CHAPTER I

INTRODUCTION

1.1. Background

Indonesia is a country having abundant natural resources, especially a diversity of plants. Indonesia has various types of medicinal plants. More than 20,000 types of medicinal plants spread throughout the territory of this country and only about 300 species of them have been used as traditional medicine. Restorative materials deriving from plants are a potential source of new traditional medicines. The use of plants as traditional medicinal ingredients requires scientific research to find out the truth of their efficacy so that they are not in doubt and can be reliable. It will further encourage people to use plants as medicinal raw materials (Atikah, 2013).

The handeuleum plant (*Graptophyllum pictum L. Griff.*) is planted mostly only as an ornamental plant. Some individuals even consider it a wild plant, so it grows unused and even they throw it away.

The results of previous studies reported that the ethanol extract of handeuleum leaves was able to inhibit the growth of *E.coli* bacteria causing diarrheal disease and inhibit the growth of *S.aureus* and *E.coli* bacteria (Riza 2010; Sitompul 2011).

The description above shows the effect of *handeuleum* leaves as antimicrobial because *handeuleum* leaves have secondary metabolites that are strong enough to be usable as medicinal ingredients. Therefore,

this study used compounds in handeuleum leaf extract to determine the antimicrobial effect inhibiting the growth of *Pseudomonas aeruginosa* and *Escherichia coli*.

1.2. Question

The question used in this study is how to extract the handeuleum leaf extract samples using ethylacetate, n-hexane solvents, phytochemical screening analysis and analysis of antimicrobial activity tests of the handeuleum leaf extract samples?

1.3. Objective

The objectives of this study were to extract samples of handeuleum leaf extract using ethylacetate and n-hexane solvents and analyze phytochemical screening and antimicrobial activity tests of the handeuleum leaf extract samples.

1.4. Benefit

The benefit of this study is as a source of scientific information about use of handeuleum leaf extract samples as natural antimicrobial ingredients.

CHAPTER II

STUDY OF LITERATURE

1.1. Medicinal Plant

Medicinal plants are plant species found to have medicinal properties, classified into the following three groups: (1) Traditional medicinal plants, namely plant species that the people know and believe have medicinal properties and have been usable as raw materials for traditional medicines. (2) Modern medicinal plants, namely plant species scientifically proven containing bioactive compounds/materials with medicinal properties and their use can be justified medically. (3) Potential plants, namely plant species containing bioactive compounds/materials with medicinal properties, but not yet scientifically proven medical or its usage as an ingredient of traditional medicine, are difficult to trace (Zuhud et al. 1994). There are 1,845 species of medicinal plants spreading across various forest formations in Indonesia. Approximately 30,000 to 40,000 species of plants spread from Aceh to Papua, from the lowlands to the highlands, from the tropics to cool areas, even plants and marine resources usable as medicine (Wijayakusuma, 2000).

Medicinal plants are plants that have medicinal properties and are usable as medicine in healing and preventing disease. The definition of efficacious medicine contains active substances that function to treat certain diseases or they do not contain certain active substances but contain a resultant effect/synergy of various substances that function to

treat. One of the medicinal plants used by the people is the handeleum leaf plant. The local people use this plant as an antidiuretic drug, hemorrhoids, constipation, wound healing, ulcers, bruises, menstrual flow (Dalimartha, 1999), anti-inflammatory, and antidiarrheal (Sumarny, 1993).

Figure 1. *Graptophyllum pictum L. Griff*.
(Collection sources 20160906_073207 and 20160906_073342)

Description of the Handeuleum Plant

Plant of handeuleum (*Graptophyllum pictum L. Griff*.) originally deriving from Irian and Polynesia can be found from the lowlands to the mountains in altitude of 1,250 masl. It is a shrub or small tree, 1.5-3 m high, woody stem. Its skin and leaves are slimy and smells bad. Its branches are obtuse-angled, pole-shaped and tightly segmented. It is single leaf, short-stemmed, oppositely crossed, ovate to lanceolate,

pointed tip and base, but wavy, pinnate bone, 8-20 cm long, 3-13 cm wide, upper surface glossy purple.

