ANTIBACTERIAL ACTIVITY OF HIMBABAO (*Broussonetia luzonica*) ETHANOLIC LEAF EXTRACT AGAINST *Staphylococcus aureus*, *Escherichia coli* AND *Klebsiella aerogenes*

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ABSTRACT

The study determined the antibacterial activity of Himbabao (*Broussonetia luzonica*) ethanolic leaf extract. Experimental test design was used in the study. The collected leaves had undergone maceration to come up with ethanolic leaf extract. It was further subjected to phytochemical screening and obtained alkaloids, saponins, phenolic compounds, and flavonoids. The researchers tested the ethanolic leaf extract through disc diffusion assay to measure the zone of inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella aerogenes*. Through paper disc diffusion assay, the average mean of the zone of inhibition against *Staphylococcus aureus* was 6 millimeter, the average mean of the zone of inhibition against *Escherichia coli* was 6 millimeter and the average mean of the zone of inhibition against *Klebsiella aerogenes* was 6 millimeter, which indicated that the Himbabao (*Broussonetia luzonica*) ethanolic leaf extract has very low zone of inhibition and compared to the positive control (amoxicillin). Using One-way ANOVA and Tukey HSD multiple comparisons, the researchers obtained a p-value of .001 for the comparison of positive control (amoxicillin) and Himbabao (*Broussonetia luzonica*) ethanolic leaf extract which indicates a significant difference. The result further shows that the p-value of 1.000 was obtained for the comparison of the negative control (distilled water) and Himbabao (*Broussonetia luzonica*) ethanolic leaf extract, which statistically shows no significant difference. In conclusion, the Himbabao (*Broussonetia luzonica*) ethanolic leaf extract does not possess antibacterial property against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella aerogenes*.

***Keywords**: Himbabao (Broussonetia luzonica), ethanolic leaf extract, antibacterial, Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes*

INTRODUCTION

Infection is caused by a foreign organism in the body. It is the invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present in the body. An infection may remain localized, or it may spread through the blood or lymphatic vessels to become systemic. Some infections are mild and barely noticeable; but others are severe and life-threatening; and some are resistant to treatment (Nordqvist, 2017). A limited number of bacterial species are responsible for the majority of infectious diseases in healthy individuals, it occurs when the harmful strain of bacteria multiply on the surface or inside the body (Bhatt P, 2017).

Antibiotics are medicines used to prevent and treat bacterial infections; they are substances that destroy or inhibit the growth of other microorganisms and are used in the treatment of external or internal infections. While some antibiotics are produced by microorganisms, most are now manufactured synthetically (WHO, 2018).

As modern medicine's wonder drug, antibiotics have eradicated a lot of diseases. However, their abundance and rampant use have led to abuse and misuse which results in antibiotic resistance. For the past years, doctors were alarmed on Antimicrobial Resistance where infectious microorganisms, previously treatable by certain drugs, eventually become resistant to treatment (Hedge, A., 2015). Antibiotic resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases (WHO, 2018). As the statistics provided by Centers for Disease Control and Prevention, 8.6% of Klebsiella species are commonly found in intensive care unit that causes nosocomial infections. *Klebsiella aerogenes* encompasses 15-25% of the majority of Klebsiella infections and bacteremia cases. A total number of 7,249 Enterobacteriaceae isolates were collected from clinical samples, among which 18.1% (1,311/7,249) were carbapenem-resistant (Li, et. al, 2018). *Staphylococcus aureus* has 80,641 cases of severe MRSA infection and deaths of 11,285 (CDC, 2013). Bacterial resistance to antibiotic represents serious problem to pharmaceutical industries. One of the solutions to this problem is the widespread screening of medicinal plants from the traditional system to get newer, safer and more effective agents that can be used to fight infectious diseases. In the Philippines, 8-65% of *Escherichia coli* associated with urinary infections presented resistance to antibiotics; a cumulative methicillin-resistant *Staphylococcus aureus* rate from the sites in the National Capital Region was recorded at 54%. (DOH, 2017)

Plants play a remarkable role in treating and preventing diseases and help lessen the active proliferation of some health conditions, it has been an important source of drug products since ancient times in every culture throughout the world. Traditionally, the community uses them as a way of preventing and managing diseases such as infections. Herbal medications are not only considered for their cost-effective way of preventing and managing infections but also, due to their abundance in the community for maintaining human health. *Broussonetia luzonica*, known as Himbabao is endemic in the Philippines, a species of flowering plant in the family of Moraceae. Leaves and flowers of this plant have biological and pharmacological properties and are locally eaten by Filipinos.

