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Potential Properties of Himbabao (*Broussonetia Luzonica B.*) As Anticancer, Antifungal and Antimicrobial

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Abstract: Plants are known for its holistic use such as producers of food, clothes, and others for animal and human consumption and a component for medicinal practices. They have been an important source of drug products since ancient times, containing secondary metabolites which are necessary for the survival of the species (Choa JB D. et al, 2016). Nowadays, plants like Himbabao still cope up to the world's metamorphosis, thus discovering their potentials to be a treatment for some diseases. According to Philippine Statistics Authority (PNA), the second highest cause of mortality was cancer with 60,470 or 10.4% recorded cases in 2016. Likewise, according to World Health Organization (WHO, 2019), pneumonia is the single largest infectious caused by a number of infectious agents, including viruses, bacteria, and fungi that led to the 808,694 death cases of children under the age of 5 in 2017. This study primarily aimed to determine possible uses of Himbabao plant leaves based on the abundance of phytochemicals as revealed by the phytochemical screening and the results of bioassay and experimental treatment on onion roots and mung beans. Thus, the results showed that there were carbohydrates, reducing sugars, flavonoids, tannins, glycosides, phytosterols being detected. Thus, based on the experiment, the 75% and 100% concentration inhibited the growth of onion roots. Based on several studies, flavonoids and phytosterols are anticancer agents while tannins are antifungal and antibacterial. Therefore, the study of Himbabao extract has been proven significant in the scientific community because of these reasons.

Keywords: Broussonetia luzonica, Himbabao, Flavonoids, Phytochemical screening, leaves, cancer, fungi, bacteria, tannins, phytosterols

Introduction

Plants are known for its holistic use, it is one of the numerous entities that life depends on. They also serve not only as producers of food, clothes, and others for animal and human consumption but also a component for medicinal practices. They have been an important source of drug products since ancient times. These plants contain secondary metabolites which are necessary for the survival of the species (Choa JB D. et al, 2016). In history, few plants already had significant roles in the field of medicine. Now in the modern day, plants still cope up to the world's metamorphosis, many plants were discovered to have potentials to be a treatment for some diseases like Himbabao.

Himbabao or *Brussonetia luzonica (Blanco) Bureau* is a medium-sized shed tree growing to a height of 15 meters with a trunk diameter of 30 centimeters. Bark is smooth. Leaves are alternate with a pointed apex and rounded base. Lower leaf surface is hairy. Flowers are very small, borne on long, slender, spike-like flowering branches. Inflorencences are pistillate and staminate borne on separate plants. It is commonly found in Indonesia and in the Philippines, in thickest and second growth forests, at low and medium altitudes.

Flowers (per 100g) yields water (86.8g), energy (52 kcal), protein (2.9g), carbohydrate (8.1g), fiber (1.5g), ash (1.3g), Ca (278mg), phosphorus (75mg), iron (4.3mg), carotene (300 µg), vitamin A (50 µg), and thiamin (0.06mg). Its extract yields alkaloid, flavonoid, unsaturated sterol and triterpene, steroid glycoside, cyanogenic glycoside, tannin and phenol (Stuart Jr, 2017). These phytochemicals are useful in curing different illnesses like cancer, fungal infections and pneumonia.

According to Philippine Statistics Authority (PNA), the second highest cause of mortality after ischemic heart disease is the infamous cancer. There are 60,470 or 10.4% recorded cancer cases in 2016. Cancer is a broad term. It describes the disease that results when cellular changes cause the uncontrolled growth and division of cells. Some types of cancer cause rapid cell growth, while others cause cells to grow and divide at slower rate. Certain forms of cancer result in visible growths like tumors, while others, such as leukemia, do not exhibit the same characteristic (Nall, 2018).

In addition, pneumonia was also seen as a lethal disease like cancer but most commonly occur on children. According to World Health Organization (WHO, 2019), pneumonia is the single largest infectious cause of death in children worldwide. Pneumonia killed 808,694 children under the age of 5 in 2017, accounting for 15% of all deaths of children under five years old. Pneumonia is caused by a number of infectious agents, including viruses, bacteria, and fungi. It can also be transmitted or spread through air-borne droplets from a cough or sneeze and through blood during and shortly after birth.

Moreover, fungal infections are also common throughout much of the natural world. In humans, fungal infections occur when an invading fungus takes over an area of the body and is too much for the immune system to handle. Fungi can live in the air, soil, water, and plant. There are also some fungi that live naturally in the human body. Like many microbes, there are helpful fungi and harmful fungi. When harmful fungi invade body, they can be difficult to kill, as they can survive in the environment and re-infect the person trying to get better (Johnson,2018).

Due to the presence of these diseases, the research for the possible remedies is a major goal in the medical field. The different phytochemicals present in the *Himbabao* leaves extract made the plant possible to have anti-cancer, antibacterial, and anti-fungal.

The researchers are interested in evaluating the potential phytochemical content of Himbabao plant leaves extract as organic anticancer agent and natural antibiotic against *Staphylococcus aureus, Escherichia coli* and *Candida albicans.*

Statement of the Problem: Specifically the researchers aims to; to determine if Broussonetia luzonica can inhibit the growth of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*; determine the presence of carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, glycosides, saponins, resins, phytosterols, anthraquinone, and proteins (peptide bonds); determine the possible uses of Himbabao plant leaves as anticancer agent based on the abundance of phytochemicals as revealed by the phytochemical analysis and on the test with the onion roots and mung beans.

Hypotheses

The researchers follow the null hypotheses; There is no significant difference between microbial and phytochemical analysis of Himbabao plant leaves extract against *S. aureus, E. coli* and *C. albicans* ;The leaf extract in different concentrations could not inhibit the growth of mung beans and onion roots, and; There is no significant difference between the results of the test in onion roots and mung beans as anticancer against the phytochemical content of the Himbabao plan leaves extract.

H_a: Himbabao plant leaves extract contain anti-fungal, anti-bacterial, and anti-cancer; and the extract could inhibit the growth of onion roots and mung beans.