It has compound flowers, out of the end of the stem, arranged in a series of bunches 3-12 cm long, purplish-red. Its fruit is a box fruit, oval in shape, brownish-purple. Its seeds are sometimes 2, round in shape, white. Handeuleum plant is often growing wild in the countryside or growing as ornamental plants and hedge plants. It grows well in open places exposed to sunlight in a dry or humid climate. There are three varieties, namely purple leaf, green leaf and white stripes. A purple-leaved variety called *Graptophyllum pictum* (L.) Griff. var *luridosguineum* Sims, has medical function. This plant flowers throughout the year, but, in Java, it rarely produces fruit. It propagates through its stem cuttings (Dalimartha, 1999).

Chemical constituents of the Handeuleum plant include alkaloids, which are organic nitrogen-containing materials as part of the heterocyclic system. At the same time, flavonoids produce yellow, red, and blue pigments in plants and protect against insects and microbes. Saponins that are surfactants are antifungal, antibacterial, and lower blood cholesterol. Tannins are polyphenolic compounds and are usable as antidiarrheal, homostatic, and anti-inflammatory. In addition, tannins also control gastric problems and hemorrhoids. Triterpenoids and steroidal saponins are included in the secondary metabolites that are toxic to insects, bacteria, and fungi. The leaves of this plant contain non-toxic alkaloids, glycosides, steroids, phenols, polyphenols, tannins, saponins,

chlorophyll and mucus. Handeuleum plant leaf stems contain calcium, potassium, sodium, magnesium, oxalate, formic acid, essential oils and fats (Dalimartha, 1999).

Benefits of handeuleum plant leaves are efficacious as laxative urine (diuretic), maturing ulcers, skin softeners (emoliens), antidiarrhea (Sumarny, 1993). The flowers are efficacious as menstrual diuretic (Dalimartha, 1999).

Hutapea (1993) said that systematics (taxonomy) of Handeuleum plants are classifiable as follows:

Sinomin: *Graptophyllum hortense*. Nees., Kingdom: Plantae, Divisio: Planta, Class: Chlorophyllum, Ordo: Tubiflorae, Families: Acanthaceae, Genus: Graptophyllum, Species: *Graptophyllum pictum* L. Griff., general name: Wungu.

1.2. Phytochemical Screening

Phytochemical screening or chemical screening is the initial stage to identify chemical compounds' content in plants. The method used in phytochemical screening has requirements. Namely, the method is fast and straightforward. The equipment used is as little as possible, selective in identifying certain compounds, and can provide additional information about the presence of certain compounds in the group of compounds studied.

Alkaloid is a group of organic compounds that are mostly in nature. Most of all alkaloid compounds come from plants and widely distribute to

various types of plants. Alkaloids found in nature have certain biological activities, some are very toxic and some are very useful in medicine.

For example, quinine, morphine and stinkyne. Alkaloids can be obtainable in various plant parts such as seeds, leaves, twigs and bark. Alkaloids are generally in small concentrations and must separate from complex mixtures of compounds derived from plant tissues (Lenny, 2006).

Saponin is naturally occurring glycosides bound to steroids or triterpenes. Saponin has broad pharmacological activities, including immunology, antitumor, anti-inflammatory, antiviral, antifungal. It can kill shellfish, hypoglycemic and hypocholestrol effects.

Saponin also has various properties such as sweet taste, bitterness. It can be in the form of foam, stabilize emulsions and cause hemolysis. In its use, saponin can be usable for many purposes. For example, they are usable to make alcoholic beverages, in the clothing industry, cosmetics, to make medicines and are usable as traditional medicines (Rustaman et al., 2000).

Flavonoid is a group of secondary metabolites that are most commonly found in plant tissues. Flavonoid belongs to the class of phenolic compounds. Phenol compounds can bind to proteins. The process of photosynthesis influences presence of flavonoids in plant leaves so that young leaves do not contain too many flavonoids. Biologically, flavonoids play an important role in plant pollination by

insects. A number of flavonoids have a bitter taste to resist certain types of caterpillars (Redha, 2010).