With the imminent threat of antimicrobial resistance in the Philippines and the world, the researchers conducted this study to discover new remedies and develop formulations that would aid in preventing the spread of these pathogens.

This research study was limited to the determination of the antibacterial activity of Himbabao (*Broussonetia luzonica)* ethanolic leaf extract against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria *(Escherichia coli* and *Klebsiella aerogenes*). This study aimed to identify the phytochemical constituents of the plant extract.

Research Questions

1. What are the phytochemical constituents present in Himbabao (*Broussonetia luzonica)* ethanolic leaf extract?
2. Is there a significant difference on the antibacterial activity against *Staphylococcus aureus;*
    a. Positive control (Amoxicillin) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract
    b. Positive control (Amoxicillin) versus negative control (Distilled water)
    c. Negative control (Distilled water) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract
3. Is there a significant difference on the antibacterial activity against *Escherichia coli;*
    a. Positive control (Amoxicillin) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract
    b. Positive control (Amoxicillin) versus negative control (Distilled water)
    c. Negative control (Distilled water) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract
4. Is there a significant difference on the antibacterial activity against *Klebsiella aerogenes;*
    a. Positive control (Amoxicillin) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract
    b. Positive control (Amoxicillin) versus negative control (Distilled water)
    c. Negative control (Distilled water) versus Himbabao (*Broussonetia luzonica)* ethanolic leaf extract

Hypothesis

- ***Ho1:*** There is no significant difference between the antibacterial results of positive control group (amoxicillin) and the experimental group with Himbabao (*Broussonetia luzonica*) ethanolic leaves extract against *Staphylococcus aureus.*
- ***Ho2:*** There is no significant difference between the antibacterial results of positive control group (amoxicillin) and the experimental group with Himbabao (*Broussonetia luzonica*) ethanolic leaves extract against *Escherichia coli*.
    - ***Ho3:*** There is no significant difference between the antibacterial results of positive control group (amoxicillin) and the experimental group with Himbabao (*Broussonetia luzonica*) ethanolic leaves extract against *Klebsiella aerogenes*.

Significance of the Study

This research study on the leaf extract of Himbabao (*Broussonetia luzonica*) will be beneficial to the community through raising the awareness of community-available alternative management for the prevention of bacterial infections. This research will also be beneficial in improving pharmaceutical knowledge and development in the Philippines through the use of an alternative source of medicine that is endemic in the country.

Literature review

Bacterial Infection

Bacterial infections differ from other infections due to the microbes that cause them. It may be transmitted through direct or indirect contact with a reservoir bacteria and may be marked by localized redness, inflammation, pain or swelling. Bacteria are single-celled microorganisms and can live in almost every conceivable environment, including in and on the human body. Certain bacteria are able to survive outside of a host and remain infective for extended periods of time. Most bacteria are harmless, and some actually help by digesting food, destroying disease-causing microbes, and providing essential nutrients. *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella aerogenes* are some of the examples of bacteria that cause infections (Nazzaro, et. al, 2013).

Infections caused by Gram Positive Bacteria

Staphylococcus aureus is a major pathogen that causes a wide range of clinical infections. It has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses, furuncles, cellulitis and serious infections such as pneumonia and bacteremia (DOH, 2010). *Staphylococcus aureus* related illness can range from mild and requiring no treatment to severe and potentially fatal. It is contagious until the infection has resolved and is spread by having direct contact

with an infected person, by using contaminated object, or by inhaling infected droplets dispersed by sneezing or coughing.

Infections caused by Gram Negative Bacteria

Escherichia coli are bacteria found in the foods and intestines of people and animals. Extraintestinal *Escherichia coli* infections, such as urinary tract infections and neonatal sepsis, represent a huge public health problem. Although, *Escherichia coli* is most closely linked to urinary tract infection; it can infect any extraintestinal site, causing meningitis, skin structure infections, myositis, osteomyelitis and epididymoorchitis (Vila, et. al, 2016).

Klebsiella aerogenes is a common gram-negative bacteria causing nosocomial and healthcare-associated infection. It is an opportunistic bacterium and has emerged as hospital-acquired from intensive care patients pathogenic, especially to those who are on mechanical ventilation (Davin-Regli, A. 2015). It causes respiratory and urinary tract infections, bacteremia, sepsis and post-chirurgical infections.

