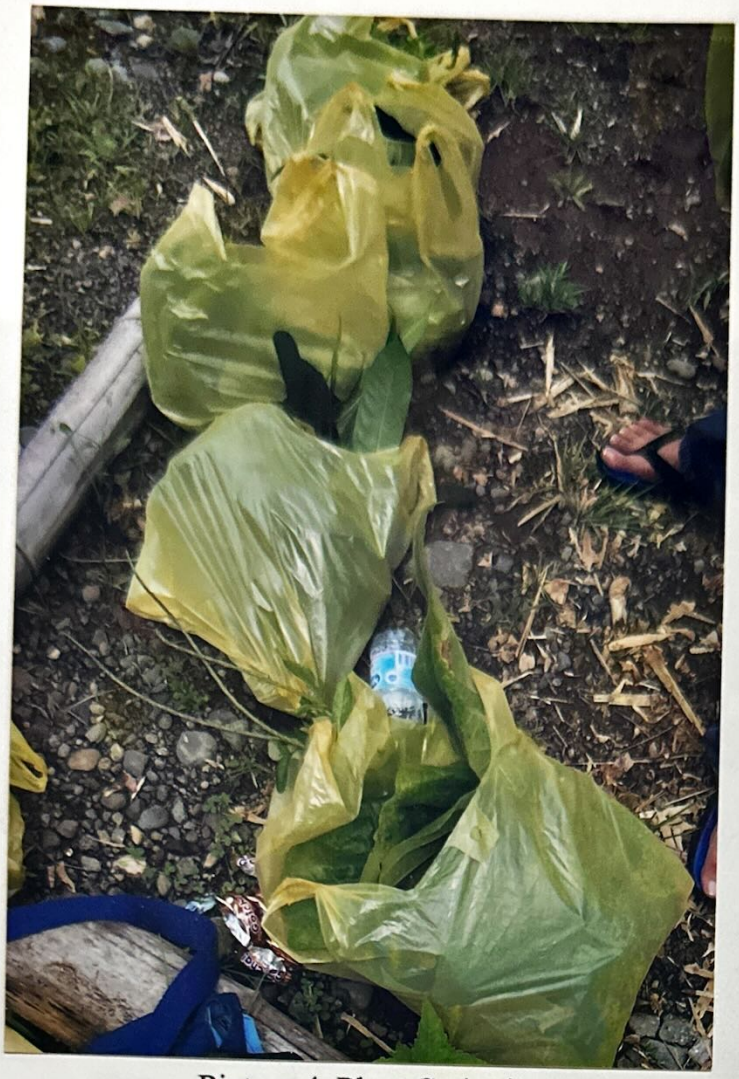
Pictures

Picture 1. Alibhon plant (False ox tongue)



Picture 2. Papaya tree

Picture 3. Mahogany or Caoba



Picture 4. Plant Gathering

Picture 5. Cut leaves soaked in Ethanol