Conceptual framework

In this study, the researchers used the Input-Process-Output (IPO) Model to present the materials, procedure, and results of the phytochemical profiling. This is a functional diagram/graph that determines the inputs, outputs, and the required processing tasks needed to transform inputs into outputs. This model is sometimes configured to include any given data that might happen in the process (Schembri, 2012)

Research Model

INPUT	PROCESS	OUTPUT
A. Gathering of Plant Source (Himbabao) B. Preparation of Extracts Washing the Leaves with Distilled Water Cutting of Leaves C. Preparation of Extract with different concentration Experimental group: Set up A -100% extract Set up B -75% extract + 25% distilled water Set up C -50% extract + 50% distilled water Set up D -Negative control group using distilled water D. Preparation of Reagents for Phytochemical Profiling D. Preparation of onion bulbs and monggo seeds	A. Microbial Test Bioassay test against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* B. Phytochemical Profiling of Himbabao Plant Extract ● Carbohydrates ● Reducing sugars ● Flavonoids ● Alkaloids ● Tannins ● Glycosides ● Saponins ● Resins ● Phytosterols ● Anthraquinone ● Proteins (peptide bonds) C. Experimentation -experimental group -control group Negative control group	Results A. Antimicrobial Property and Possible uses of Broussonetia luzonica plant leaves. Results showed that Himbabao plant leaves did not inhibit the growth of *Staphylococcus aureus, Escherichia coli,* and *Candida albicans.* B. Phytochemical Analysis Phytochemical Profiling of plant leaves revealed the presence of : ● Carbohydrates ● Reducing sugars ● Flavonoids ● Tannins ● Glycosides ● Resins ● Phytosterols However, alkaloids, saponins, resins, anthraquinone and protein are absent. The chemical compounds present prove that Broussonetia luzonica (Himbabao) can be a source of medicine. C. Experimentation Result based on experiment among the 4 set ups for:

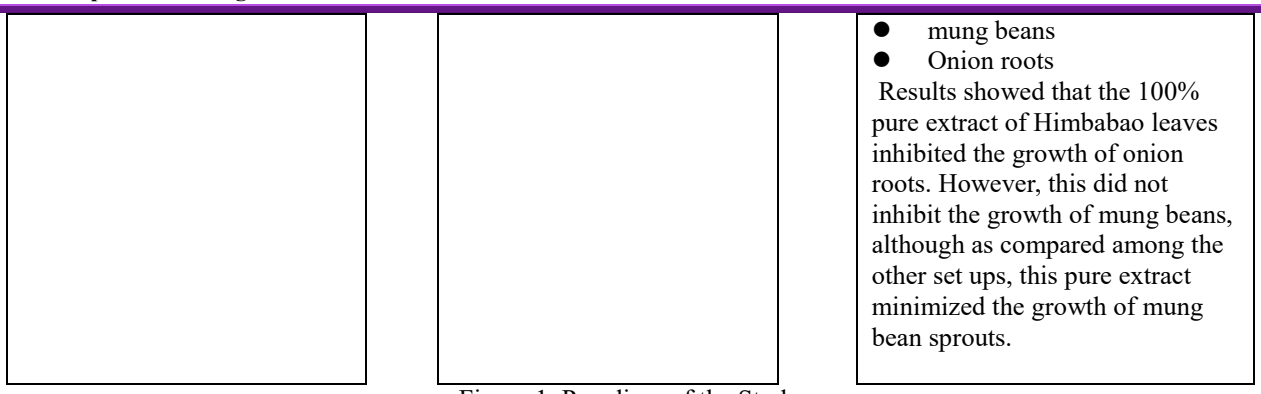


Figure 1. Paradigm of the Study

The first frame shows the inputs which include the independent variables of the research, starting the gathering of plant sources and preparation of plant extracts with different concentration. The second frame shows the process which includes phytochemical profiling of Himbabao plant extract, antibacterial assay, antifungal assay and experimentation of the different concentration of the extracts, these serve as the moderating variables of the research. The third frame reflects the outputs which describe the results of phytochemical analysis, antibacterial assay, antifungal assay and the experimental results, these serve as the dependent variables of the research.

Significance of the Study

The aim of this study is to discover a new herbal plant that could prevent/treat the fungal infections, bacterial infections, and cancer. Himbabao plant leaves will serve as the answer for the continuous fight against the said diseases. Other beneficial goal of this research is to utilize the gift of nature as organic alternatives to synthetic and commercialize medicinal products. The result of the study is seen to benefit the following:

To the Humans. This study ought to benefit the humans especially those who are experiencing the said diseases. This could raise awareness that plants can still be used as a medium of medicine for minor and even for serious illnesses.

To the Medical Field. This study can be a big help to the medical field as it provides a new information about the possible herbal plant that can prevent/treat particular diseases with the presence of

Other Researchers. This study may open a new door for further researches. This may serve as a reference for the future researchers as they conducted another study related to this research.

Scope and Limitation

This study is limited to the use of Himbabao plant leaves as a source of the extract to be tested. The researchers will not use the flowers and fruits because this plant is seasonal and only blooms during summer. Antimicrobial assay was done at MSRI at UP Diliman. This test was done to determine the potential of the extract against *S. aureus, E. coli and C. albicans.* The phytochemical properties of this plant were tested to determine only the presence of carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, glycosides, saponins, resins, phytosterols, anthraquinone, and proteins (peptide bonds).

The B. luzonica leaves were gathered in the mountain area of San Ildefonso particularly at Akle San Ildefonso, Bulacan. While the extraction was held at the Science Laboratory of Calawitan National High School. This research was started last May 06, 2019 and will end until September 25, 2019 for the school year of 2019-2020.

The extracting solvent to be used is ethyl alcohol. The extract will be tested using Phytochemical Screening and appropriate test in the College of Chemistry at UP Diliman, Quezon City. The result of the rota-evaporator from the College of Chemistry was transferred to the University of the Philippines Manila for the phytochemical analysis.