WHO against AMR

Antimicrobial resistance is the ability of a microorganism to stop antimicrobial from working against them. The antimicrobial resistance occurs naturally over time, usually through genetic changes. However, the misuse and overuse of antimicrobials are accelerating. The antibacterial drugs become ineffective and infections persist in the body, increasing the risk of spread to others (WHO, 2018).

World Health Organization ensures the plan that can be beneficial in promoting successful treatment and prevention of infectious diseases, to reduce the incidence of infection, to optimize the use of antimicrobial agents and increase the investment in new medicines and other interventions (WHO, 2018). They want to promote the awareness and understanding of antimicrobial resistance and take action by observing the use of antimicrobial agents and minimizing the incidence of infection through proper development to antimicrobial resistance.

Administrative Order 42

Administrative Order 42; Creating an inter-agency committee for the formulation and implementation of a national plan to combat antimicrobial resistance in the Philippines, encourage every health care association or individual to engage in developing and implementing a plan that can be a help in controlling incidence regarding antimicrobial resistance (AMR). Antibiotic resistance is becoming dire worldwide (WHO, 2018). Surveillance and monitoring the use of antibiotic, promoting proper hygiene and improving infection control and prevention are the major approaches in managing this threat (Uchil, et.al, 2014).

Traditional Medicine Act of 1997

Republic Act 8423 (RA 8423) also known as the Traditional and Alternative Medicine Act of 1997, focuses on improving the quality and delivery of traditional and alternative health-related management in the country. Drugs used for the prevention and management of signs and symptoms, and maintaining a healthy lifestyle which is cost-effective is needed to be researched and developed.

This law encourages indigenous people to be motivated in discovering the possible use of alternative medicines present in their locality. Thus, people should be aware and be encouraged to study those essential alternative medicinal products to acquire safe, efficient, and cost-effective medicines. By that, medical practitioners should know the proper use of every alternative medicinal product including evidence of efficacy in order to meet health care needs and to give proper advice to patients (Nolledo, 2015).

Profile of Himbabao (*Broussonetia luzonica*)

Himbabao (*Broussonetia luzonica*) also known as Alukon in Ilocano from the family Moraceae is widely distributed to Cagayan Valley (Ruma, 2015). Himbabao (*Broussonetia luzonica*) is a medium-sized shed tree growing to a height of 10 meters with a trunk diameter of 40 centimeters. Flowers are very small, borne on long, slender, spike-like flowering branches. Leaves are alternate, oblong, membranous, 15 cm in length and 7 cm in width (Choa, et.al, 2016).

Folkloric and Medicinal use of Himbabao (*Broussonetia luzonica*)

In Cagayan Valley, the whole tree is used as a source of living; trunks used in making furniture and cabinetries, while its fibrous bark is used in making ropes. The leaves which have biological and pharmacological properties are commonly eaten by Ilocanos

The leaves can be used as a tea or a decoction and are said to have an anti-inflammatory property, good for digestion and have the ability to detoxify the body (Seward, 2017). The leaves have also been proven to have an anti-obesity property.

Anti-infective property of Himbabao (*Broussonetia luzonica*)

A recent study showed that Himbabao (*Broussonetia luzonica*) had undergone phytochemical screening for antimicrobial activities using its leaf. In its phytochemical analysis, the extract of Himbabao (*Broussonetia luzonica*) shows the presence of carbohydrates, reducing sugars, flavonoids, tannins, alkaloids, and sterols. Leaf extracts of Himbabao (*Broussonetia luzonica*) also exhibit a wide range of activity such as anti-inflammatory, antiviral, antibacterial, anti-osteoporotic, anti-allergic, and anti-hepatic action (Choa, et.al, 2016).

Amoxicillin

Amoxicillin is a FDA approved and commercially available antibiotic that is used in the treatment against wide range of infections caused by wide range of Gram-positive and Gram-negative bacteria in both humans and animals. It is an acid stable, semi-synthetic drug that belongs to a class of antibiotics called the Penicillin (B-lactam antibiotics). This penicillin-type antibiotic works by stopping the growth of bacteria.

