REPUBLIC of the PHILIPPINES
City of Makati
UNIVERSITY OF MAKATI
COLLEGE OF ALLIED HEALTH STUDIES
CENTER OF PHARMACY

PHYTOCHEMICAL SCREENING OF RAIN TREE (SN: *Albizia saman;* FN: Fabaceae)

LEAF ETHANOLIC EXTRACT

A Research Paper Presented to the Faculty of

College of Allied Health Studies

University of Makati

In Partial Fulfillment of the Requirements

for the Course Pharmaceutical Botany with

Introduction to Pharmacognosy

By

CAMACHO, AZEL JOY

CARITOS, SHEILA MAE

GADEN, HANNALHAY

ISAGUIRRE, ANGELINE

LARIOZA, JAMES VALDWIN

RIZALDO, ALYSSA

October 2015

ACKNOWLEDGEMENT

We would like to express our special thanks of gratitude to our professors Ms. Estela De Vera and Ms. Mery Joyce Samonte in *Pharmaceutical Botany with Introduction to Pharmacognosy* as well as our center dean, Dr.Maria Fay Nenette M. Cariaga who gave us the golden opportunity to conduct this study on the phytochemical screening and who also helped us in making this study successful as well as on doing our researches. We came to know about so many new things that we are really thankful to them.

Secondly we would also like to thank our groupmates and classmate who helped us a lot on finalizing this project within the limited time frame and for giving their effort and time.

ABSTRACT

The objective of this study is to know the active constituent of *Albizia saman* and to know what are the indications or uses by phytochemical screening. Before our study began we did some researches about our plant sample, *Albizia saman*.

First we do the authentication to know the scientific name of the plant. And we do the moisture determination to determine the moisture content of the plant drugs sample and understand it's significant. Also we did drug extraction; we use the maceration method to get an extract from our plant also to determine the active constituents of the plant. And lastly we do the phytochemical screening to know the different active constituent present in *Albizia saman*. We determine the secondary metabolites that are present in our plant. And to know what are the possible uses of *Albizia saman*.

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Chapter I

Introduction

1.1 Background of the Study

All of us need drugs. Drugs help us maintain or restore our health, prevent a disease, injury and other damage to our body or mind. It can cure illness, boost immunities, and provide extra nutrients and vitamins that the body can't produce or consume on a daily diet. It is incredibly important but the unfortunate truth is not everyone can afford the right one but we have a saying that in every problem, there is a corresponding solution. What is then the solution? They say it is medicinal plants but what if this solution also has a problem?

Drugs started from plants. We have so many plants in this world that's why pharmacists are still making drugs out of it and especially, that is why we have so many herbal drugs. Using a plant, we can make drug and with drug, we can be cured and be protected from any possible diseases. As future pharmacists, we should know and be aware of these things. In this new generation many plants have been discovered to have a potential use as a drug but not all of us know it and not all of us know how to take it. We also believe that there are still thousand of plants that have important uses but are still undiscovered. These common problems weaken our solution.

In our phytochemical screening, we are aiming to find a drug that can help everyone with a specific or multiple diseases at a very simple way. We chose Rain tree (*Albizia saman)* because of some personal experiences and interesting things on our researches. We can see many of this in Bonifacio Global city (BGC), its origin here in Asia. At the first time I saw it, it can already give me relaxation that I know we all need. At that time, I have no idea what tree that is.

I don't know how but it attracted me. That is why when we are going to choose a plant to examine, this tree was the first thing that came in my mind. Based on our research about the plant that we examined, studies from China shows that the bark of rain tree can really relieve anxiety and depression. Those studies motivate us to conduct our own study.

In terms of our global problem, depression is the most common. Many people with depression are trying to kill themselves and it is because of too much thinking on something that bothers them. In the record there were 350 million around the world who suffered from depression and women are more likely to experience major depression than men. And depression also will lead to suicide. According to WHO over 800 000 people all over the world die due to over depression. Although there are known effective treatments for depression, fewer than half of those affected in the world (in many countries, fewer than 10%) receive such treatments. Barriers to effective care include a lack of resources, lack of trained health care providers, and social stigma associated with mental disorders. Another barrier to effective care is inaccurate assessment. In countries of all income levels, people who are depressed are often not correctly diagnosed, and others who do not have the disorder are too often misdiagnosed and prescribed antidepressants. The burden of depression and other mental health conditions is on the rise globally. In some studies, there is also growing evidence that untreated depression can contribute to or worsen other medical problems. "Heart disease is the one that has been most linked to depression, but research also suggests a link between depression and metabolic issues such as obesity, diabetes, and diseases such as Alzheimer's and cancer".

A pharmaco-epidemiologic study in 2002 found that rain tree was the third most commonly prescribed Chinese herbal medicine for treating insomnia. A study in rats pretreated with rain tree documented anxiolytic-like effects potentially mediated by changes in the

serotonergic nervous system, especially 5-hydroxytryptamine 1A receptors. Another study in chronically stressed rats found that rain tree alleviated growth inhibition caused by stress and regulated levels of monoamine brain neurotransmitters. Rain tree is native to Asia, occurring from Iran to Japan.

In traditional Chinese Medicine, rain tree (bark and flower) is classified as sweet with a neutral energy and enters the heart and liver organ meridians. According to the Chinese Herbal Medicine Materia Medica, rain tree is used primarily for insomnia, poor memory, irritability and angry feelings due to constrained emotions especially when accompanied by epigastric pain and feelings of pressure in the chest. Secondarily, the bark is regarded as one of the most important herbs for the treatment of external trauma and injuries. It promotes blood circulation, reduces pain and swelling, promotes the regeneration of flesh and facilitates the healing of bone fractures.

The relaxation that it gives us whenever we are seeing this tree is our very own motivation. What if it is not only its physical appearance is relaxing? What if it really has a chemical property that can be a medicine? With this, we want to prove that there is really something in this tree that may help many people living. We are also motivated to finish this study because of many interesting things that we knew in our researches. We also want to help those people who don't have enough money to buy a product or medicines. We want to offer them an alternative medication for their illness. Because people nowadays are getting poorer and poorer that they don't have the sufficient money to buy the right and enough medication for their own health. We want to discover another solution; another medicinal plant. Most especially, we want to do something about the case of depression which is a global problem.

Just like what we already stated, depression connects or worsens any other diseases or medical problems like cancer, insomnia and heart disease. Because of these things, it makes and commands us to strive more and harder. It is because if this will be successful, it means that if this will be successful we are not just hitting one but two or more than birds in one stone. Because of the studies, we are looking positively forward on the positive results that we are hoping to have.