Flavonoids are plant pigments with yellow, orange, yellow, and red colors that can be obtainable in fruits, vegetables, nuts, seeds, stems, flowers, herbs, spices, food, and medicinal products from plants such as olive oil, tea, chocolate, red wine and herbal medicine. These compounds play an important role in determining the color, taste, smell and nutritional quality of food. For plants, flavonoid compounds play a role in self-defense against pests, interactions with microbes, seed dormancy, protection against UV radiation, signal molecules in various transduction pathways, and signal molecules in pollination and male fertilization (Mulyaningsih, 2014).

Tannins widely distribute to vascular plants. In angiosperms, they are especially obtainable in woody tissues. Tannins have properties such as soluble in water or alcohol because tannins contain much phenol having OH group, can bind heavy metals, and the presence of termite and fungal substances (Rustaman et al., 2000).

1.3. Extraction Process

Extract is the extraction of nutritious substances or active substances from medicinal plants, animals and several types of fish, including marine biota. The active substances are present in the cells, but plant and animal cells are different and their thickness. Hence, an extraction method using certain solvents is necessary to extract them.

Extraction of chemical content in plants is executable to attract chemical substances contained in simplicia, namely natural ingredients found in plants.

This extract is under the principle of mass transfer of the components of the substance into the solvent, where the transfer begins to occur at the interface layer and then diffuses into the solvent. The fragrant *pandanus* plant contains several active substances where properties depend on the type of solvent used to extract the leaves (Aisyah, 2015).

Mukhriani, 2014 suggested the manufacture of extracts, especially for materials derived from plants, in the stages as follows:

(1) Grouping of plant parts (leaves, stems, flowers etc.), drying and grinding of plant parts,

(2) Solvent selection is usable to separate or isolate the active substance. The solvent selected is selectively dependent on the desired active substance,

(3) Separation and purification, these are the separation of active substances that are expectable to obtain pure extract,

(4) Extract drying aims at removing the solvent from the material to produce a cloudy dry mass,

(5) Yield is the ratio between the extract obtained and the initial simplicia.

The types of extraction methods that can be usable are as follows:

a. Maceration

Maceration is a simplicia extraction process using a solvent in several times of shaking or stirring at room (chamber) temperature. Maceration aims at attracting nutritious substances that are heat-resistant and non-heat-resistant. Maceration is the simplest extraction method (Istiqomah, 2013).

This method is executable by inserting plant powder and suitable solvent into an inert container that tightly close at room temperature. The extraction process stops when equilibrium reaches the concentration of the compound in the solvent and the concentration in the plant cell. After the extraction process, the solvent separates from the sample through filtration. The main disadvantage of this maceration method is that it takes a lot of time, the solvent used is quite a lot and it is possible that some compounds are lost. On the other hand, this method can avoid the destruction of thermolabile compounds (Mukhriani, 2014).

b. Percolation

Percolation is filtration by flowing the filter fluid through the moistened simplicia powder. The name of tool used to extract is a percolator, and name of the collected extract is a percolate (Ibtisam, 2008).

In the percolation method, the sample powder is slowly moistened in a percolator. The solvent is added to the top of the sample powder and allowed to drip slowly to the bottom. The advantage of this method is that the sample is always flooded with new solvents. While the disadvantage is

that if the sample in the percolator is not homogeneous, the solvent will be challenging to reach the entire area. In addition, this method requires many solvents and takes longer time (Mukhriani, 2014).

c. Soxhlet

Soxhletation is extraction using solvent that is always new, which is generally carried out with special tools so that continuous extraction occurs in relatively constant amount of solvent in the presence of back cooling (Istiqomah, 2013). The advantage of this extraction is that the extraction process is continuous. The sample extraction uses condensed pure solvent so it does not require a lot of solvent and does not take much time.

The disadvantage of this extraction is that thermolabile compounds can degrade because the extract obtained is constantly at the boiling point (Mukhriani, 2014).

d. Reflux and Steam Distillation

In the reflux method, the sample is put with the solvent into a flask connected to a condenser. The solvent is heated until it reaches the boiling point. The vapor condenses and returns to the flask. Steam distillation has the same process and is commonly usable to extract essential oils. During heating, the steam condenses and the distillate collects in a container contained in the condenser. The disadvantage of these two methods is that thermolabile compounds can degrade (Mukhriani, 2014).