Research paradigm

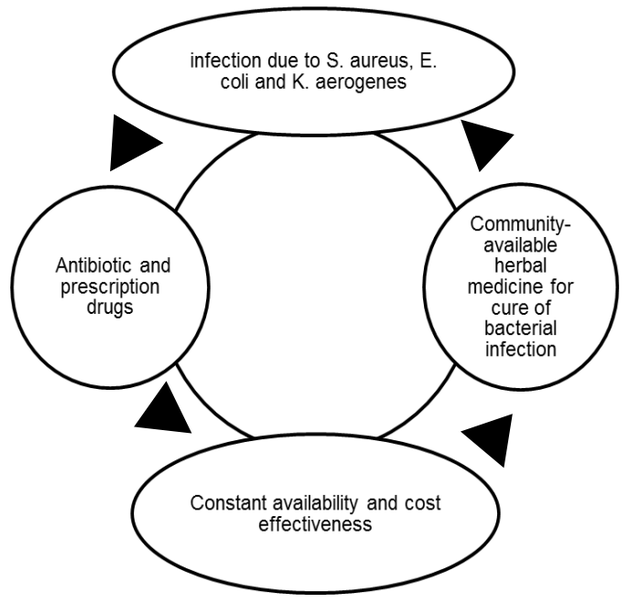

Figure 1 Research Paradigm

The paradigm above shows that due to bacterial infections caused by *Staphylococcus aureus, Escherichia coli, and Klebsiella aerogenes* individuals settle for antibiotics and other prescription drugs to cure the infection.

The paradigm shows that with the continuous antibiotic usage constant availability and antibiotic resistance might occur. Considering also the cost effectiveness of the medication, herbal medicines should be considered for the cure of the infections caused by *Staphylococcus aureus, Escherichia coli, and Klebsiella aerogenes.*

METHODS

This part of the research paper discusses the methods, materials, processes and procedures that were used in conducting the research entitled “The Antibacterial Activity of Himbabao (*Broussonetia luzonica*) leaf extract against *Staphylococcus aureus, Escherichia coli* and *Klebsiella aerogenes”.*

The research methodology, subject of the study, sampling technique and the procedure of gathering the statistical treatment used are shown below.

Research Design

Experimental method was used in this study. The experiment was conducted at the Pharmacy laboratory of University of Saint Louis Tuguegarao, Saint Louis University Baguio and at the Department of Science and Technology Provincial laboratory.

Methodological Framework

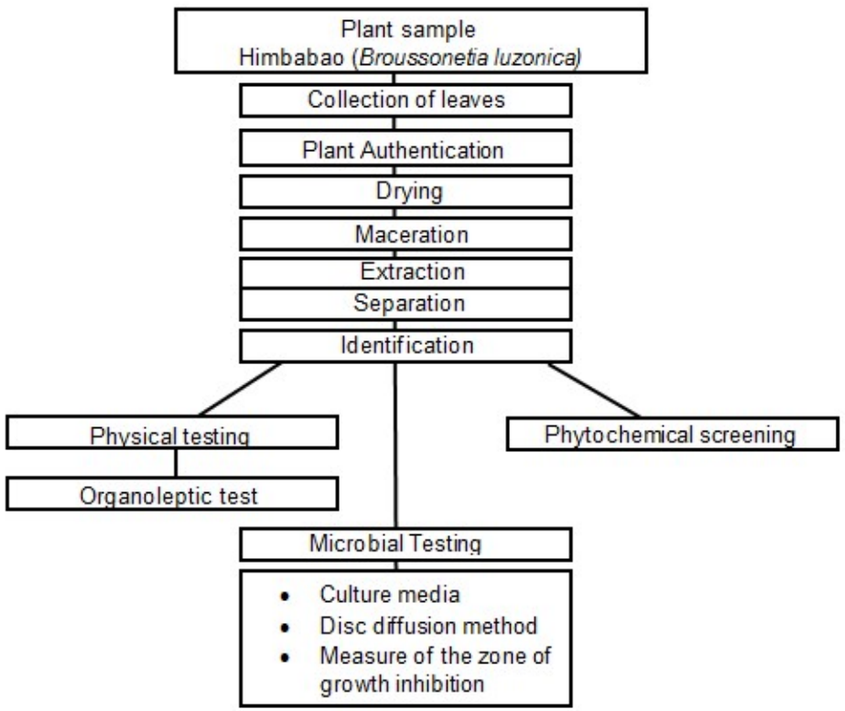

Figure 2 Methodological Framework

This methodological flow chart shows the general methods that the researchers used in the study of the Antibacterial activity of Himbabao (*Broussonetia luzonica)* ethanolic leaf extract.

Plant Authentication/Classification

The plant sample, Himbabao (*Broussonetia luzonica*), was collected in a clean bag within Atulayan, Tuguegarao City. Botanical verification and authentication of the plant material was held in the Department of Agriculture, Carig, Tuguegarao City. The plant sample was washed with distilled water and air-dried for 48 hours to remove the moisture of the plant sample.