1.2 Conceptual Framework and Theoretical Framework

Rain tree is known to be the tree of happiness in China because they believe that the tree of Albizia, especially the bark and flower can relieve the stress and depression of people. The bark as to “anchor” the spirit while flower is to lighten up the mood. To support that, there are several people who tested the said plant and they said that it is effective; they use it before they go to sleep and they said that there are no signs of drowsiness. This is also effective for patients who are taking some anti-depressant drugs because this can be used as an alternative, also because of its natural sources that the cost is cheap in the market.

There are broadly three classes of antidepressant medications, Tricyclic Antidepressants (TCA’s), Monoamine Oxidase inhibitors (MAOI’s) and the most popular, Serotonin Selective uptake inhibitors (SSRI’s).TCA’s enhance concentrations of the neurotransmitter chemicals norepinephrine (stimulating) and serotonin (the happiness hormone) in the brain. These are known as monoamines and they must be inactivated and reuptaked by the secreting cells. TRI’s block this reuptake, allowing the monoamines to remain active in the body much longer.MAOI’s not only enhance the same neurotransmitters, norepinephrine and serotonin, but dopamine as well (dopamine is the reward, or satisfaction hormone). This is the most dangerous and least prescribed class of antidepressants, because it may also inhibit the reuptake of tyramine, which can cause dangerously acute hypertension.SSRI’s (Selective Serotonin Reuptake Inhibitor’s) include the popular drugs Luvox, Paxil, Prozac, Celexa, and Zoloft and work by blocking serotonin reabsorption. Specifically, they prolong the effects of serotonin, with an accompanying sense of prolonged well-being.

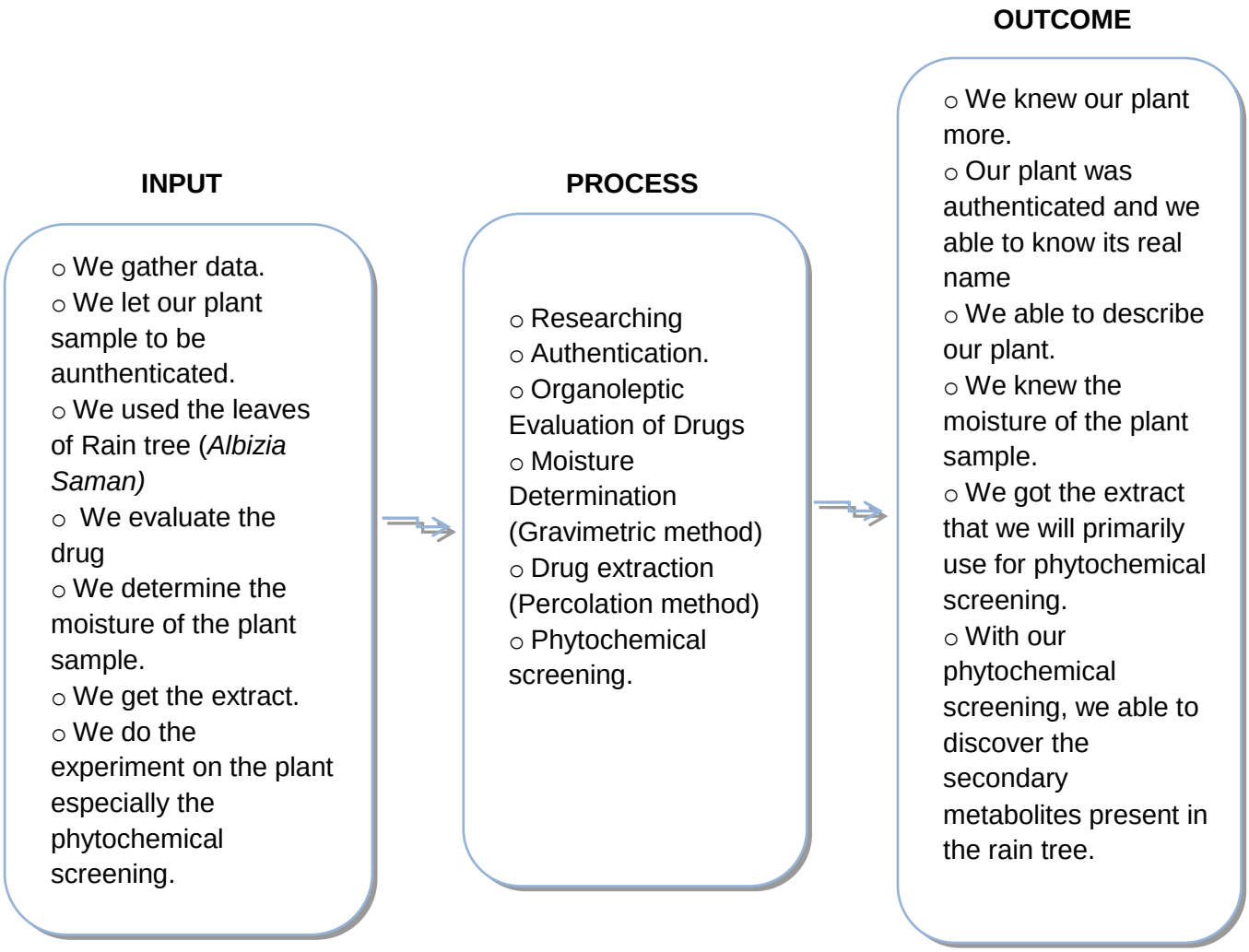
All three of these drug catalories list a plethora of possible and, in many cases, likely side effects. These include abnormal weight gain, headache, excessive sweating, upset stomach,

diarrhea, sleep disturbances, drowsiness, tremor, weight loss more often than weight gain, and decreased libido. In addition some may predispose one to feelings of apathy, cognitive impairment, sudden irrational bursts of violent rage, and suicide. Expectant mothers generally should avoid their use during the 3rd trimester of pregnancy because of adverse reactions on the infant after birth.Albizia is thought to enhance all aspects of neurotransmitter secretion and regulation. With thousands of years of traditional use, albizia is a terrific antidepressant and anti-anxiety herb with no known side effects. However, because of its blood-moving properties, it is contraindicated for use during pregnancy.

Theory:

Albizia is used as antidepressant, though there no research tells that albizia has an adverse reaction to people.