1.4. Antimicrobial Activity

Antimicrobial testing method of a substance, the method that is often usable is the diffusion method. This method can executable by using a *disc* where the antimicrobial is inserted in a specific glass and placed on solid media inoculated with indicator bacteria after incubation. There will be a saturated area around the well or *disc* and the diameter of the barrier is a measure of the resistance strength of the antimicrobial substance against the bacteria used.

The width of the zone formed, which is also determined by the concentration of the effective compounds used, is the basis for quantitative testing. It indicates that these compounds can freely diffuse throughout the medium (Rochani, 2009).

Bacterial Growth Phase

Microbiologists know the phases in bacterial growth. There are four (4) phases of bacterial growth when grown in culture, namely the adaptation phase (*lag phase*), the *exponential phase*, the static phase (*stationary phase*), and the *death phase*.

Table 1. Characteristics and phases of bacterial growth (Source: Brock & Madigan 1991)

Growth Phase	Characteristic

Lag phase	After inoculation, there is an increase in cell size, starting at a time when the cells have no or minor division. This phase is characterized by an increase in macromolecular components, metabolic activity, and susceptibility to chemical substances and physical factors. The *lag phase* is a significant adjustment period for the addition of metabolites to the cell group, towards a level commensurate with maximum cell synthesis.
Exponential phase	In the exponential or logarithmic phase, the cell is in a state of balanced growth. During this phase, cell mass and volume increase by the same factor in that the mean cell composition and relative concentrations of metabolites remain constant. During this period of balanced growth, the rate of increase can be expressed by a natural exponential function. Cells divide at a constant rate determined by the intrinsic nature of the bacteria and environmental conditions. There are variations in the growth rates of various microorganisms.
Stationer phase	When normal culture conditions are usable, accumulation of waste products, nutrient deficiencies, changes in pH, and other unknown factors will overwhelm and disrupt the culture, resulting in decreased growth rates. During this phase, the number of living cells remains constant for different periods, depending on the bacteria, but eventually leads to a period of population decline. In some cases, cells present in a culture in which the cell population is not growing may elongate, swell abnormally, or undergo aberrations, a manifestation of imbalanced growth.
Death phase	When the medium runs out of nutrients, the bacterial population will decrease in number. At this time, the number of dead cells is more than the living cells.

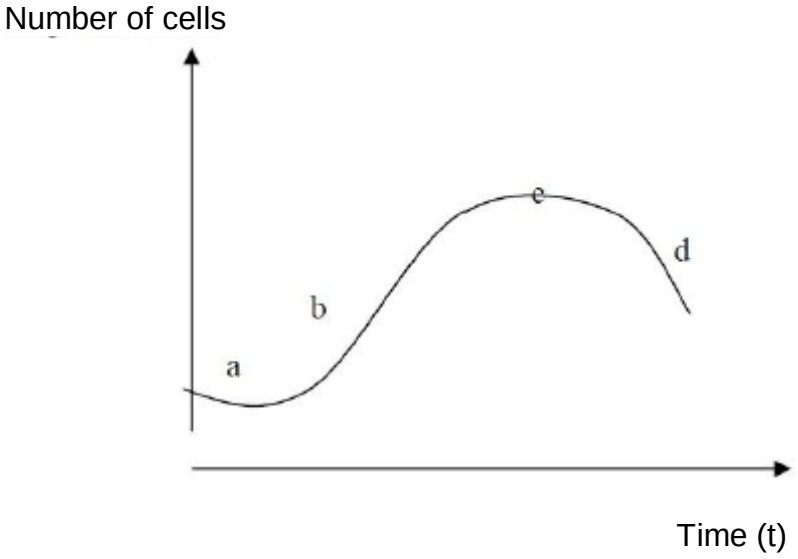

Figure 2. Bacterial Growth Curve: a. *lag phase*; b. *exponential phase*; c. *stationary phase* and d. *death phase* of population (source Brock & Madigan, 1991)