The screening shall determine the presence of each phytochemical component as absent, with traced amount, slightly detectable or strongly present. The plant was authenticated at the College of Biology at the University of the Philippines Diliman.

Review of Related Studies

The radical scavenging activity and effect on liver function enzyme marker alanine aminotransferase of the flavonoid extract from the leaves of himbabao (broussonetia luzonica) moraceae

The study conducted by Batu & De la Merced (2016) investigated the radical scavenging activity and effect in liver function enzyme markers alanine aminotransferase (ALT) of flavonoid extract from the leaves of himbabao. The antioxidant activity of the flavonoid extract of himbabao was determined through in vitro tests. The flavonoid extract of himbabao showed an antioxidant activity in concentration dependent manner when tested directly using DPPH, nitric oxide and superoxide scavenging assays. The three doses (40mg/kg/, 100mg/kg, 200mg/kg)of the flavonoid extract of himbabao were able to lower down the ALT level of the CCl4- induced rats in similar effect. Therefore, the flavonoid extract of himbabao possesses antioxidant and hepatoprotective activities.

Chemistry and Biological Activities of Flavonoids: An Overview

Most recent researches have focused on the health aspects of flavonoids for humans. Many flavonoids are shown to have

antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities. In plant systems, flavonoids help in combating oxidative stress and act as growth regulators. For pharmaceutical purposes cost-effective bulk production of different types of flavonoids has been made possible with the help of microbial biotechnology (Kumar S., and Pandey A., 2013).

Tannins and human health: a review.

Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins (Chung KT,et al.,1998)

Levels of Tannins and Flavonoids in Medicinal Plants: Evaluating Bioprospecting Strategies

To establish a quantitative relationship between the species popularly considered to be antimicrobial, antidiabetic, and antidiarrheal, the contents of tannins and flavonoids were determined. The plant selection was based on an ethnobotanical survey conducted in a community located in the municipality of Altinho, northeastern Brazil. For determination of tannin content was utilized the technique of radial diffusion, and for flavonoids, an assay based on the complexation of aluminum chloride (Siguera C. Et al., 2011).

Anticancer effects of phytosterols

In summary, mounting evidence supports a role for phytosterols in protecting against cancer development. Hence, phytosterols could be incorporated in diet not only to lower the cardiovascular disease risk, but also to potentially prevent cancer development (T A Woyengo, V R Ramprasath, and P J H Jones, 2009).

Flavonoids: Important Biocompounds in Food

Flavonoids are secondary metabolites in plants that show some desirable characteristics. These compounds can be grouped in different classes on the basis of their basic structure. It has been reported that flavonoids are important for human health because of their antioxidant, antibacterial, antiviral, and anti-inflammatory activities and because they act as free radical scavengers as they are potential reducing agents that protect from oxidative damage, which are conferred by the content of hydroxyl groups (Cruz et al, 2017).

Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins

Among the activities determined on a molecular basis are the chemical, biological and pharmacological actions such as superoxide anion scavenging, apoptosis, antitumor, anti-EVB, anti-MRSA and anti-plasmin inhibitory activities, etc., in addition to their fundamental activities, i.e., binding to proteins, large molecular compounds and metallic ions, and antioxidant activities (Okuda T., and Ito H., 2011).

Phytochemical screening of Broussonetia luzonicus (Moraceae) leaves

Other studies of other Broussonetia spp. have been reported to have antibacterial, antioxidant, antitumor, and pancreatic lipase inhibitory activity. This study sought to identify the phytochemical that the Broussonetia luzonicus leaves contain (Choa J B, et al., 2016).

Phytosterols as anticancer dietary components: evidence and mechanism of action

Epidemiologic and experimental studies suggests that dietary phytosterols (PS) may offer protection from the most common cancers in Western societies, such as colon, breast, and prostate cancer. This review summarizes the findings of these studies and the possible mechanisms by which PS offer this protection. These include the effect of PS on membrane structure and function of tumor and host tissue, signal transduction pathways that regulate tumor growth and apoptosis, immune function of the host and cholesterol metabolism by the host. (Awad & Fink, 2000).

Anticancer properties of cardiac glycosides

Epidemiologic evidence suggests that breast cancer patients who were treated with digitalis have a significantly lower mortality rate, and their cancer cells have more benign characteristics than those from patients not treated with digitalis. Interestingly, the concentrations of cardiac glycosides used for cancer treatment are extremely closed to those found in the plasma of cardiac patients treated with the same drugs, suggesting that the anticancer effects of these drugs are exerted at non-toxic concentrations. Furthermore, studies have suggested that cardiac glycosides target cancer cells selectively (Pongrakhanon V.,2013).

Evaluation of Phytochemical and Anti-mitotic Potential of Poly-herbal extract by using Onion Root Model

Anti-mitotic assay by onion root tip method was selected due to comprehensive results. Phytochemical investigation was carried out by standard procedures for investigation of phytoconstituents. Significant reduction in mitotic index was observed in Poly-herbal extract in high concentration in comparison to standard Vincristine as well as in comparison to individual plant with P value <0.01 (Annntoniya R., Kelvin B., and Ranajit DT, 2017).

Germinating seeds of the mung bean, Vigna radiata (Fabaceae), as model for the preliminary evaluation of cytotoxic effects of drugs

Cytotoxic properties of plant extracts and drugs being developed for cancer treatment are usually evaluated by a variety of in vivo nd in vitro tests carried out in animal or plant based models. In the present study we have evaluated the possibility of using the germinating mung beans (Vigna radiata), for rapid and inexpensive screening of drugs exhibiting cytotoxic properties. Mung beans were allowed to germinate either in tap water or in different rug solutions, and parameters like percent germination, increase in radicle length, change in seedling weight and mitotic index of apical root meristems were determined at two time intervals coinciding with the time at which the radicle length in control group was 1.0 to 1.5 cm (time 0,T0) and 48 h later (T48) (Kumar and Singhal, 2009).