1.3 Paradigm of the Study



1.3 Figure 1: Paradigm of the study

1.4 Statement of the Problem

1. Are there any secondary metabolites present in Rain tree (*Albizia saman*)?

2. Is alkaloid present in *Albizia saman* ethanolic leaf crude extract?

3. Is unsaturated sterols and triterpenes present in *Albizia saman* ethanolic leaf crude extract?

4. Is flavonoid present in *Albizia saman* ethanolic leaf crude extract?

5. Is saponin present in *Albizia saman* ethanolic leaf crude extract?

6. Is anthraquinone present in *Albizia saman* ethanolic leaf crude extract?

7. Is cynogenic glycoside present in *Albizia saman* ethanolic leaf crude extract?

1.5 Hypothesis

For the screening of alkaloids:

For modified mayers test, the result is negative because there's no production of precipitate. And also for valser's the result is negative same as the first test there has no production of precipitate. Even in the wagners test, there's no production of precipitate.

Screening for unsaturated sterols and triterpenes.

Liebermann test, the result is negative, it because the color is same as the original color of the plant sample. In short there's no color change. In salkowski, the result is negative it because there's no color change same as the first test. The original color turned to other color that not related to the positive result.

Screening for steroids.

The kedde reaction- there no available reagent, although on the keller kelliani test, the result is negative because there is no color change that occured.

For the screening of saponins, the first test is the froth test, the positive result is formation of froth and foam, but there no formation of it thatoccured. On the hemolysis test, there's no reagent available.

For the screening of tannins and phenolic compounds.

The test A and B's reagents are unavailable. And unfortunately, it is also not researchable. Although in test C, the positive result is greenish black, there is no change in color.

1.6 Significance of Study

Why are we making this experiment or specifically phytochemical screening? This study is aiming to produce or discover a plant that would help everyone. We are making it to make our plant sample more important than anyone can imagine that it can be a medicinal drug that would help everyone living. We would like to know if it contains some secondary metabolites. By doing so, we will know its properties that may be cure to a specific or multiple disease.

Every secondary metabolite corresponds to a specific use or maybe an important medicinal use. One of the secondary metabolites that we will test is alkaloids. If it will be positive, then it may be a stimulant or anti-depressant. They are some of the phamacologic uses of alkaloids that take an important role as we live. The findings of the study will rebound to the benefits of society considering that drugs play an important role in this generation. Just like what I've said earlier, in terms of our global problem, depression is the most common. Many people with depression are trying to kill themselves and it is because of too much thinking on something that bothers them. If this study will be successful, it means that we'll be able to do something.

The aim of this study is to examine the level of knowledge of the student of University of Makati from College of Allied Health Studies, Center of Pharmacy about Phytochemical screening that would determine the secondary metabolites present in our plant sample that may be an anti depressant, anti insomnia or for heart diseases treatment.

Some of study will also be beneficial to the following:

To the Pharmacist, this study will serve as an information material for them to become aware about the possible herbal drug to be used for insomnia, depression or heart diseases for the society. This will also prompt them to a comprehensive patient counseling as one of the ways to prevent the said diseases by an alternative medicine and to motivate and educate the patient for full compliance.

For the Future Pharmacists, this study will give insights and awareness about the possible herbal drugs for depression, insomnia and heart diseases, who in turn can help the country to prevent the increasing rate of patients with tumor, depressed persons, patients with heart disease and persons with insomnia.

For the Public, this study will aid on designing a strategy to address the issues on their knowledge and awareness about an alternative medicine that can help them if they have the said diseases like depression and insomnia.

For the Community, this study will serve as a basic study for them to become aware of the medicinal plant or herbal drug that may help them

For the Future Researcher, this will serve as a simple guide to their study that is related to phytochemical screening. With this, they'll know the right process.

1.7 Scope and Limitation

This study was conducted in the University of Makati specifically in the university laboratory. The duration of the study for doing the phytochemical screening is almost ten days. This study aims to discover if there is any secondary metabolite present in *Albizia saman* that can have a corresponding pharmacological use.

We are doing our very best but we will not deny that we and this study itself have weaknesses. One and the major problem or weakness that we encountered is the lack of reagents that we need for phytochemical screening. Because of this, we unable to discover if there is any metabolite present.

For our plant, our problem is that our plant is a tree that even if it is easy to grow, it will still take time.

CHAPTER II

Related Literature and Studies

We have researched 3 screenings about *Albizia saman*. The first screening is entitled "The study of Antioxidant and Organ Protective Effects of Leaves of *Albizia saman*" This study was designed to determine the concentration of total polyphenolic and flavonoidal contents, antioxidant, hepatoprotective, nephro-protective and gastro-protective effects of bark of *Albizia saman* and it is conduct in AMERICA.

The second one is entitled "Chemical and Biological Investigation of *Albizia saman*" These support the traditional uses of this plant in various infectious diseases. The plant can be further screened against various diseases in order to find out its unexplored and it conduct in INDIA.

And the last one is "Toxicity and Tolerance of *Albizia saman*" Germination rate of *Albizia saman* showed that increased concentration of different metals from 25 to 100 ppm. Tolerance indices determined for different metal illustrated that increasing concentrations of metals reduced the tolerance of *A. saman* but this reduction was more prominent for Pb and Cd as compared to Cu and Zn treatments and it conduct in PAKISTAN.

Between our study and the other 3 studies that we got, there are similarities, differences and uniqueness.

If we will compare our study in their studies, they have some similarities like doing the screening of flavonoids, to establish the pharmacological study of plants and finally are to successfully make a drug for us. In terms of differences, there are lots of contrasts between

our study and in the 3 studies that we had researched. First one is the screening of alkaloids, unsaturated sterols and triterpenes, steroid, saponin, tannins and phenolic compounds, anthraquinones heterosides and screening of cynogenic glycosides, the other one is their objectives, procedures and result and the last one is the part of the plant we used in the screening. The uniqueness between our studies is that we exploit lots of screening with a positive result than what they did.