Data Gathering

1. Preparation of Plant Extract

1.1 One hundred grams of the fresh plant sample was minced and soaked in 80% ethyl alcohol and was covered with tightly-closed container, which was autoclaved for sterility of the apparatus.

1.2 Macerated for twenty-four hours. The filtrate was placed in a tightly-closed container.

1.3 Filtered using a muslin cloth and filter paper.

1.4 The sample was weighed and kept in a refrigerator to preserve the potency.

1.5 The separation of the desired extract from ethyl alcohol was performed using rotary evaporator not exceeding 60 degree Celsius.

$$\text{Percentage yield} = \frac{\text{weight of the residue}}{\text{weight of the sample}} X$$

$$\text{Percentage yield} = \frac{5.6\ g}{150\ g} X$$

=3.73%

2. Physical Testing

Since the extract drug from the plant was not purified and will not be so, only a limited amount of procedure was done. Physical testing was based on the Organoleptic Analysis of Herbal Ingredients (Dentali, 2013).

Table 1. *Physical testing*

Shape	Symmetrical
	Asymmetrical
Size	Coarse

	Fine
Clarity	Clear
	Translucent
	Opaque
Color	Red, Orange, Yellow, etc.
	Pearlescent
	Shiny, Sparkly, Glossy
	Dull, Matte
	Bright, vibrant
	Pale, light
	Clear, light
	Muddy
	Uniform, consistent
	Swirled, speckled, spotted, blotched

Table 1 shows the basis on how the physical evaluation was done on the leaf sample.

2.1 Organoleptic evaluation The color, odor, and appearance of the ethanolic extract were examined based on the Organoleptic Analysis of Herbal Ingredients (Dentali, 2013).

Table 2*. Organoleptic evaluation*

Texture	Hard-solid
	Soft-solid
	Semi-solid
	Liquid
Odor	Aromatic/spicy aroma
	Fragrant, pleasant, sweet
	Foul/strong aroma
Color	Red, Orange, Yellow, etc.

Table 2 shows the basis on how the organoleptic evaluation was done on the leaf sample.

3. Phytochemical Screening

The Himbabao (*Broussonetia luzonica*) ethanolic leaf extract was subjected to phytochemical screening to determine the constituents present in the leaf extract. The extract was submitted at Saint Louis University, Baguio.

3.1 Detection of Alkaloids: extracts were dissolved individually in dilute hydrochloric acid and filtered.

3.1.1 Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide).Formation of a yellow colored precipitate indicated the presence of alkaloids.

3.1.2 Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloid.

3.1.3 Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

3.2 Detection of Carbohydrates: Extracts were dissolved individually with 5milliliter distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

3.2.1 Molisch's test: Filtrates were treated with 2 drops of alcoholic o-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of Carbohydrates.

3.2.2 Fehling's test: Filtrates were hydrolysed with diluted HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicated the presence of reducing sugars

3.3 Detection of Glycosides: Extracts were hydrolyzed with diluted HCl, and were subjected to test for glycosides.

3.4 Modifies Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonium solution. Formation of rose-pink color in the ammoniacal layer indicated the presence of anthranol glycoside.

3.5 Detection of Saponins

3.5.1 Froth Test: Extracts were diluted with distilled water to 20milliliter and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimeter layer of foam indicated the presence of saponins.

3.5.2 **Foam Test:** 0.5 gram of extract was shaken with 2 milliliter of water. If foam produced persists for 10 minutes, it indicated the presence of saponins.

3.6 Detection of Phenols

3.6.1 **Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of blush black color indicated the presence of Phenols.

3.7 Detection of Tannins

3.7.1 **Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitates indicated the presence of Tannins.

3.8 Detection of Flavonoids

3.8.1 **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of Flavonoids.

3.9 Detection of Proteins and Amino Acids

3.9.1 **Xanthoproteic Test:** The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow color indicated the presence of Proteins.

3.10 Detection of Sterols

3.10.1 **Salkowski Test:** Few milligrams of residue of each extract were taken in 2 milliliter of chloroform and in it 2 milliliter of concentrated sulfuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layer indicated the presence of sterols.

3.10.2 **Liebermann-Burchard Reaction:** Few milligrams of residue were dissolved in chloroform. To this, few milliliter of acetic anhydride was added. Then two drops of concentrated sulfuric acid was added from the side of the test tube. The greenish transient color indicated the presence of sterols.

4 Determination of the antibacterial activity of the Himbabao (*Broussonetia luzonica)* leaf extract

The Himbabao leaf extract was submitted to Department of Science and Technology for the determination of its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella aerogenes.*

4.1 Preparation of Assay Plate (Mueller –Hinton Agar)

4.1.1 17.5 grams of Acid Casein Peptone, 2 grams of Beef Infusion, and 1.5 grams of Corn Starch was suspended to a 1 liter of distilled water.