The purpose of antimicrobial testing is to obtain an effective and efficient treatment system. There are various antimicrobial testing methods, including: (Pratiwi, 2008)

1. Diffusion Method

There are five diffusion methods, namely *disc diffusion* (Kirby & Bauer test), *ETest, ditch-plate technique, gradient-plate technique, and cup-plate technique*. The method commonly used is the *disc diffusion* method (Kirby & Bauer test). The plate containing the antimicrobial agent is placed on an agar medium that has been planted with microorganisms that will diffuse into the agar medium. The clear area indicates the inhibition of the growth of microorganisms by antimicrobial agents on the surface of the agar medium.

2. Dilution Method

The dilution method is divided into two, namely the liquid dilution method and the reliable dilution method.

a. **Liquid dilution method**. This method is usable to determine the minimum inhibitory concentration (MIC) and minimal killing concentration (MBC) of the antibacterial material tested against the test bacteria. The trick is to dilute the test antibacterial material in a liquid medium until several concentrations are obtainable, then add the test bacteria to each concentration.
b. **Solid dilution method**. This method is the same as the liquid dilution method, but it uses a solid medium. The advantage of this method is that one concentration of the tested antibacterial agent can test several other bacteria.

A scientist from France stated that the agar diffusion method of the Kirby-Bauer procedure is often usable to determine bacterial resistance. The principle of this method is the inhibition of the growth of microorganisms, namely the zone of inhibition will be viewable as a clear area around the paper disc containing antibacterial substances. The diameter of the zone of inhibition of bacterial growth indicates the sensitivity of bacteria to antibacterial substances. Furthermore, it is said that the wider the diameter of the inhibition zone formed by the bacteria, the more resistant it is (Chambers, 2004).

In general, the method used in the bacterial resistance test is the Agar Diffusion method, namely by observing the inhibition of the growth of

microorganisms through available extracts from the area around the paper disc that is not overgrown by microorganisms. This growth inhibition zone indicates the sensitivity of bacteria to antibacterial agents (Ermilla, 2006).

The Zone of Inhibition is a place where the growth of bacteria is inhibited due to antibacterial or antimicrobial. The zone of inhibition is an area to inhibit the growth of microorganisms on agar media by antibiotics. For examples: tetracycline, erythromycin, and streptomycin. Tetracycline is an antibiotic having a broad spectrum so that it can inhibit the growth of bacteria widely (Novillia, 2008).

One of the methods used is the diffusion method consisting of several ways, including:

1. *Disc diffusion* method (Kirby and Bauer test) is to determine the activity of antimicrobial agents. The plate containing the antimicrobial agent is placed on an agar medium planted with microorganisms that will diffuse into the agar medium. The clear area indicates the inhibition of the growth of microorganisms by antimicrobial agents on the agar surface.
2. The *E-test* method is useable to estimate the MIC (minimum inhibitory concentration) or MIC (minimum inhibitory concentration), which is the minimum concentration of an antimicrobial agent to inhibit the growth of microorganisms. In this method, plastic strips containing antimicrobial agents from the lowest to the highest levels are used and placed on the surface of the agar media planted with microorganisms.

Observations were made on the resulting clear area showing the levels of antimicrobial agents that inhibit the growth of microorganisms on agar media.

3. *Ditch-plate technique*. In this method, the test sample in the form of an antimicrobial agent is placed in a trench made by cutting the agar medium in a petri dish in the middle longitudinally and the test microbe (maximum 6 kinds) is streaked towards the trench containing the antimicrobial agent.
4. The *Cup-Plate Technique* method is similar to the disc diffusion method, where a well is made on agar media that has been planted with microorganisms and the well is given an antimicrobial agent to test.
5. *Gradient-Plate Technique*. In this method, the concentration of antimicrobial agent on agar media theoretically varies from 0 to maximum. The agar medium is melted and the test solution is added. The mixture is then poured into a petri dish and placed in an inclined position. The second nutrient is then calculated on top of it (Ermilla, 2006).