CHAPTER III

METHODOLOGY

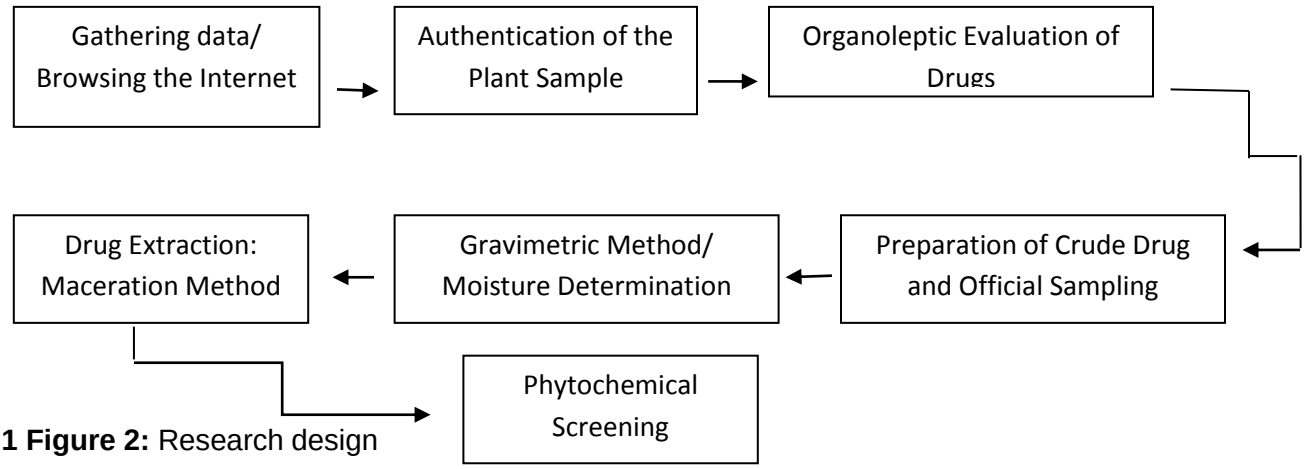
3.1 Research Design

How did we really start this experiment? Of course, like every other experiments, we started by making several researches. We started by using some of the studies made by others from our chosen plant sample that may be and hopefully be a drug someday. By doing so, we knew our plant more. We saw its real characteristics more than what we are seeing and more than what we know about them. Making or doing a research is really important for this is the very start of any experiment. It is to have knowledge and to know what you are aiming to do. We are aiming to have an effective drug; a crude drug. But before that, we should have enough knowledge. We should know what we should do. We should know what our plant is. We should know what a crude drug is.

We should have a good research but the very big question there is “HOW?”? How will we have a good research? How will we research? Now what we do is what I’ll talk about. We started collecting information by having an authentication for our chosen plant sample. We went to our very own National Museum for us to confirm the information that we have about the plant. After that, we collected information in the internet. It is already 2015 and the technology is really beyond our expectations before; because of this, we able to collect enough information about our plant that we really didn’t know before. All of us, members of the group were assigned by the leader to collect information and to have enough of it. It is because if only one of us researched, I bet we will not have enough information for our experiment. This also helped us to know our plant more. Now, how did we collect?

We firstly search for the background of our plant sample. We searched for its official name, other names, botanical source of the plant. Cultivation and distribution of the plant, description of the plant, reported constituents, and especially reported uses. With this plant, we are hoping to make a crude drug so we also research information about crude drugs and how to make them. We should know its definition, description and process on doing so. We searched about the steps involved in the preparation of a drug for commercial uses. We also collect information about the methods of drying drugs. We did all of these researches though we all know that it is not the end. We should not just rely on those. We should not be contented about the others' study that we just got in the internet about our plant that will hopefully be a drug. We should also collect information by actually doing it.

By actually doing it, we firstly observe the plant personally or by doing an organoleptic evaluation of drugs. Then, we had moisture determination or the Gravimetric method. By doing so, we able to determine the moisture content of the plant sample and understand its significance. After those things, we then did our very own experiment using ethanolic extract by maceration method. With these, finally we proceeded to phytochemical screening.



3.1 Figure 2: Research design

3.2 Research Methodology

We firstly did the maceration method to get an ethanolic extract out of our drug or plant sample that we need for the phytochemical screening. By doing so, we did the following procedure with the help and guide of our professor.

1. First, we weighed the powdered sample accurately.
2. Second, we moistened the powdered sample with 90% ethyl alcohol in order to permit the drug to absorb the liquid and swell (we make sure that the volume added is enough to permit total swelling without yielding a liquid extractive)
3. Third, we leave the moistened plant material covered for 15 minutes.
4. Fourth, we packed the moistened plant material in the percolator.
5. Fifth, we carefully added 90% ethyl alcohol (about 200 mL). Then, we macerated it for less than 48 hours.
6. Sixth, at the end of the maceration period, we collected the percolate in a tared evaporating dish.
7. After that, we concentrated the extract to about 40 ml.
8. Then, we measured the volume of the extract.
9. Finally, we determined the percentage of the extractive obtained.

After having the ethanolic extract, we then proceeded to phytochemical screening to be able to know the different secondary metabolites present in our chosen plant sample. We did the following tests or procedure; of course, still with the help of our professor.

Screening for Alkaloids

For the screening of alkaloids, we firstly evaporated 70 ml of the 95% ethanolic extract to dryness on a steam bath. Then we dissolved the residue in 7mL of 1% hydrochloric acid, aided by warming on the steam bath for 1-2 minutes. After that, we cooled, filtered and then adjusted the volume of the filtrate to 7mL by washing the residue on the filter paper with a sufficient quantity of 1% hydrochloric acid. Then, we add a few grains of powdered sodium chloride to the filtrate. After doing so, we shake it then refilter.

We then placed 1mL of the filtrate into each 4 small test tubes. To the first test tube, we added 3 drops of Modified Mayer's reagent (Mercury Potassium Iodide t.s); to the next, we added 3 drops of Valser's reagent (Mercury iodide t.s); to the third, we added 3 drops of Wagner's reagent (Iodine and Potassium iodide t.s) and to the last, we added 3 drops of Bouchardat's Reagent (2% iodine in 4% potassium iodide).

Positive Result: Production of precipitate

C. Screening of Unsaturated Sterols and Triterpenes.

We evaporated 30 mL of the 90% ethanolic extract to dryness on a water bath. Then we cooled the residue to room temperature and added 15 mL of light petroleum ether. We mixed it well then filtered it. After that, we repeated with additional volumes of petroleum ether as needed until the last volume of petroleum ether is colorless. We then combined the ethereal filtrates. We also set aside the defatted residue for screening for flavonoids and leucoanthocyanins.

We evaporated the combined ethereal filtrates to dryness and then dissolved the residue in 15 mL chloroform. Then, we dried the chloroformic solution over anhydrous sodiium sulfate, filtered and divided the filtrate equally into three dry test tubes. These tests are essentially dehydration reactions and therefore moisture must be excluded in each experimental step.

1. **Liebermann-Burchard test-** To 5 mL of the filtrate in suitably dry test tube, we added 0.3 mL of acetic anhydride and then, we mixed it gently. We added one drop of concentrated sulfuric acid. After observing the color change over 60 minute period, we run the test concurrently with 5 mL portions of standard solutions prepared from plants known to contain unsaturated sterols and triterpenes.

Positive Result: Color changes

2. **Salkowski Test-** We transferred 5 mL of the filtrate to a dry test tube and performed a ring test with concentrated sulfuric acid. Then, we shook it after 1-2 minutes and noted the color change. A cherry-red color is indicative of the presence of unsaturated sterols.