4.1.2 The mixture was dissolved by heating with frequent agitation.

4.1.3 The mixture was boiled for 1 minute until complete dissolution. It was dispensed into appropriate containers and was sterilized in the autoclave at 121°C for 15 minutes.

4.1.4 The mixture was stored at 2-8°C. (Guevarra et al, 2005)

4.2 Sterilization method

Using an autoclave machine placed the prepared culture media and clean dry petri dish and flask (properly wrapped) that was used in the assay method. To operate the autoclave:

4.2.1 The autoclave was covered and the air outlet was open. The autoclave was heated and the air outlet was closed as soon as the steam came out. The pressure was allowed rising until the desired pressure or temperature was reached (121 degree Celcius or 15LBS).

4.2.2 The temperature or pressure was maintained from 15-20 minutes.

4.2.3 After 15- 20 minutes, the pressure went down to zero to escape valve opened before opening the autoclave.

4.3 Paper Disc Diffusion Method

4.3.1 A pair of forceps was sterilized using flame until the blue alcohol flame disappeared

4.3.2 With the forceps, a paper disc was immersed into the extract for assay.

4.3.3 The moistened disc was on the seeded agar plate.

4.3.4 With the use of forceps the sides of the disc was tapped to ensure maximum full contact of the disc with agar medium.

4.3.5 The plates were incubated. (Guevarra et al, 2005)

4.4 Inoculation of Microorganism

4.4.1 15 milliliter of melted Mueller- Hinton agar was poured into dry and sterile petri dishes.

4.4.2 The medium was left to solidify.

4.4.3 A sterile cotton swab was moistened into the test organism/inoculum.

4.4.4 The moistened swab was aseptically swabbed onto a solidified Mueller-Hinton agar plate by streaking the swab over the entire surface of the agar plate 3 times.

4.4.5 The plates were rotated approximately 60 degrees after each application to ensure an even distribution of the inoculum on the surface of the medium. (Guevarra et al, 2005)

Data Analysis

The tests were done three times and computed the mean of the zone of inhibition of test extract. One-way Analysis of Variance (ANOVA) with 0.05 level of significance was used to identify if there are any significant difference obtained. Tukey HSD test was further used to identify which among the Himbabao (*Broussonetia luzonica*) ethaolic leaf extract, positive control and negative control have significant difference in antibacterial activity *against Staphylococcus aureus, Escherichia coli* and *Klebsiella aerogenes.*

Waste Disposal

Waste disposal was conducted at the Department of Science and Technology located at Carig Sur, Tuguegarao City Cagayan. The proper observation of waste disposal was done by the staff.

The agar plates used were decontaminated by an autoclave with the temperature of one hundred twenty one degrees Celsius and fifteen PSI within 30 minutes.

Ethical Considerations

The researchers asked permission from the Vice President for Academics, Dr. Emmanuel James P. Patagguan, the Academic Dean, Dr. Therese May G. Alejandrino, and the Associate Dean, Dr. Dindo V. Asuncion through a letter that was submitted during the research study on the second semester. In addition, the researchers ensure that this research endeavor had undergone the evaluation of the University Research Ethics Board (UREB). Upon experimentation, researchers did not use animals to conduct the said test.

RESULTS

The findings of the study from the experiments conducted to the plant sample are discussed in this part of the research paper. All the data from the previous parts of this research paper is presented here together with the phytochemical screening and the biological testing result.

Table 3. *Result of the Physical Testing of the Himbabao (Broussonetia luzonica) Leaves.*

Leaves	Observation
Shape	Asymmetrical
Size	Coarse
Clarity	Translucent
Color	Bright green - dark green
	Glossy

Table 3 shows the shape, size, clarity and color of the leaves of Himbabao (*Broussonetia luzonica*). It appeared to be asymmetrical shape, coarse, translucent and has a bright to dark green glossy leaves.

Table 4. *Result of the Organoleptic Evaluation of the Himbabao (Broussonetia luzonica) Leaf Extract.*

Extract	Observation
Appearance	Liquid
Color	Deep green
Smell	Foul/Strong aroma

Table 4 showed the color, odor and physical appearance of the Himbabao *(Broussonetia luzonica)* leaf extract. It has a physical appearance of a deep green liquid with a characteristic odor.