Definition of Terms

Anti-cancer, a substance present in Himbabao leaves extract that ought to use against or tending to arrest or prevent cancer.
Anti-fungal, a property that the researchers anticipated to be present in Himbabao leaves extract to destroy fungi or to inhibit their growth.
Anti-microbial, a natural substance of Himbabao plant leaves used in destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms.
Escherichia coli, also known as E. coli, commonly found in the lower intestine of warm-blooded organisms. It is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia.
Flavonoids, are group of plant metabolites which contain 15 carbon atoms and are soluble in water. These chemicals are also used for cell cycle inhibition and said to have anticancer property.
Glycosides, are molecules formed when sugar is bound with non-carbohydrate modify, most commonly a small organic molecule that plays numerous significant role in living organisms. It is used as medications since plants store inactive glycosides that can be activated through enzyme hydrolysis that causes sugar to break apart.
Himbabao, is a medium-sized shed tree growing to a height of 15 meters with a trunk diameter of 30 centimeters. Bark is smooth. Leaves are alternate with a pointed apex and rounded base. It is usually found in mountain areas of Philippines.
Microbial Assay, bioassays designed to analyse different compounds or substances that impact micro-organsms
Phytochemicals, are chemical compounds naturally occurring in plants with health-related benefits but are not considered essential nutrients.
Phytochemical screening, process of tracing plant constituents. There is standard procedure which is usually involving color changes as indicators.
Phytosterols, related to cholesterol that are present in vegetable oils, cereals, nuts, and vegetables which reduce the cholesterol absorption and lower the plasma LDL.
Pneumonia is an infection caused by variety of organisms such as bacteria, viruses, and fungi that results to an inflammation of the air sacs of maybe one or both lungs. It may caused cough with phlegm, fever, chills, and difficulty in breathing once the air sacs was filled with fluid or purulent material or pus.
Staphylococcus aureus is a bacterial human pathogen that is commonly found in the environment or in normal human flora, located on the skin and mucous membranes, usually in the nasal area, of most healthy individuals that causes a wide variety of clinical manifestations.

Methods

A. Materials

The materials used in the study are the following:

Himbabao leaves	empty containers	petri dish	mung beans
Ethyl alcohol	mortar/pestle	plastic cups	tissue
Distilled water	dropper	sticks	weighing scale
Strainer	graduated cylinder	strings	

Procedure:

1. Authentication of plant

Himbabao plant leaves were gathered, washed, cleaned and dried for proper authentication in the College of Biology at UP Diliman, Quezon City.

2. Preparation of plant for phytochemical screening.

Fresh Himbabao leaves were gathered and a bottle of alcohol was also prepared. The leaves were chopped and weighed 1 kg of plant leaves were mixed with 1L 70% of ethyl alcohol then soaked it for 24 hours. After soaking, it was squeezed using clean piece of cloth or strainer and then the extract was set aside in a clean and sterilized empty bottle.

The leaves of *Broussontia luzonica* , also known as Himbabao, were harvested in Akle, San Ildefonso, Bulacan. Himbabao plant leaves were cleaned, washed and dried in preparation for proper authentication to be submitted to a plant taxonomist in College of Biology at University of the Philippines, Quezon City and confirmed the species as *Broussontia luzonica (Blanco) Bureau.* Another set of Himbabao leaves were cleaned washed for extraction of the plant for microbial assay. From 1 Kg of plant leaves, the researchers obtained 50 mL of pure extract by squeezing out the juice with a cheesecloth. In addition, another set of fresh Himbabao leaves were gathered and a bottle of alcohol was also prepared. The leaves were chopped and weighed 1 Kg of plant leaves which was mixed with 1L of 70% of ethyl alcohol then soaked for 72 hours. After soaking, it was squeezed by using a cheesecloth or strainer then extracted in a clean empty bottle. The extract was submitted to the College of Chemistry Analytical Analysis Division for rotary evaporation. After 10 days, the extract was submitted to the phytochemical Analysis Division of University of the Philippines, Manila for phytochemical analysis.

3. Preparation of experimental groups and negative control group

3.1 Extracts with Onion roots

The onion bulbs with an average masses and sizes were bought from the public market of San Ildefonso, Bulacan. These onion bulbs were attached in a stick through string and set in four different clean cups. Fresh Himbabao leaves were gathered, washed and dried for the preparation of extracts in different concentrations. The leaves were weighed into 200 g, 150 g and 100 g.

These leaves were chopped and set aside. The 200g of leaves was squeezed using cheesecloth to obtain pure extract of about 20mL, this was the Set up A. The 150g of leaves were chopped and mixed with 50mL of distilled water, then extracted using a cheesecloth to obtain 50mL of extract that served as Set up B. The 100g of leaves were chopped and mixed with 100mL of distilled water then was squeezed using a cheesecloth to obtain 100mL of extract that served as Set up C. For the Set up D, a negative control of distilled water was prepared of about 20mL. After preparing and measuring of 20mL of each extract, these are poured in the cup containing the onion bulb, making sure that the root part would only be dipped in the solution just to observe the effect of the extract in the growth of onion roots. The set ups were being set aside for every 24 hours observation.

3.2 Extracts with mung beans

The mung beans with an average sizes were bought from the public market of San Ildefonso, Bulacan. These mung beans were counted for 15 pieces per set and placed in a tissue in four different clean petri dish. Fresh Himbabao leaves were gathered, washed and dried for the preparation of extracts in different concentrations. The leaves were weighed into 200 g, 150 g and 100 g.These leaves were chopped and set aside. The 200g of leaves was squeezed using cheesecloth to obtain pure extract of about 20mL, this was the Set up A. The 150g of leaves were chopped and mixed with 50mL of distilled water, then extracted using a cheesecloth to obtain 50mL of extract that served as Set up B. The 100g of leaves were chopped and mixed with 100mL of distilled water then was squeezed using a cheesecloth to obtain 100mL of extract that served as Set up C. For the Set up D, a negative control of distilled water was prepared of about 20mL. After preparing and measuring of 5 mL of each extract, these are dropped using a dropper in the petri dish containing the mung beans after being subjected in the solution, the beans are covered within the tissue to avoid the contact with the air. These set ups are being set aside for every 24 hours observation.