Pathogenic Microbes

Microbes are a group of microorganisms that cannot be viewable using the naked eye. Therefore, tools are necessary to view them such as microscopes, loupe and others. Pathogenicity is the ability of an organism

to cause disease (Pelczar and Chan, 1988). Thus, pathogenic microbes are microbes that have the ability to cause disease in humans. Pathogenic microbes in humans include *Escherichia coli* and *Pseudomonas aeruginosa*.

Bacteria and microorganisms adapt to the environment, including humans and animals, where microorganisms normally live. At work, microorganisms increase the ability to survive and increase the likelihood of spread. By producing asymptomatic infection or mild disease, and without causing the death of the host, microorganisms that normally live in the human body are likely to spread from one person to another (Jawetz et al., 2001). The microorganisms that can cause infection are *Pseudomonas aeruginosa* and *Escherichia coli*.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a significant pathogen for humans. These bacteria occasionally colonize humans and cause infection when the host defense function is abnormal. Therefore, *P. aeruginosa* is called an opportunistic pathogen, namely, it exploits damage to the host defense mechanism to initiate an infection. These bacteria can also live in normal humans and act as saprophytes in the normal intestine and on human skin (Boel et al., 2004).

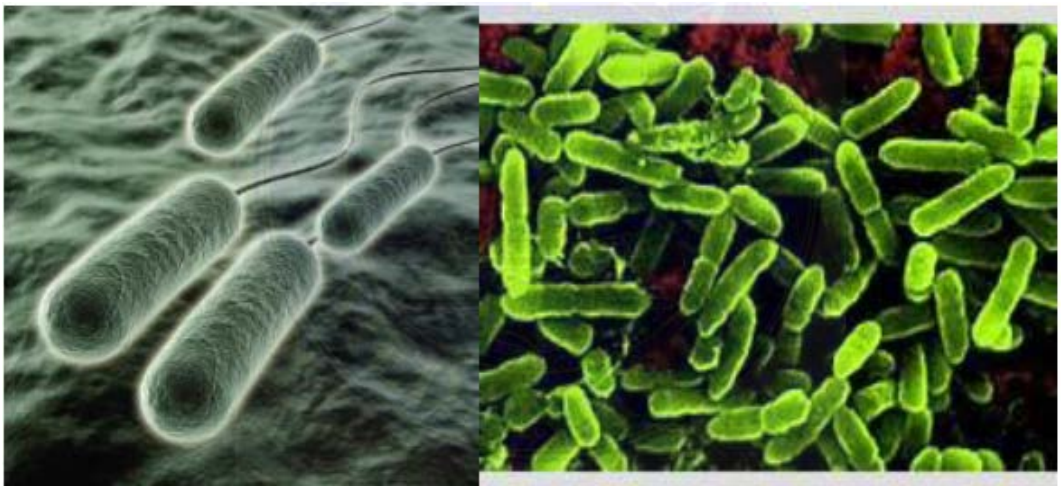

Figure 3. Morphology of *Pseudomonas aeruginosa* (source: bioquell.com)

Mayasari (2006) suggested that taxonomy *P. aeruginosa* is classifiable as follows; Kingdom : Bacteria, Phylum : Proteobacteria, Class : Gamma Proteobacteria, Ordo : Pseudomonas, Family : Pseudomonas, Genus : Pseudomonas, Species : *Pseudomonas aeruginosa*. The morphology of *P. aeruginosa* bacteria is rod-shaped in size of about 0.6 x 2 µm. These bacteria are seen as single bacteria, in pairs, and sometimes form short chains. *P. aeruginosa* includes gram-negative bacteria. These bacteria are aerobic, catalase positive, oxidase positive, unable to ferment but can oxidize glucose/other carbohydrates, do not have spores, have no sheath and have a monotric flagellum (single flagellum at the poles) so they are always moving. The optimum temperature for the growth of *P. aeruginosa* is 42 ^{o}C.

Escherichia coli

Escherichia coli is commensal bacteria that can be pathogenic, acting as a significant cause of morbidity and mortality worldwide.