Table 5. *Phytochemical Screening Result for Himbabao (Broussonetia luzonica) Ethanolic leaf Extract*

Constituents	Results
Alkaloids	Positive
Carbohydrates	Positive

Glycosides	Negative
Saponins	Positive
Phytosterols	Positive
Phenolic Compounds	Positive
Flavonoids	Positive
Proteins	Positive

Table 5 shows that alkaloids, carbohydrates, saponins, phytosterol, phenolic compounds, flavonoids and proteins can be found in the Himbabao (*Broussonetia luzonica*) ethanolic leaf extract while glycosides are absent.

Table 6 *Antibacterial activity of Himbabao (Broussonetia Luzonica) Ethanolic Leaf Extract, Amoxicillin (positive control) and Distilled Water (negative control) against Staphylococcus aureus, Escherichia coli and Klebsiella aerogenes*

	Zone of Inhibition											
	S. aureus				***E.coli***				***K. aerogenes***			
	R1	**R2**	**R3**	**Mean**	**R1**	**R2**	**R3**	**Mean**	**R1**	**R2**	**R3**	**Mean**
	6	6	6	**6**	6	6	6	**6**	7	6	6	**6**
	63	65	65	**64**	37	36	36	**36**	18	18	19	**18**
	6	6	6	**6**	6	6	6	**6**	6	6	6	**6**

A= Himbabao (Broussonetia Luzonica) ethanolic leaf extract
B= Amoxicillin
C= Distilled water
Legends:
**Zone of inhibition interpretation*
<10 millimeter= inactive
10-13 millimeter= partially active
14-19 millimeter= active
>19 millimeter= very active

Table 6 shows the zone of inhibition pattern that amoxicillin (B) has the highest mean of 64 millimeter and 36 millimeter which indicates very active against *Staphylococcus aureus* and *Escherichia coli*, respectively. While a mean of 18 millimeter which indicates active against *Klebsiella aerogenes*. Plant extract and Negative control showed a mean of 6 millimeter, thus inactive, against *Staphylococcus aureus, Escherichia coli* and *Klebsiella aerogenes.*

Table 7. *One-Way Analysis of Variance for the Zones of Inhibition for Staphylococcus aureus.*

	Sum of Squares	**Df**	**Mean Square**	**F**	**p-value**	**Interpretation**
Between Groups	6805.556	2	3402.778	7656.25	.001	Reject Ho
Within Groups	2.667	6	.444			
Total	6808.222	8				

Table 7 shows that with a p-value of .001 there is a significant difference on the computed zone of inhibition of different treatment groups against *Staphylococcus aureus*.

Table 8. *Tukey HSD Multiple Comparisons Test of Himbabao* (*Broussonetia luzonica*) *Extract against the Positive and Negative Control under Staphylococcus aureus.*

Extract	**Control**	**Mean Difference (I-J)**	**Std. Error**	**p-value**	**Interpretation**
Himbabao	Amoxicillin (Positive)	-58.33*	0.544	.001	Reject Ho
	Distilled Water (Negative)	0.00	0.544	1.000	Accept Ho

*. The mean difference is significant at the 0.05 level.

Table 8 shows that with a p-value of 1.000 there is no significant difference on computed zone if inhibition of Distilled water and Himbabao (*Broussonetia luzonica*) ethanolic leaf extract against *Staphylococcus aureus*

Table 9. *One-Way Analysis of Variance for the Zones of Inhibition for Escherichia coli*

	F-value	**p-value**	**Decision**
	8281.00	.001	Reject Ho

Table 9 shows that with a p-value of .001 there is a significant difference on the computed zone of inhibition of different treatment groups against *Escherichia coli*.

Table 10. *Tukey HSD Multiple Comparisons Test of Himbabao* (*Broussonetia luzonica*) *Extract against the Positive and Negative Control under Escherichia coli.*

Extract	Control	Mean Difference (I-J)	Std. Error	p-value	interpretation
Himbabao	Amoxicillin (Positive)	-30.33*	0.272	.001	Reject Ho
	Distilled Water (Negative)	0.00	0.272	1.000	Accept Ho

Table 10 shows that with a p-value of 1.000 there is no significant difference on computed zone if inhibition of Distilled water and Himbabao (*Broussonetia luzonica*) ethanolic leaf extract against *Escherichia coli.*

Table 11. *One-Way Analysis of Variance for the Zones of Inhibition for Klebsiella aerogenes*

	Sum of Squares	df	Mean Square	F	p-value	Interpretation
Between Groups	296.222	2	148.111	666.50	.001	Reject Ho
Within Groups	1.333	6	0.222			
Total	297.556	8				