These experimental groups were being observed within five days to record the number of seeds and onion roots that sprouted in each set up.

4. Data Analysis

The average number of monggo seeds that was inhibit the growth of sprouts as well as in the onion roots were determined in each treatment. The data were subjected to Analysis of Variance (ANOVA) two-factor without replication for both 0.05 level of significance.

Results

I. Antimicrobial assay of *Broussontia luzonica* leaves extract

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

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

The NA plates were incubated at 35ºC and observed after 24 hours. The clearing zone was measured in millimeters and the

average diameter of the clearing zone was calculated. The antimicrobial index (AI) was computed using the following formula.

AI= Diameter of clearing zone-diameter of the well
Diameter of the well

The calculated AI was 0 which means that the Himbabao leaves did not inhibit the growth of *S.aureus and E.coli*. The Chloramphenicol served as the positive control.

Table 1. Summary of the results of the Bioassay analysis against *S.aureus and E.coli*

Test Organism	Sample	Clearing zone, mm			AI
E.coli		1	2	3	
	Himbabao leaves	-	-	-	0
	Chloramphenicol disc[a]	27			3.5
S. aureus	Himbabao leaves	-	-	-	0
	Chloramphenicol disc[b]	33			4.5

In table 1, result showed that Himbabao leaves did not inhibit the growth of *E.coli and S. aureus.*

B. Antimicrobial assay of *Broussontia luzonica* leaves extract

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

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

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

AI= Diameter of clearing zone-diameter of the well
Diameter of the well

The calculated AI was 0 which means that the Himbabao leaves did not inhibit the growth of *Candida albicans*. The Canesten solution, 100µl, served as the positive control.

Table 2. Summary of the results of the Bioassay analysis against *C.albicans*

Test Organism	Sample	Clearing zone, mm			AI
C.albicans		1	2	3	
	Himbabao leaves	-	-	-	0
	Canesten solution, 100µl	32			2.2

In table 2,result showed that Himbabao leaves did not inhibit the growth of *C.albicans.*

II. Phytochemical Analysis of Himbabao leaves extract using ethanol as extracting medium

1. Test and Results: Phytochemical Analysis

Table 3. Summary of the results of Phytochemical analysis

Phytochemical Analysis of Himbabao Leaves			
TESTS	POSITIVE RESULTS	ACTUAL RESULTS	INDICATION
For CARBOHYDRATES			
Molisch Test	Violet ring at the junction	Violet ring at the junction	positive
For REDUCING SUGARS			

Fehling's Test	Formation of brick red precipitate	Brick red precipitate	positive
For FLAVONOIDS			
Alkaline Reagent Test	Yellow coloration which disappears upon addition of dilute acid	Yellow coloration disappears after adding dilute acid	positive
Lead Acetate Test	Presence of yellow turbidity or precipitate	Yellowish-white precipitate	positive
For ALKALOIDS			
Hager's Test	Yellow precipitate or turbid solution	Clear light-yellow solution	negative
Mayer's Test	White precipitate or turbid solution	Clear light-yellow solution	negative
Wagner's Test	Reddish brown or turbid solution	Clear reddish-brown solution	negative
For TANNINS			
Ferric Chloride Test	● Blue solution---presence of gallic tannins ● Greeen to black solution---presence of catecholic tannins	Greenish black colored solution	positive
For GLYCOSIDES			
Keller killani Test	Reddish brown/purple ring at the junction	Reddish brown ring at the junction	positive
For SAPONINS			
Froth Test	Froth greater than 2cm even after 30 seconds	Froth formation that disappears after 30 seconds	negative
For RESINS			
Test for Resins	Turbid solution	Clear light-yellow solution	negative
For PHYTOSTEROLS			
Liebermann-Burchard Test	Brown ring at junction, ● green upper layer---presence of sterols ● Deep red---presence of triterpenoids	Purple ring at junction	positive
For ANTHRAQUINONE			
Test for Anthraquinone	Red color	Clear yellow-brown solution	negative
For PROTEINS (Peptide Bonds)			

Biuret Test	Purple or violet color	Clear yellow-brown solution	negative

Table 4. Summary of Phytochemical analysis/result of Himbabao leaves extract

Tests for the Presence of the following:	Broussonetia luzonica leaves
For CARBOHYDRATES	
Molisch Test	+
For REDUCING SUGARS	
Fehling's Test	+
For FLAVONOIDS	
Alkaline Reagent Test	+
Lead Acetate Test	+
For ALKALOIDS	
Hager's Test	-
Mayer's Test	-
Wagner's Test	-
For TANNINS	
Ferric Chloride Test	+
For GLYCOSIDES	
Keller killani Test	+
For SAPONINS	
Froth Test	-
For RESINS	
Test for Resins	-
For PHYTOSTEROLS	
Liebermann-Burchard Test	+
For ANTHRAQUINONE	
Test for Anthraquinone	-
For PROTEINS (Peptide Bonds)	
Biuret Test	-

III. Experimental Design and Treatments

Table 5.A Number of mung beans which the growth of sprouts was inhibited in various treatments

Treatment on Leaves extract	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
50%	14	11	8	0	0
75%	15	13	10	0	0

100%	15	15	11	1	0
Distilled water	7	3	0	0	0

Table 5.B Average length of the sprouts in mung beans in various treatments

Treatment on Leaves extract	Average length of sprout (mm) after five days
50%	41.07
75%	9.83
100%	5.5
Distilled water	85.07

Table 6.A Number of onion roots which the growth was not inhibited in various treatments

Treatment on Leaves extract	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
50%	0	0	0	0	6
75%	0	0	0	0	0
100%	0	0	0	0	0
Distilled water	0	0	5	0	2

Table 6.B Average length of the onion roots in various treatments

Treatment on Leaves extract	Average length of onion roots (mm) after five days
50%	1.42
75%	0
100%	0
Distilled water	2.07