Positive Result: Color change/ cherry-red ring color.

3. **Color control-** we added 5 ml of the filtrate to the third test tube. We added no reagents. We used the test tube to serve as color control for both tests.

D. Screening for Flavonoids

Note: Because of lack of reagents, we UNABLE to perform this screening. However, we'll still indicate its procedure.

Dissolve the defatted residue from section C in 30 ml of 50% ethanol filter and place 1-2 ml of the filtrate in each of three test tubes.

To test tube #1, add 0.5 ml of concentrated hydrochloric acid and warm it in a steam bath for about 5 minutes observe the color changes. The development of a red-violet color is indicative of the presence of leucoanthocyanins. Color formation may be slow. If the color is not immediately apparent, allow the test solution to stand at room temperature for 1 hour before recording the result as negative.

Positive Result: Color change/ development of red-violet color

To test tube #2, add 0.5 ml of concentrated HCl and 3-4 magnesium turnings. Observe carefully for a color change (to green, red, etc.) within 10 min which is indicative of the presence of flavonoids. If a definite color is formed, cool and dilute with an equal volume of water and add 1.0 ml of octyl alcohol. Shake and allow to separate. The color in the octyl layer is due to aglycones while the color in the aqeous layer is due to glycosides.

Positive Result: Color change/ green, red, etc.

E. Screening for Steroid (Cardioactive) Glycosides

1. Presence of unsaturated sterols (Liebermann- Burchard test) - we used the result in section C.1.

2. Presence of unsaturated lactones- since the following three test involve color reactions, it is necessary to run concurrent test with the control sample.

a. Kedde reaction

(Note: Because of lack of reagents, we UNABLE to perform this screening. However, we'll still indicate its procedure..)

To 5 ml of the 95% etahanolic extract in the evaporating dish, add 5 ml of kedde reagent (2g to 3,5dinitrobenzoic acid in 100 ml of ethanol) and mix well with a glass stirring rod. To the mixture, add 2 ml of 1N NaOH. Mix and observe color development. A purple color is positive indication of the presence of the unsaturated lactone ring.

3. Presence of 2-deoxysugars (Keller Kelliani test)- We placed 10 ml of the 95% ethanolic extract in an evaporating dish and dried it in a steam bath. Then we added 3ml of the ferric chloride reagent (mixed 0.3 mL of 10% ferric chloride solution with 50 ml of glacial acetic acid). After that, we stirred it to mix well, and transferred to a small test tube. With the test tube held at 45^ angle, layer 1 ml concentrated sulfuric acid by allowing it to the down the inside wall of the test tube. We avoided shaking and agitating to the test tube. Then we observed for a purple ring at the interface which would indicate the presence of 2- deoxysugars.

Positive Result: Purple ring color

F. Screening of Saponin

a. Froth test- We took a volume of the alcoholic extract. For control, we used 2ml of 10% gugo extract (prepared by extracting 1g gugo bark with 10ml of ethanol in a separated test tube). Then we added 10 ml of distilled water to each test tube. After that, we put a stopper and shake the tubes vigorously. We alllowed it to stand for 30 minutes before observing it.

Positive Result: Formation of froth and foam

b. Hemolysis Test

Note: Because of lack of reagents, we UNABLE to do this test. However, I'll still indicate its procedure.

Obtain the blood agar plate, using a small test tube, remove a minicup of blood agar from three areas of the plate which are equidistant from one another. Number each agar cup at the bottom of the inverted plate with a marking pencil. With a small pipette, add enough plant extract (the aqueous solution prepared in the froth test may be used) to one of the agar cups to fill it. Fill the second agar cup with the same volume of gugo extract, and the third agar cup with distilled water. Allow the plate to stand undisturbed. After an hour, observe the agar plate for any clear zone and the three agar cups. Measure the diameter of the halo in mm.

G. Screening for tannins and phenolic compounds.

We evaporated 100 ml of 95% ethanolic extract to dryness on a steam bath. Then, we removed the evaporating dish from the steam bath and added 25 ml of hot distilled water to the residue. We mixed it well with a stirring rod and allowed it to cool to room temperature spontaneously. We centrifuged the cooled extract for several minutes and decanted the upper half from each tube used. After that, we added 3 drops of 10% sodium chloride solution to the decanted supernatant. Precipitation at this point is indicative of salting out reaction probably due to non-tannin components. Filter off any precipitate. Add 3 ml of filtrate to each of three test tubes. To tube #1, add 3 drops of 1% gelatin solution, to tube #2, add same amount of gelatin salt reagent (1% gelatin, 10% sodium chloride); and tube #3 add several drops of ferric chloride T.S.

The absence of a reaction with ferric chloride indicates the absence of tannins and phenolic compounds. A greenish-black color after the addition of ferric chloride is and correlated with precipitation on the gelatin-salt block test indicates the presence of tannins of the cathecol type. A blue black color after addition of ferric chloride is and correlated with precipitation on the gelatin salt- block test indicates the presence of tannins of the pyrogallotype. A negative gelatin salt-block test associated with color production after the addition of ferric chloride indicates the absence of tannins and the presence of other phenolic plant constituents.

Positive Result: A. 1% gelatin- production of precipitate

B. Gelatin-salt reagent- production of precipitate

C. Ferric Chloride test- greenish blue or greenish black

I. Screening for Anthraquinone heterosides

A. **Borntrager test**- transfer 5 ml of the 95% ethanolic extract to an evaporating dish and dry over a steam bath. Defat the residue in the dish with 5-10 ml of petroleum ether. Add 50 ml distilled water to the defatted residue, mix well, and filter into small separatoryfunenel. Add 10 ml of benzenes, shake to mix well, allow the two phases to separate. Drain out the aqueous layer (bottom layer) and transfer the benzene phase (upper layer) to a test. Introduce 5 ml of ammonia t.s mix well and observe the benzene layer for color change.

Positive Result: color change/ red color

B. **Modified Borntrager Test**- heat 0.3 g of the plant powder with 10 ml of 0.5 N. Potassium hydroxide and 1 ml of diluted hydrogen peroxide for 10 min. cool, filter and acidity 5 ml of the filtrate with approximately 10 drops of glacial and partition with 10 ml of benzene. Filter the benzene phase and transfer 5 ml to a test tube containing 2.5 ml of ammonia. Mix well and observe for color changes.

