CHAPTER I
INTRODUCTION

1.1. Background

Currently, infectious diseases require great attention in the health sector and are the most common diseases found in everyday life. Infection is the entry of microorganisms into the body and can cause illness. Cases of infection are usually caused by several microorganisms, such as bacteria, parasites, viruses, and fungi. The bacteria that often cause infection in humans are *Staphylococcus aureus* and *Staphylococcus epidermidis.*

Efforts to prevent and treat infectious diseases caused by bacteria can be made by utilizing plants with antibacterial properties, such as Chinese Betel Plants (*Sirih China*) (*Peperomia pellucida L.*).

The Chinese betel plant (*Peperomia pellucida L.*) is a herbaceous plant that belongs to the Piperaceae family. This plant grows in not-so-dry areas. This plant is generally found in not very fertile areas, such as on rocks, damp walls, fields and yards, even on the edges of ditches.

The Chinese betel plant (*Peperomia pellucida L.*) has traditionally been used by the community in treating several diseases. The ability of the Chinese betel plant (*Peperomia pellucida L.*) as a medicinal plant is thought to be related to the antioxidant content of the plant. From the phytochemical screening results conducted by Angelina et al. (2015), the Chinese betel plant (*Peperomia pellucida L.*) contains alkaloids, flavonoids, saponins, tannins and triterpenoids. With compounds contained in the Chinese betel plant (*Peperomia pellucida L.*), it can be assumed that this plant can inhibit the growth of bacteria.

Based on the description above, this study was conducted to determine whether the Chinese betel plant (*Peperomia pellucida L.*) has antibacterial activity

against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

1.2. Formulation of the Problem

Based on the description above, the problem in this study is whether the Chinese betel plant (*Peperomia pellucida L.*) extract has antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

1.3. Objective of the Study

The objective of this study is to determine the presence or absence of antibacterial power of the Chinese betel plant (*Peperomia pellucida L.*) extract against *Staphylococcus aureus* and *Staphylococcus epidermidis* and to determine the best inhibitory concentration against the test bacteria.

1.4. Hypothesis

H_0: Chinese betel plant (*Peperomia pellucida L.*) extract does not have antibacterial activity against the test bacteria.

H_1: Chinese betel plant (*Peperomia pellucida L.*) extract has antibacterial activity against the test bacteria.

1.5. Significance of the Study

For the author, the significance of the study is to increase the author's knowledge about the benefits of a Chinese betel plant (*Peperomia pellucida L.*) as an antibacterial. For science, the significance of the study is to add information on the Chinese betel plant (*Peperomia pellucida L.*) usage as an antibacterial, as a reference source for practitioners who are interested in conducting microbiological research, and as data and information for conducting further research.

CHAPTER II

LITERATURE REVIEW

2.1. Description of the Chinese Betel Plant (*Peperomia pellucida L.*)

The Chinese betel plant is a herbaceous plant originating from the United States. However, this plant grows wild and is easily found in Indonesia. This plant is often found in the yard, the edge of a trench, and humid places. This plant has a height of 10-20 cm with erect, soft and light green stems. This plant has a single leaf with a spiral position, oval shape, 1-4 cm length, 1.5-2 cm wide, pointed tip, incised base, flat edge, curved bones, smooth surface, soft, and green. This plant has compound flowers, grain-shaped, located at the end of the stem or in the axilla leaf, 2 – 3 cm long-grain, soft stem, and yellowish-white. The roots of this plant are fibrous, white, and shallow roots (Heyne, 1987).