Table 7. A Summary of Result showing the number of mung beans which the growth of sprouts was inhibited in each treatment
ANOVA: Two Factor without Replication
Himbabao leaves extract

Treatment on Leaves extract	*Count*	*Sum*	*Average*	*Variance*
50%	5	33	6.6	40.8
75%	5	38	7.6	51.3
100%	5	42	8.4	54.8
Distilled water	5	10	2	9.5
Day 1	4	51	12.75	14.91666667
Day 2	4	42	10.5	27.66666667
Day 3	4	29	7.25	24.91666667
Day 4	4	1	0.25	0.25
Day 5	4	0	0	0

Table 7.B Summary of computation: ANOVA: Two Factor without Replication

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	122.95	3	40.98333333	6.124533001	0.009063578	3.490294819
Columns	545.3	4	136.325	20.37235367	2.78606E-05	3.259166727
Error	80.3	12	6.691666667			
Total	748.55	19				

Table 7.C Summary of Result showing the number of mung beans which the growth of sprouts was inhibited in each treatment (Graph 1)

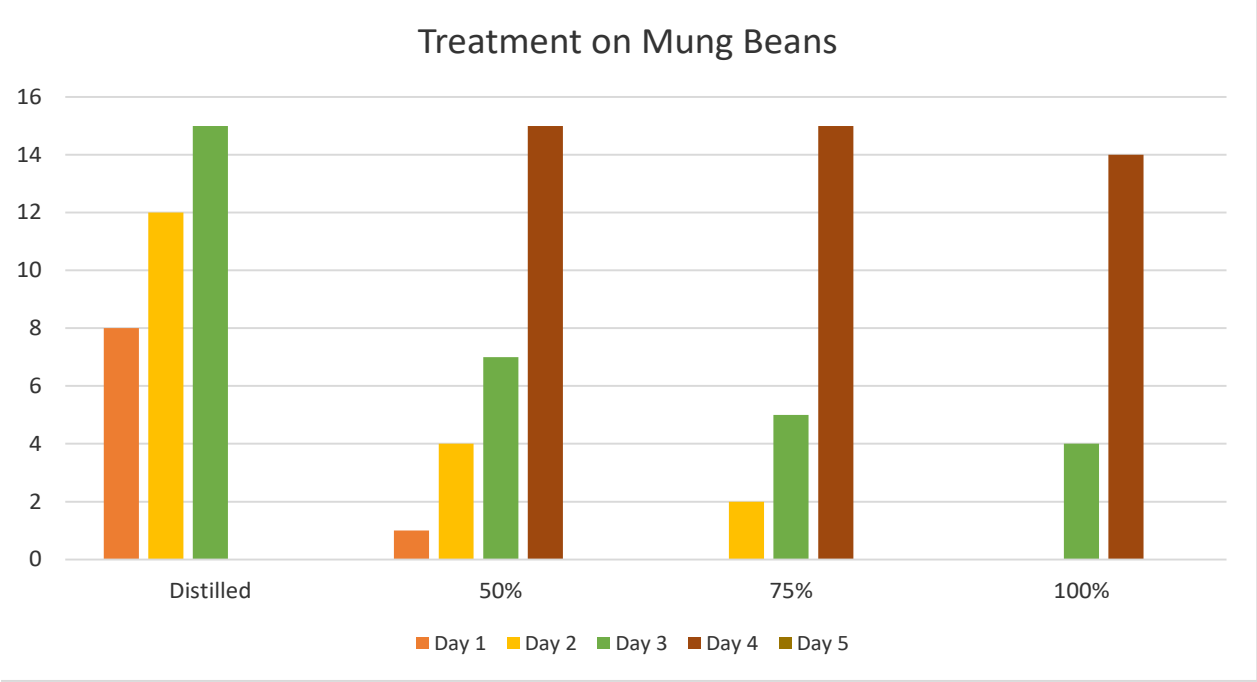


Table 8.A Summary of Result showing the number of onion roots which the growth was not inhibited in each treatment
ANOVA: Two Factor without Replication
Himbabao leaves extract

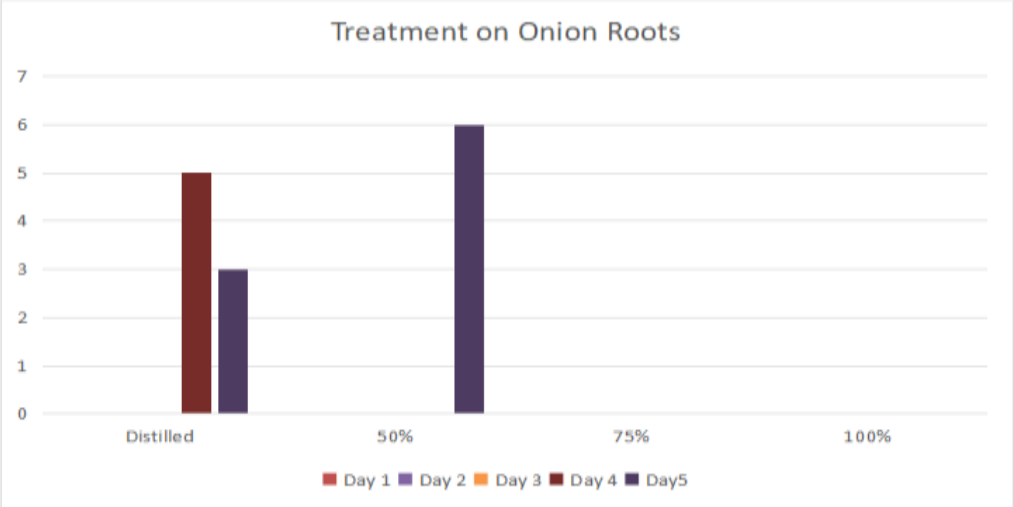
Treatment on Leaves extract	*Count*	*Sum*	*Average*	*Variance*
50%	5	6	1.2	7.2
75%	5	0	0	0
100%	5	0	0	0
Distilled water	5	7	1.4	4.8
Day 1	4	0	0	0
Day 2	4	0	0	0
Day 3	4	5	1.25	6.25
Day 4	4	0	0	0
Day 5	4	8	2	8