Positive Result: color change/ pink color

J. Screening for Cynogenic Glycosides

Guignard Test- place 2-5 g of the crushed plant sample in a test tube. Moisten with water and add few drops of chloroform to enhance enzyme activity for a firm stopper on the tube, use cork from which is suspended a piece of picrate paper. The paper strip must nottouch the inner sides of the test tube. Warm the tube at 35-40^c or keep it at room temperature for 3 hours. Observe any change in color of the paper. The

appearances of various shade of red within 15 minutes is a measure of relative concentration of cynogenic glycosides. If no color is observed after 3 hours, absence of the glycosides is indicated.

Positive Result: appearance of various shades of red of the picrate paper within 15 minutes

3.3 Data Gathering Tools

We used internet, books and other studies rendered to collect data.

Laboratory apparatuses are used such as

- Erlenmeyer flask,
- test tube,
- evaporating dish,
- water bath,
- desiccator,
- oven
- etc.

In gathering our materials, we first authenticated our plant at the National Museum of the Philippines. Healthy plant samples were gathered at Bonifacio Global City, Philippines on October 2015.

3.4 Data Gathering Procedure

It is really hard to collect data especially if you don't even have a single idea though just like everyone is telling, nothing is impossible if you really want to know something; if you really want to do it. In this experiment, in this study that we are conducting, it is important that you know how to gather data. You should know how to collect data, what data you should collect, and most importantly, you should know where will you get your data and how will you gather it.

The number 1 tool that we are using is our laboratory manual. It is because this manual will teach us things that we should do. It shows the procedures that we should make. Aside from those, we are considering it as a very essential thing for this is our guide on doing the experiment and on understanding it.

For gathering information, we mostly rely by proper and wise researching in the internet. What is proper and wise researching? It is the proper and wise way of getting information. Just like what I've said technology is already beyond our expectations before. We can now easily find and get information in the internet in just one click but the question is, will you trust that information? Can we really rely on those things? We don't really know but we can do this proper and wise way of getting information. We are not sure if the things that we are researching are all true that is why we should thoroughly observe, know and conclude things before we get that information.

3.5 Treatment of Data

After we finally collected all of the data that we need, we compile it and briefly analyzed it. We made statistical representations for us to properly make our conclusions that will be the essence of our research.

CHAPTER IV

RESULTS AND DISCUSSIONS

SCREENING FOR ALKALOIDS	RESULTS
Mayer's reagent	Positive
Valser's reagent	Positive
Wagner's reagent	Positive
Bouchardat's reagent	UNAVAILABLE
SCREENING FOR UNSATURATED STEROLS AND TRITERPENES	RESULTS
Liebermann-Burchard test	Positive
Salkowski test	Positive
SCREENING FOR FLAVONOIDS	RESULTS
Test tube #1	UNABLE
Test tube #2	UNAVAILABLE
SCREENING FOR STEROID (CARDIOACTIVE) GLYCOSIDES	RESULTS
Presence of Unsaturated Sterols	Positive

Presence of unsaturated lactones:	
Kedde Reaction	UNAVAILABLE
Presence of 2-deoxysugars (Keller Kelliani Test	Positive
SCREENING FOR SAPONIN	RESULTS
Froth test	Positive
Hemolysis Test	UNAVAILABLE
SCREENING FOR TANNINS AND PHENOLIC COMPOUNDS	RESULTS
1% gelatin	UNAVAILABLE
gelatin-salt reagent	UNAVAILABLE
Ferric chloride	Positive
SCREENING FOR ANTHRAQUINONE HETEROSIDES	RESULTS
Borntrager test	UNAVAILABLE
Modified Borntragers test	UNAVAILABLE

SCREENING FOR CYNOGENIC GLYCOSIDES	RESULTS
Guignard test	UNAVAILABLE

4.0 Table 1: Results

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Exercise 13
(Organoleptic evaluation of Drugs)

(underground parts)

Shape: VASE, SPREADING
Odor: NONE
Taste: BITTER

(stem)

Shape: branches droop; not showy; typically multi-trunked; thorns
Size: Height: 15 to 25 feet
Spread: 25 to 35 feet
Odor: unknown
Taste: acid taste (sour)

(leaves)

Size: less than 2 inches
Shape: oblong, lanceolate
Color: green
Odor: unknown
Taste: bitter

We defined the parts of our plant to be use on the phytochemicals specifically the odor, taste, color, and size.

Exercise 14

(Backround of the Plant, Preparation of Crude drug and Official Sampling)

Backround of the plant:

The official name of our plant sample is Rain tree and its other name is Mimosa tree. The part of a plant that use for our phytochemical screening is its leaves.

Scientific name is *Albizia saman* from family of fabaceae.

We collect the plant part we will use in our phytochemical screening which is its leaves and we wash it to remove the extraneous parts (dust, dirt). We wipe the leaves with a clean cloth to make it dry. We place it on oven for five hours at 105˚c until the leaves turns to brittle.

Exercise 15

(Moisture Determination)

We determine the moisture content of the plant drug sample and understand its significance.

Procedures:

Weigh the pulverized sample 5grams in an evaporating dish. Then we record the weigh. We dry the drug for one hour, put on a desiccator and weigh again. Then repeat the drying and weighing at an interval of one hour until the difference of two successive weights does not exceed on 0.25% (0.0025).

Exercise 16

(Drug extraction: Percolation Method)

This exercise aims to get an extract from our plant sample. We learn about this activity the principle of percolation as a method of drugs extraction. The procedures are: weighing the powdered sample accurately. Then, we put the 80% ethyl alcohol on a graduated cylinder then we add water to make an 80% ethyl alcohol solution. Then to the 80% ethyl alcohol solution add 100mg plant powder and leave the moistened plant for 15 minutes. After 15 minutes. We carefully added 80% ehtylalcohol (about 100ml) and allow to macerate for less than 48 hours. After the maceration, collect the percolate in a tared evaporating dish. And concentrate the extract to 40ml and above.

We have 2 way of extraction: first is the maceration and the second is percolation.

Exercise 17

(Phytochemical Screening)

We learned about the different active constituents present in our plant sample which is the *Albizia saman.*

Here are the results of all the screening test:

Screening for alkaloids:

Modified Mayer's: the result is positive, It is because there is a production of precipitate.

Valser's: the result is positive, It is because there is a production of precipitate

Wagners: the result is positive, It is because there is a production of precipitate.

Bouchardat's: NO REAGENT AVAILABLE.

Screening for unsaturated sterols and triterpines:

Liebermann: the result is positive. It is because of the color changes

Salkowski: the result is positive, It is because of the color change, the orginal color is dark green but when we put the concentrated sulfuric acid it produce a cherry-red ring color.

Screening for flavonoids- UNABLE TO DO THE TESTS

Screening for steroid:

Kedde reaction: UNABLE TO DO THE TEST

Keller kelliani: the result is positive; it is because of the color changes. The original color is dark green, then when we mix 0.3ml of 10% ferric chloride solution with 5oml of glacial acetic acid. The dark green turns to purple ring color.