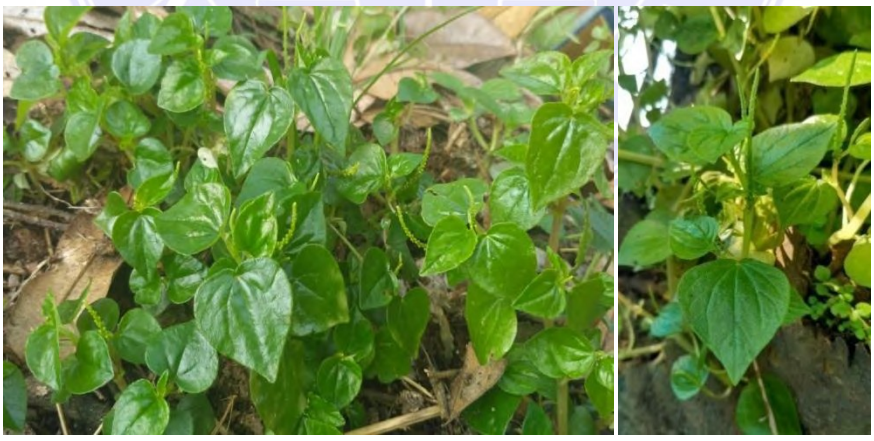

Figure 1. Chinese Betel Plants (*Peperomia pellucida L.*).
(Source: Private Collection)

The classification of the Chinese betel plant is as follows: Kingdom: Plantae; Subkingdom: Trachebionta; Superdivision: Spermatophyta; Division: Magnoliophyta; Class Magnoliopsida; Subclass: Magnoliidae; Order: Piperales; Family: Piperaceae;

Genus: Peperomia; Species: Peperomia pellucida L. Chinese betel plants have different names in each region, such as *Suruhan; Sladanan; Rangu-rangu* (Java), *Saladaan* (Sundanese), *Ketumpangan ayer* (Sumatra), and *Gofu doroho* (Ternate). (Heyne, 1987).

2.2. Chinese Betel Plant Chemical Compounds

This plant contains many chemical compounds that have been studied previously. In a study conducted by Xu et al. (2005), this plant contains essential oil compounds, especially *carotol dillapiole* and *β-carophyllene*. In a study conducted by Majumder and Kumar (Majumder and Kumar, 2011), this plant has steroid compounds, flavonoids, carbohydrates, alkaloids, flavonoids, saponins, tannins, and triterpenoids (Irsyad, 2013). From the phytochemical results conducted by Angelina et al. (2015), the Chinese betel plant (*Peperomia pellucida L.*) contains alkaloids, flavonoids, saponins, tannins and triterpenoids. With the compounds contained in this plant (*Peperomia pellucida L.*), it can be assumed that this plant can inhibit the growth of bacteria.

In their research, Nwokocha et al. (Nwokocha et al., 2012) stated that tannin and flavonoid compounds have antiseptic and antimicrobial activity. Tannins act as antibacterials through a complex formation with microbial enzymes or substrates entering through the cell membrane. Flavonoids work as antimicrobials by forming extracellular protein complexes and cell walls. Flavonoids are lipophilic, which can damage cell membranes.

2.3. Benefits of the Plant

The Chinese betel plant (*Peperomia pellucida L.*) has traditionally been used in treating several diseases, such as abscesses, boils, acne, skin inflammation, kidney disease and stomach pain. Other benefits of the Chinese betel plant (*Peperomia*

pellucida L.) include headache and fever medicine (Oloyede, 2011).

According to Sio Susie O (2001), this plant is used as an alternative treatment for gout. Meanwhile, according to Mappa et al. (2013), this plant is used as a wound-healing drug. The potential of this plant as an anticancer, antimicrobial and antioxidant compound has been reported by Wei et al. (2011). In their research, Seikh et al. (Sheikh et al., 2013) stated that this plant has analgesic, anti-inflammatory, and hypoglycemic activities. According to Nwokocha (Nwokocha, 2012), this plant can be used as an antimicrobial, anticancer, antibacterial, and antihypertensive.

2.4. Extraction and Extract

2.4.1. Extraction

Extraction is a technique for separating a compound based on the difference in solutes between two miscible solvents. In general, the extracted solvent is insoluble or slightly soluble in one solvent but easily soluble in other solvents. According to the Ministry of Health of the Republic of Indonesia (2000), extraction is the activity of withdrawing the content of soluble chemical compounds to be separated from insoluble materials with liquid solvents. Extraction can be carried out by various methods depending on the purpose of extraction, the type of solvent used, and the desired compound.

The extraction methods used in this study include:

Maceration

Maceration is a simplicia filtering process by immersion using a solvent with stirring at room temperature. Maceration carried out with continuous stirring is called kinetic maceration. Meanwhile, maceration carried out by repeated addition of solvent after filtering the first macerate and so on is called remaceration. In this study, the

method used is the maceration method since this method is simpler. This method can attract both heat-resistant and non-heat-resistant compounds. Ministry of Health of the Republic of Indonesia, 2000).