Table 8.B Summary of computation:
ANOVA: Two Factor without Replication

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	8.55	3	2.85	1	0.426221379	3.490294819
Columns	13.8	4	3.45	1.210526316	0.356513286	3.259166727
Error	34.2	12	2.85			
Total	56.55	19				

Table 8.C Number of onion roots which the growth was not inhibited in various treatments(Graph 2)



Discussion

A. Antimicrobial Test

Bioassay or antimicrobial test of Himbabao (*Broussontia luzonica)* leaves was conducted to determine its phytochemical conponents *against Escherichia coli, Staphylococcus aureus and Candida albicans.* The Himbabao pure leaves extract had been submitted in Natural Science Research Institute at Miranda Hall, UP Diliman, Quezon City. Results showed that the Himbabao leaves was not capable of inhibiting the growth of E.coli, S. aureus and C. albicans.

B. Phytochemical Analysis

Phytochemical Analysis of Himbabao (*Broussontia luzonica)* leaves was conducted to determine the phytochemical constituents which are anticancer, antifungal and antimicrobial agents..

In this study, the extract *Broussontia luzonica* underwent the process of pythochemical screening test. The fresh leaves were gathered in Akle , San Ildefonso, Bulacan. The gathered leaves were cut into small pieces and then washed with distilled water then were extracted using cheesecloth. In addition, another set of leaves was prepared for the process of bioassay. It was soaked into Ethanol for 72 hours. After soaking, it was subjected to rotary evaporation in Analytical Research at the College of Chemistry Research Building and bioassay in Natural Science Research Institute at Miranda Hall UP Diliman, Quezon City. The phytochemical test was done in the College of Chemistry Research Building at the UP Manila, Metro Manila. The phytochemical screening test included the test of the presence of .carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, glycosides, saponins, resins, phytosterols, anthraquinone, and proteins (peptide bonds). After the pythochemical screening was done, the results showed that there were carbohydrates, reducing sugars, flavonoids, tannins, glycosides, phytosterols being detected while the alkaloids, saponins, resins , anthraquinone and proteins were absent. The researchers based their results in the changes in colors, precipitates formed, and the bubbles /rings formed. For possible better result other researchers may try to use other extracting solvent to produce the extract of Himbabao leaves.

Most recent researches have focused on the health aspects of flavonoids for humans. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities.For pharmaceutical purposes cost-effective bulk production of different types of flavonoids has been made possible with the help of microbial biotechnology (Kumar S., and Pandey A., 2013).

The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation.The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins (Chung KT,et al.,1998)

Epidemiologic and experimental studies suggests that dietary phytosterols (PS) may offer protection from the most common cancers in Western societies, such as colon, breast, and prostate cancer.(Awad & Fink, 2000). In addition, mounting evidence supports a role for phytosterols in protecting against cancer development (T A Woyengo, V R Ramprasath ,and P J H Jones, 2009).

The results of phytochemical analysis showed that the chemical components such as flavonoids and phytosterols of Himbabao leaf could be used for treating cancer , while the tannins could be used for inhibiting the growth of fungi and bacteria.

C. Experimental Treatment

Results on Table 5A and 5B on the treatment of Himbabao leaf extract on monggo seeds showed that in five (5) days the following results were observed and listed. For 50% concentration, all the mung beans grew with an average length of 41.07 mm. This indicated that in this concentration, the Himbabao leaf extract will just minimize the growth of sprouts but not necessarily inhibiting it.

While in 75% concentration, all mung beans grew but with a lesser value for its average length of 9.83 mm after the five-day observation. This showed that this concentration could also minimize the growth of the sprouts better than the 50% set up,

When the beans were subjected with 100% concentration, sprouts only grew simultaneously on the fifth day with an average length of 5.5mm. This indicated that this concentration was not able to inhibit the growth of the mung beans but had the greatest effect in minimizing the rate of growth.

Meanwhile, the Tables 6A and 6B showed the results on the treatment of Himbabao leaf extract on the growth of onion roots within the five-day observations, these are being observed and listed. For the 50% concentration, there were six (6) roots that grew on the fifth day with the average length of 1.42 mm. This indicated that the concentration was not effective in inhibiting the growth of onion roots. However, this set up had minimized the rate of growth as compared to the results in negative control with a resulting eight (8) onion roots with an average length of 2.07mm .

In contrary, the 75% and 100% concentration showed that there were no onion roots that grew from the bulb after the five-day observations. This indicated that these concentrations had effectively inhibit the growth of onion roots.

Based on Table 5A, it determined whether there was a significant difference in Himbabao extract in mung beans at different concentration, the Two Factor without Replication (ANOVA) was used and tested at 0.05 level of significance.

On the other hand, in Table 7A the results of the ANOVA computation showed that there was significant differences in the level of effectiveness of Himbabao (*Broussontia luzonica*) extract between 100% concentration and negative control since they obtained higher than 0.05. Therefore, the null hypothesis was accepted. The results showed that this concentration was not able to inhibit the growth of mung beans but had only minimized its rate.

Table 7B showed the summary that is in two parts. The first part provided summary statistics for the rows. The second part provided the summary statistics for the columns. Summary statistics include the SS, df, MS, F, p-value and Fcrit. The summary of scores shown is 748.55. The degrees of freedom for the rows is 3, 4 for the column and 12 for the error. The mean score for the rows is 40.98333, while 136.325 for column, and 6.691667 for the error. The frequency shows 6.124533 for rows while 20.37235 for the column. The p-value revealed 0.009064 for the rows and 2.79E-05 for the columns. The Frequency critical shows the result of 3.490295 for the rows and 3.259167 for the columns.

Based on Table 6A, it determined whether there was a significant difference in Himbabao extract in onion roots at different concentration, the Two Factor without Replication (ANOVA) was used and tested at 0.05 level of significance.