Screening of saponin:

A. **Froth test**: the result is positive it is because of formation of froth and foam.

B: **Hemolysis test**- UNAVAILABLE REAGENT

Screening for tannins and phenolic compounds.

A. 1% gelatin- UNAVAILABLE REAGENT

B. gelatin-salt reagent- UNAVAILABLE REAGENT

C. Ferric chloride test- The result is positive since after the test, it turned to greenish black color

We UNABLE to do the screening for anthraquinone heterosides and screening for cynogenic glycosides because of unavailable reagents.

Note: We want to just research the results of the tests that we unable to do but they are not researchable.

5.2 CONCLUSION

The objective of phytochemical screening is to know if there is any secondary metabolite present in *Albizia saman* ethanolic leaf crude extract and to discover what are they.

By doing the experiment, we knew that in *Albizia saman* ethanolic leaf crude extract, alkaloids, unsaturated sterols and triterpenes, steroid glycosides, saponin, and tannins and phenolic compunds are all present as secondary metabolites.

With all of these information and results that we got with our experiments, we therefore conclude that there are active secondary metabolites present in *Albizia saman* ethanolic leaf crude extract.

5.3 RECOMMENDATION:

The bark and the flowers of albizia are used as a calming sedative in Oriental traditional medicine.Categorized in the Chinese MateriaMedica as a calming spirit herb, the bark is thought to 'anchor'the spirit, while the flowers lighten it. The flowers have also been used for the treatment of insomnia, amnesia, sore throat, and contusion in Oriental traditional medicine (Kang, et al) as well as depression, melancholy and anxiety.

Considering the proliferation of antidepressant drugs throughout the Western world with their increasingly recognized adverse effects, it's wonderful that nature has, in abundance, a safer and better alternative probably growing in close proximity to one's doorstep. In my opinion, albizia offers a more profound effect in treating depression and anxiety than the two most commonly promoted herbs, St Johnswort (*Hypericumperforatum*) and Kava (*Piper methisticum*) and thus should be more widely used. Also natural remedies such as this can be a game changer when it comes to the pharmaceutical world. if utilized properly this plant can revolutionize the remedies for anti-depressants.

Case Reports:

I have given a 5 to 1 alcoholic extract of albizia to many patients for depression, anxiety and insomnia. Usually I prescribe about a half-teaspoon of the extract three times a day and, for more severe cases, up from a teaspoon to a tablespoon three times daily. I also prescribe a 5 to 1 dried powdered extract.

A woman age 54 who had suffered from severe feelings of negativity and depression most of her life, was recommended by a friend, who was already taking antidepressives, to see his psychiatrist. This woman certainly had many reasons for her depression and would benefit from psychotherapy. However, many patients go to a particular psychologist as this woman did, expressly to be evaluated for antidepressant medication.

Unfortunately, like many psychiatrists, this therapist had a reputation for clearing clients, for spending little time with his patients and sending them off with an antidepressant prescription. Case in point, this woman spent a total of seven minutes with the psychiatrist and was sent home with a prescription for one of the Serotonin uptake inhibitor antidepressants. No advice was given as to whether counseling and some lifestyle changes might be a better choice than the drug.

I suggested that she hold off on taking the drug, focus on a few tough decisions and lifestyle changes and that she take a half teaspoon three times daily of a 5:1 extract of Albizia. She reported that after three or four days, she noticed a marked improvement with greater mood stability, and has continued to use the albizzia flower extract rather than use the drug.

Another man, age 43, had a history of episodes of chronic depression, with accompanying morbid thoughts. His wife asked if there was anything I could give him that would help. I gave the same albizia flower extract, but started with a teaspoon of the extract three times daily and after a week, stepping it down to a half teaspoon. He has been on the albizia extract for several months and has reported that while he occasionally has some down moments, they are not nearly as severe as they were previously.

As well as giving albizia to many patients suffering from acute and chronic depression and anxiety I've also given it to those who complain of high stress, with noticed marked improvement '"even after a single day of use. While there are undoubtedly many individuals who will require stronger medication (and for these pharmaceuticals may be of value), albizia is a good choice for probably greater than 50% of those who are presently taking a pharmaceutical drug. At a mere fraction of the price, albizia is devoid of the adverse side effects of the drugs and can be easily stopped at anytime. It seems reasonable to conclude that before one resorts to the use of drugs, that nature's own gift from the 'tree of collective happiness should be given a try instead

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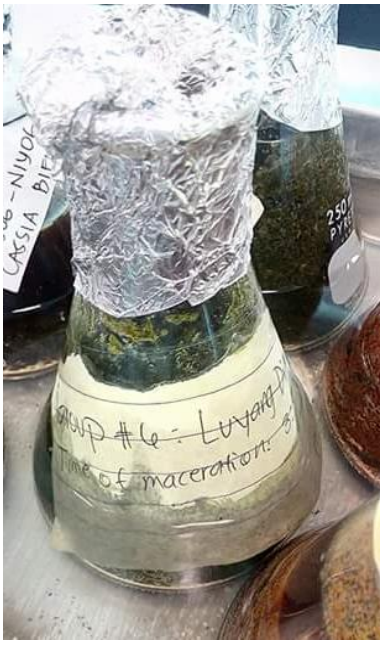
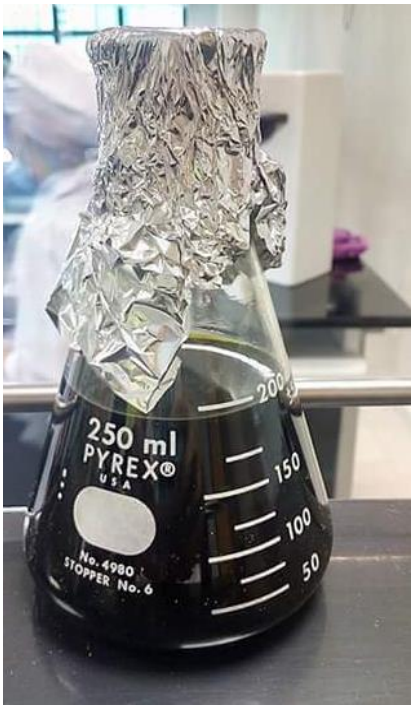
APPENDIX A