2.4.2. Extract

Extracts are dry, viscous and liquid preparations made by filtering simplicia, outside the influence of direct sunlight (Ministry of Health of the Republic of Indonesia 2000). Extracts are grouped based on their properties, namely:

1. Dilute extract;
2. Thick extract;
3. Dry extract;

2.5. Sterilization

Sterilization is an attempt to free tools or materials from all unwanted microorganisms. The investigation of a pure culture species is based on the investigation of the pure culture properties of that species. In order to maintain pure cultures, sterile tools and media are needed. Several ways can be used for sterilization, namely physical sterilization and chemical sterilization. In this study, the sterilization used was physical sterilization, namely, sterilization carried out in the following ways:

- Incandescent Sterilization. This method is used to sterilize an inoculated wire (Ose Needle) by burning the device over a spiritual lamp until it glows.
- Dry Heat Sterilization (Hot Air).

  This method is used to sterilize glassware. The tool used is an oven with a temperature of 170°C - 180°C for 2 hours.
- Pressed Steam Sterilization (Wet).

  This method is used to sterilize tools and materials that are resistant to high-

pressure temperatures. The tool used is an autoclave with a temperature of 110°C-121°C (Kristanti, 2014).

2.6. Bacteria Used In the Research

In this study, the test bacteria used were *Staphylococcus aureus* and *Staphylococcus epidermidis*.

2.6.1. *Staphylococcus Aureus* Bacteria

The name *Staphylococcus aureus* comes from the word *staphele* that means a collection of grapes, and the word *Aureus* that means gold. The name is based on the shape of the golden-coloured bacterial cells. These bacteria are gram-positive bacteria that are spherical (coccus) with a size of about 1 µm and are arranged in irregular groups, do not form spores, and do not move. The cells are shaped like grapes. However, in liquid culture, this type of bacteria may exist separately (singular), paired in the form of a tetra (4 cells), also in the form of a chain. The colonies are gray to dark golden yellow (Jawetz, 1996). The metabolism of these bacteria is aerobic and anaerobic.

Classification of *Staphylococcus aureus* bacteria:

Domain : Bacteria

Kingdom : Eubacteria

Division : Firmicutes

Class : Bacilli

Order : Bacilles

Family : Staphylococcaceae

Genus : Staphylococcus

Species : Staphylococcus aureus

These bacteria can grow well at 37°C but form the best pigments at 20°C - 25°C. The optimum temperature for the growth of *Staphylococcus aureus* bacteria is 35°C - 37°C, with a minimum temperature of 6,7°C and a maximum temperature of 45,5°C. *Staphylococcus aureus* can grow in a pH range of 4,0 – 9,8 with an optimum pH of around 7,0 – 7,5. *Staphylococcus aureus* is relatively resistant to drying, heat and is resistant to 9% NaCl. However, the growth of these bacteria is easily inhibited by certain chemicals (Jawetz, 1996).

2.6.2. Staphylococcus Epidermidis Bacteria

The classification of *Staphylococcus epidermidis* is as follows:

Kingdom : Bacteria

Phylum : Firmicutes

Class : Bacili

Order : Bacilles

Family : Staphylococcaceae

Genus : Staphylococcus

Species : Staphylococcus epidermidis

Staphylococcus epidermidis is an opportunistic bacterium that attacks individuals when the body's system is weak. The critical characteristics of *Staphylococcus epidermidis* bacteria are cocci-shaped and 0,5-1,5 m in diameter. *Staphylococcus epidermidis* colonies in clusters resembling grapes. The colonies are usually white or cream in color. These bacteria are Gram-positive. *Staphylococcus epidermidis* is a facultative aerobic (Jawetz, 1996).

2.7. Antibacterial Testing Method

Antibacterial is a substance or compound used explicitly for a group of bacteria. Antibacterial can be distinguished based on its work mechanism, namely antibacterial that inhibits cell wall growth and antibacterial that causes changes in cell membrane permeability. Antibacterial activity can be divided into two types: bacteriostatic activity (inhibits growth but does not kill pathogens) and bactericidal activity (can kill pathogens in a wide range).