On the other hand, in Table 8A the results of the ANOVA computation showed that there was significant differences in the level of effectiveness of Himbabao (*Broussontia luzonica*) extract between 75% as well as 100% concentration and the negative control since they obtained lower than 0.05. Therefore, the null hypothesis was rejected. Also, in this concentrations the growth of onion roots was inhibited.

Table 8B showed the summary that is in two parts. The first part provided summary statistics for the rows. The second part provided the summary statistics for the columns. Summary statistics include the SS, df, MS, F, p-value and Fcrit. The summary of scores shown is 56.55. The degrees of freedom for the rows is 3, 4 for the column and 12 for the error. The mean score for the rows is 2.85, while 3.45 for column, and 2.85 for the error. The frequency shows 1 for rows while 1.210526 for the column. The p-value revealed 0.426221 for the rows and 0.356513 for the columns. The Frequency critical shows the result of 3.490295 for the rows and 3.259167 for the columns.

Conclusion

Results of the antimicrobial and antifungal tests showed that the extract was not capable of inhibiting the growth of S.aureus, E.coli and C.albicans. However, based on the phytochemical analysis, it indicated the presence of tannins , a compound which is proven in the recent studies that could inhibit the growth of fungi and bacteria. Thus, the significant difference between the results of appropriate tests might be rooted from the concentration of the extract and amount of water present in the extract used in the bioassay tests. As the growth of fungi and bacteria might be triggered with the presence of water in the extract, this could be the reason why the effect of the Himbabao was turned to be negative.

The phytochemical screening test included the test of the presence of .carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, glycosides, saponins, resins, phytosterols, anthraquinone, and proteins (peptide bonds). After the pythochemical screening was done, the results showed that there were carbohydrates, reducing sugars, flavonoids, tannins, glycosides, phytosterols being detected while the alkaloids, saponins, resins , anthraquinone and proteins were absent.

These results were used as basis in formulating initial conclusion that Himbabao leaves contain anticancer agents, more particularly, the phytosterols and flavonoids which could be categorized into different classes such as flavones which are said to be antitumors and could aid the cancer.

The results of the experiments also indicated the effect of the Himbabao on the growth of mung beans and onion roots. These samples were already used in other studies focusing in anticancer tests. As the experiment, particularly in mung beans, suggested that the extract could only minimize the growth of sprouts. On the other hand, in the concentrations of 75% and 100% tested with the onion roots, it showed that the plant extract could inhibit the growth of onion roots. Thus, these results could be a potential in formulating organic medicine in preventing cancer as it could inhibit the mitotic activity in the said sample.

Therefore, the study of Himbabao extract has been proven significant in the scientific community because of these reasons.

Recommendation

Based on the findings of this investigation, the researchers hereby recommended that;

1. Preparation of plant as lowering of cholesterol. Possible uses of plant in chemical industry should be investigated.
2. Length of observation for the experiment might be taken in a longer time .
3. Phytochemistry of other plants, maybe of the same genus or family, and the plant bark and flower should also be analyzed.
4. The extract might be used for further research for an actual testing to organisms to maximize its effectiveness as alternative remedy for cancer.
5. Preparation of extract for bioassay must be controlled more effectively to avoid being diluted.

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References

Website

Annntoniya R., Kelvin B., and Ranajit DT. (2017). Evaluation of Phytochemical and Anti-mitotic Potential of Poly-herbal extract by using Onion Root Model**.** Retrieved from https://irjponline.com/admin/php/uploads/2652_pdf

Batu & De la Merced. (2016). The radical scavenging activity and effect on liver function enzyme marker alanine aminotransferase of the flavonoid extract from the leaves of himbabao (broussonetia luzonica) moraceae. Retrieved from research.uic.edu.ph/ojs/index.php/IJER/article/view/476

Chung KT, et al. (1998). Tannins and human health: a review. Retrieved from https://www.ncbi.nlm.nih.gov /pubmed/9759559/

Choa JB D., Lu R. V., Nombrado M. A., Rayos GK. R., et al. (2016). Phytochemical screening of Broussonetia luzonicus (Moraceae) leaves. Retrieved from https://www.researchgate.net/publication/296443651_Phytochemical_screening_of_Broussonetia_luzonicus_Moraceae_leaves

Cruz S. R., Hernández S. C., Ruiz K. L. H., Chávez L. A. C., et al. (2017). Retrieved from https://www.intechopen.com/books/flavonoids-important-biocompounds-in-food
Johnson J. (2018). What you need to now about fungal infections. Retrieved from https://www.medicalnewstoday.com/article/317970.php

Kumar S. and Pandey A. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. Retrieved from https://www.hindawi.com › tswj/2013/162750

Kumar and Singhal. (2009). Germinating seeds of the mung bean, Vigna radiata (Fabaceae), as model for the preliminary evaluation of cytotoxic effects of drugs. Retrieved from https://www.researchgae.net/publiction/26269972-_Germinating_seeds_of_the_mung_bean_Vigna_radiata_Fabaceae_as_model_for_the_preliminary_evaluation_of_cytotoxic_effects_of_drugs

Nagy M. (2017). Cardiac Glycosides in Medicinal Plants. Retrieved from https://www.intechopen.com/books/aromatic-and-medicinal-plants-back-to-nature/cardiac-glycosides-in-medicinal-plants

Nall R. (2018). What to know about cancer. Retrieved from https://www.medialnewstoday.com/article/323648.php

Okuda T. and Ito H. (2011). Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins. Retrieved from https://www.mdpi.com/1420-3049/16/3/2191

Philippine Statistics Authority (PSA). (2016). Deaths in the Philippines, 2016. Retrieved from https://psa.gov.ph/ontent/deaths-philippines-2016

Stuart Jr. (2017). Himbabao. Retrieved from stuartxchange.com/Himbabao

T A Woyengo, V R Ramprasath & P J H Jones (2009). Anticancer effects of phytosterols. Retrieved from https://www.nature.com/articles/ejcn200929