ORGANOLEPTIC EVALUEATION OF

MOISTURE DETERMINATION

DRUG EXTRACTION: PERCOLATION METHOD



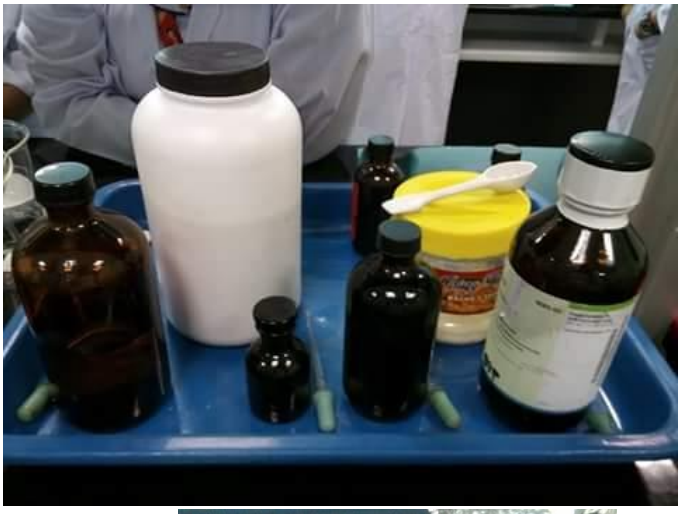


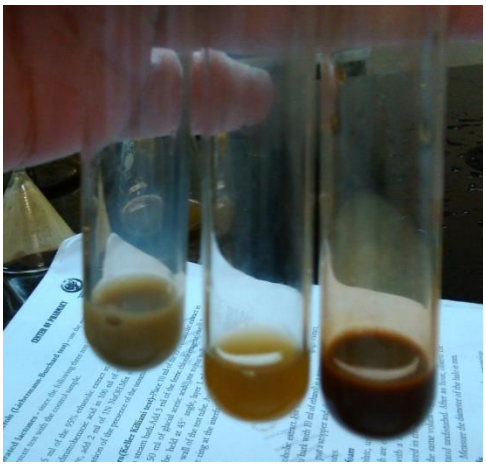

200 ml
±5%
150
100
50
250 mL
PYREX
200
150
100
50

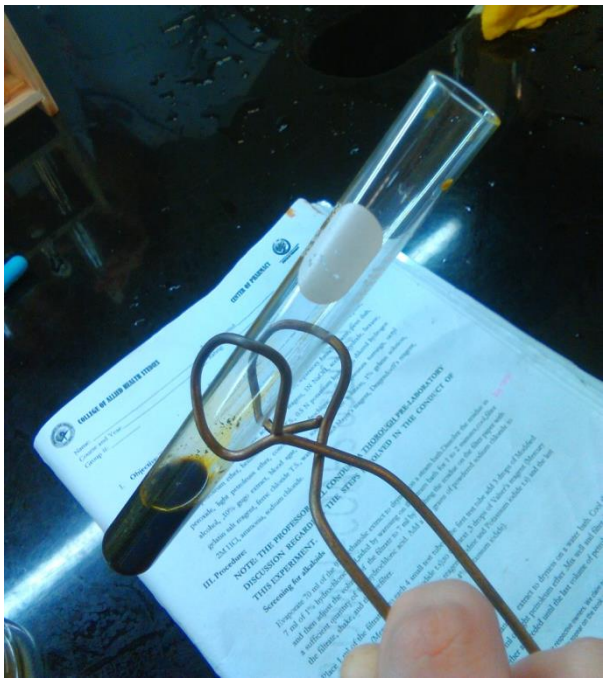

TRIPLE BEAM BALANCE
OHAUS

REPUBLIC of the PHILIPPINES
City of Makati
UNIVERSITY OF MAKATI
COLLEGE OF ALLIED HEALTH STUDIES
CENTER OF PHARMACY

PHYTOCHEMICAL SCREENING







APPENDIX B

NATIONAL MUSEUM
PAMBANSANG MUSEO

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 : **AZEL JOY CAMACHO**
JAMES VALDWIN LANOZA
SCHOOL/OFFICE/INSTITUTION : University of Makati
ADDRESS : Makati City
PURPOSE : Research

Specimen Number	Family	Scientific Name
01	FABACEAE / LEGUMINOSAE	*Albizia saman* (Jacq.) Merr. (Syn: *Samanea saman* (Jacq.) Merr.)

☐ Determined by:
■ Verified by:

DANILO N. TANDANG
Museum Researcher II
Botany Division

Date: Oct. 9, 2015
Control Number: 15-10-539
O.R. No.: 6075571

CURRICULUM VITAE

Personal information:

Name: Larioza James Valdwin A.

Birthdate: May 31,1998

Birthplace: Makati city

Address: 178 Molave St. Cembo Makati City

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name: Fe Larioza ,

Jaime Larioza

Academic Background:

Tertiary: University of Makati

Secondary: Fort Bonifacio High School

Primary: Cembo Elementary School

CURRICULUM VITAE

Personal information:

Name: Camacho,Azel Joy

Birthdate: January 06, 1999

Birthplace: Makati City

Address: Southside Makati City

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name: Maricel Camacho ,

Adel Camacho

Academic Background:

Tertiary: University of Makati

Secondary: Fort Bonifacio High School

Primary: Andres Bonifacio High School

CURRICULUM VITAE

Personal information:

Name: Isaguirre, Angeline Kaye

Birthdate: November 15, 1998

Birthplace: Taguig City

Address: #18-a Mangga St. Zone 1 North Signal Village Taguig City

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name: Evangeline Isaguirre,

Ardel isaguirre

Academic Background:

Tertiary: University of Makati

Secondary: Signal Village National High School

Primary: Em's Signal Elementary School

CURRICULUM VITAE

Personal information:

Name: Hannalhay Gaden

Birthdate: June 16, 1999

Birthplace: Pasay City

Address:#74 St. Joseph Barangay 201 Pasay City

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name:

Academic Background:

Tertiary: University of Makati

Secondary: Kalayaan National High School

Primary: Kalayaan Elementary School

CURRICULUM VITAE

Personal information:

Name: Shela Mae Caritos

Birthdate: September 25, 1997

Birthplace: Bicol

Address: Western Bicutan Taguig City

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name: Corazon Caritos,

Arnold Caritos

Academic Background:

Tertiary: University of Makati

Secondary: San Ramon High School

Primary: San Agustin Elementery School

CURRICULUM VITAE

Personal information:

Name: Alyssa Rizaldo

Birthdate: September 02,1998

Birthplace: Cavite City

Address: Blk 18 Lot 12 p2b Elisa Homes, Molino 4 Bacoor Cavite

Civil status: Single

Religion: Catholic

Nationality: Filipino

Parent's Name:

Academic Background:

Tertiary: University of Makati

Secondary: Nergos Occidental High School

Primary: Rizal Elementary School