One of the antibacterial tests can be done by a disk diffusion test. This is done by measuring the diameter of the clear zone, which indicates a response to the inhibition of bacterial growth by antibacterial compounds in the extract. The disk diffusion test is one method that is often used. This method can be done in 3 ways: the cylinder method, the hole/well method, and the disc method. (Eli, 2017).

According to the general standard for drugs from the Ministry of Health of the Republic of Indonesia (1998), bacteria are sensitive to antibacterials from plants if they have an inhibitory zone of 12-24 mm. Meanwhile, according to Greenwood (1995, in Ibrahim, 2013), the effectiveness of antibacterial can be classified in the following table:

Table 2.7. Bacterial Growth Inhibitory Response Classification

Inhibition Zone Diameter	Growth Barrier Response
<10 mm	None
10-15 mm	Weak
16-20 mm	Moderate
>20 mm	Strong

Source: Ibrahim, 2013.

CHAPTER III

RESEARCH METHODOLOGY

3.1. Methods of the Research

This research was conducted using experimental laboratory methods. An antibacterial test was carried out using an agar diffusion method using a blank disk to determine the diameter of the inhibition zone. The tests were arranged in a Completely Randomized Design (CRD) with five treatments and four replications using various extract concentrations, namely; 0%, 25%, 50%, 75% and 100%. The data obtained were then analyzed using the ANOVA (Analysis Of Variance) method.

3.2. Implementation of the Research

This research was conducted in March – June 2019 at the North Sumatra Regional Health Laboratory.

3.3. Population and Sample

Sampling was done randomly on Jl. Kolam no.7 Medan Estate as much as ± 4 kilograms. The bacterial cultures used were obtained from the USU Pharmaceutical Microbiology Laboratory and the North Sumatra Regional Health Laboratory.

3.4. Tools and Materials

The tools and materials used to conduct this research were: petri dish, dropper pipette, micropipette, petri disk, microscope, funnel, plastic, tissue, 250 ml Erlenmeyer

flask, and 100 ml measuring cup, glass rod, filter paper, ose needles, sterile cotton swabs, label paper, aluminum foil, plastic wrapping, autoclave, water bath, and Bunsen.

The materials used were Chinese betel plants (*Peperomia pellucida L.*). The chemicals used in this study were 70% ethanol (technical), distilled water, spirit, Mc. Farland standard solution, chloramphenicol antibiotics and blank disk. The media used in this study were Mannitol Salt Agar (MSA) and Mouler Histone Agar (MHA) media. The bacteria used in the study were *Staphylococcus aureus* and *Staphylococcus epidermidis cultures*.

3.5. Procedures of the Research

The working procedure used in this study consisted of several stages, namely:

3.5.1. Sample Preparation

Chinese betel plants were obtained from Medan City on Jl. Kolam No.7 Medan estate as much as ± 4 kilograms. First of all, the author took the plant parts (leaves, stems and flowers), then dried them at room temperature (not exposed to direct sunlight) until the water content was reduced by 10% (±2 days).

3.5.2. Making China Betel Plant Extract

The plant parts that have been dried and crushed using a mortar to form a powder were weighed as much as 200g. After that, they were macerated using 70% ethanol (Technical) solvent as much as 1200 ml (1:6). The plants were then allowed to stand for 3x24 hours by changing the solvent every 24 hours. Then, the plants were filtered using filter paper to obtain the filtrate. The result was a filtrate evaporated using a vacuum rotary evaporator to obtain a thick extract and evaporated using a water bath

at a temperature of 70°C-80°C to evaporate the ethanol solvent. Thenceforth, a pure extract of Peperomia pellucida L. would be obtained (Mulyani, Isbiantoro, & Fatimah, 2017).

3.5.3. Tool Sterilization

Tool sterilization was carried out by the dry heat method using an oven. Meanwhile, media sterilization was carried out with moist heat, namely, using an autoclave. Before the remaining test sample was removed, an inactive process using the moist heat method was carried out. The remaining test sample was then disposed of at the waste treatment plant.

3.5.4. Making Concentration Variable Stock

The variables used in this study amounted to 7 variables. The negative control was distilled water, while the positive control used a chloramphenicol disc. The variations in extract concentration were 25%, 50%, 75% and 100%. Making 25% concentration: 0,25 g of sample was added with 9,75 ml of distilled water; making 50% concentration: 0,5 g of sample was added with 9,5 ml of distilled water; making 5% concentration: 0,75 g of sample was added with 9,25 ml of distilled water; making 100% concentration: the concentration was not added with distilled water.