Table 11 shows that with a p-value of .001 there is a significant difference on the computed zone of inhibition of different treatment groups against *Klebsiella aerogenes.*

Table 12. *Tukey HSD Multiple Comparisons Test of Himbabao* (*Broussonetia luzonica*) *Extract against the Positive and Negative Control under Klebsiella aerogenes*

Extract	Control	Mean Difference (I-J)	Std. Error	p-value	Interpretation
Himbabao	Amoxicillin (Positive)	12.000	0.384	.001	Reject Ho
	Distilled Water (Negative)	0.333	0.384	0.679	Accept Ho

Table 12 shows that with a p-value of 0.679 there is no significant difference on computed zone if inhibition of Distilled water and Himbabao (*Broussonetia luzonica*) ethanolic leaf extract against *Klebsiella aerogenes.*

DISCUSSION

This research study determined the antibacterial activity of the Himbabao (*Broussonetia luzonica*) by undergoing organoleptic testing, phytochemical screening and biological testing.

The phytochemical analysis of the Himbabao (*Broussonetia luzonica*) ethanolic leaf extract revealed the presence of saponins, alkaloids, phenolic compounds and flavonoids. According to the study of Bhat (2014) secondary metabolites identified in the test samples like flavonoids, saponins, tannins, steroids and alkaloids have importance in medicinal value due to the production of a defined physiological action on human body with antioxidant, antibacterial, anti-inflammatory, antiviral, immune system stimulant and detoxification activities. The presence of flavonoids in many research findings shows an evident response of antibacterial activity as a defense mechanism against invasion of microorganisms by creating complexes with extracellular soluble proteins and polypeptides in the cell wall of microorganisms, which will disrupt the function of cell membrane of microorganisms (Karaman, 2012). With increasing prevalence of untreatable infections induced by antibiotic resistant bacteria, flavonoids have attracted much interest because of the potential to be substitutes for antibiotics (Xie, et. al, 2015). Moreover, the secondary metabolites present in the plant sample could be a source of potential pharmaceutical agent which can be used to prevent and treat infectious diseases caused by pathogens (Dae & Gerald, 2016).

The average mean of zone of inhibition of Himbabao (*Broussonetia* luzonica) ethanolic leaf extract against *Staphylococcus aureus* was 6 millimeter which is inactive compared that of the positive control (amoxicillin), which showed an average mean of zone of inhibition of 64 millimeter indicating a very active activity against the bacteria. The average mean of zone of inhibition against *Escherichia coli* was 6 millimeter which is not effective compared to the positive control that showed an average mean zone of inhibition of 36 millimeter, and the average mean zone of inhibition against *Klebsiella aerogenes* was 6 millimeter, which is not effective compared to the positive control that showed an average mean zone of inhibition of 18 millimeter. Furthermore, statistical analysis has shown that Himbabao (*Broussonetia luzonica*) and the positive control (amoxicillin) have no equal efficiency in inhibiting and treating the bacterial infections. With the zones of inhibition of Himbabao (*Broussonetia luzonica*) ethanolic leaf extract against *Staphylococcus aureus, Escherichia coli* and *Klebsiella aerogenes* of less than 10 mm, extract is inactive in terms of antibacterial activity. In comparison with the studies made by Ruma, et. al. (2015), 95% ethanolic leaf extract of Himbabao (*Brusonica luzonica*) exhibited partial activity against S. aureus with zone of inhibition mean of 11 millimeter and inactive against E. coli with zone of inhibition of 9 millimeter. This study utilized only 80% of ethanolic leaf extract; therefore it is fitting to say that different solvent ratio used in extracting the plant sample will yield different extraction of compounds. According to Azwanida (2015), basing on the polarity of the compounds, plants do not give the same range of secondary metabolites using different solvent concentration. This condition may have caused this study to obtain a different result in biological testing using disc diffusion assay.

CONCLUSION

Based on the data gathered and analyzed, the researchers therefore conclude that the Himbabao (*Broussonetia luzonica*) ethanolic leaf extract does not possess antibacterial property against *Staphylococcus aureus, Escherichia coli* and *Klebsiella aerogenes*.

RECOMMENDATIONS

Based on the results of this research study, the following are recommended:

1. To utilize 95% ethanolic leaf extract of Himbabao (*Broussonetia luzonica*).
2. To test other parts of Himbabao (*Broussonetia luzonica*) for their antibacterial activities.
3. To test for other possible medicinal property of Himbabao (*Broussonetia luzonica*) leaf.

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