3.5.5. Pure Culture Rejuvenation of Test Bacteria

A total of one pure culture colony of test bacteria obtained from the USU Pharmaceutical Laboratory was taken using a sterile tube from the pure culture. Next, the colonies were inoculated in Nutrient Agar (NA) medium and incubated in an incubator at 37°C for 1x24 hours. Observations of test bacteria were carried out,

including colony morphology and gram staining (Kristanti, 2014).

3.5.6. Making Bacterial Suspension

Bacterial suspension is a pure culture of test bacteria that has been reproduced in a Nutrient Agar (NA) medium for 24 hours at a temperature of 25-30°C. 1 ose was taken from the bacterial cultures and then transferred in a 0,9% NaCl solution. The bacterial suspension was balanced using a nephelometer (BD Phoenix) with a standard of 0,5 Mc Farland (estimated at $1,5x10^8$ bacterial cells/mL).

3.5.7. Anti-Bacterial Test

Effective testing of antibacterials was carried out using a method with several concentrations. The concentrations used were: 0%, 25%, 50%, 75%, 100%. The test was carried out by preparing the bacterial test suspension and preparing the Mueller Hinton Agar (MHA) media to be used.

A total of 10 mL of Mueller Hinton Agar (MHA) medium was put into a petri dish and allowed to solidify. After solidification, 1 ose of bacteria was taken, which had been measured based on the Mc. Farland 10^8CFU/ml standard. After that, it was smeared using a cotton bud evenly on the surface of the compacted Mueller Hinton Agar (MHA) media. Henceforth, blank disks that have been extracted using a micropipette with a predetermined concentration were inserted into the surface of the media, with a distance of 1-2 cm from one another to the edge of the petri dish. Chloramphenicol acted as a positive control (+), while distilled water acted as a negative control (-). Then, it was incubated at 44°C for 1x24 hours. Furthermore, the inhibition zone formed was observed, and the inhibition zone diameter was measured

using a caliper. The author performed four repetitions at each concentration of extract.

Barrier diameter measurement can be done with a caliper using the following formula (Kristanti, 2014):

$$R(\%) = \frac{d1+d2}{2}$$

Note:

R = Resistance (mm)

D1= Diameter of the longest Inhibitory Zone (mm)

D2= Diameter of the shortest inhibition zone (mm)

CHAPTER V

CONCLUSION AND SUGGESTION

5.1. Conclusion

Based on the research that has been done, it can be concluded that in this study, the Chinese betel plant extract (*Peperomia pellucida L.*) does not have antibacterial activity since it has not been able to inhibit the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

5.2. Suggestion

After knowing the results that the Chinese betel plant (*Peperomia pellucida L.*) extract shows zero results, further research should be carried out using different types of bacteria. In the extraction process, it is recommended to use a larger solvent concentration.

PROOFREADING

1.	done	:	made
2.	that have	:	with
3.	Chinese betel plant	:	The Chinese betel plant
4.	also	:	and
5.	long grain	:	long-grain
6.	yellowish white	:	yellowish-white
7.	was	:	is
8.	kumar	:	Kumar
9.	titerpenoids	:	triterpenoids
10.	which enter	:	entering
11.	of	:	for
12.	wound healing	:	wound-healing
13.	et al	:	et al.
14.	solvent,	:	solvent
15.	so that they can	:	to
16.	Preparations,	:	preparations
17.	high pressure temperature	:	high-pressure temperature
18.	-	:	is
19.	laboratory experimental	:	experimental laboratory
20.	Antibacterial test	:	An antibacterial test
21.	micro pipette	:	micropipette
22.	Nutrient Agar (NA) medium	:	a Nutrient Agar (NA) medium
23.	while	:	, while
24.	showes	:	shows
25.	technique	:	a technique
26.	extracts	:	extract
27.	maceration method	:	the maceration method
28.	solvent	:	a solvent
29.	was	:	is
30.	Gram positive	:	Gram-positive
31.	so that	:	, so that
32.	high-salt	:	high salt
33.	Acd	:	Acid