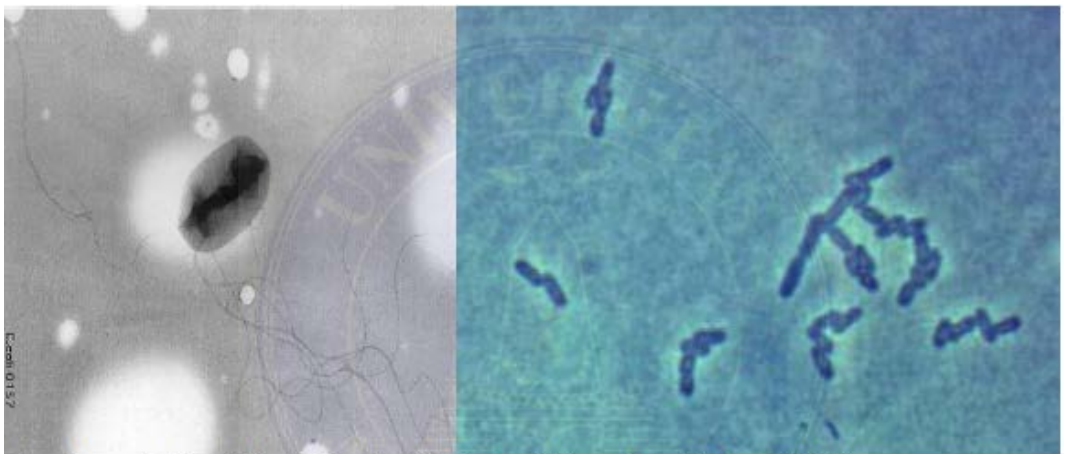

Figure 4. Morphology of *Escherichia coli* (source: Todar 2008)

According to (Tenailon et al., 2010), the taxonomy *E.coli* is classifiable as follows; Kingdom: Bacteria, Division: Proteobacteria, Class: Gamma Proteobacteria, Ordo: Enterobacteriales, Family: Enterobacteriaceae, Genus: Esherichia, Species: *Esherichia coli*.

The growth of *Escherichia coli* bacteria indicates that *Escherichia coli* was firstly isolated by Theodore Escherich in 1885 from the feces of an infant (Merchant and Parker, 1961). *E.coli* is a short rod-shaped Gram-negative bacterium that has length of about 2 m, diameter of 0.7 m, width of 0.4-0.7 m and it is a facultative anaerobe. *E.coli* forms round, convex, smooth colonies with marked edges (Smith-Keary, 1988). In general, bacteria require high humidity of around 85% (Madigan and Martinko, 2005).

Escherichia coli is a group of mesophilic bacteria, namely bacteria at which optimum growth temperature is 15-45°C and can live at pH 5.5-8.

Escherichia coli grows optimally at a temperature of 27°C. According to research conducted, *Escherichia coli* has a maximum growth temperature of 40-45°C, above that temperature, the bacteria will experience inactivation. *Escherichia coli* bacteria that cause diarrhea are prevalent throughout the world.

1.5. Antibiotics

Antibiotics, also known as anti-bacterial drugs, are drugs used to treat infectious diseases caused by bacteria. In 1927, Alexander Fleming discovered the first antibiotic, namely penicillin. In 1940, antibiotics could be said to change the world of medicine and dramatically reduce morbidity & mortality caused by infectious diseases (Ganiswarna, 1995).

The definition of antibiotics originally referred to compounds produced by fungi or microorganisms that can kill disease-causing bacteria in animals & humans. Currently, several types of antibiotics are synthetic compounds (not produced from microorganisms) but can also kill or inhibit bacterial growth. Technically, substances that can kill bacteria in the form of synthetic or natural compounds are called antimicrobial substances, but many people call them antibiotics. Antibiotics have many benefits. Excessive use of antibiotics can also trigger antibiotic resistance (Wasitaningrum, 2009).

Antibiotics are chemical substances produced by fungi and bacteria that have the property of killing or inhibiting the growth of germs while their toxicity to humans is relatively small. Researchers around the world have

obtained many other substances with antibiotic properties, but due to their toxicity to humans, only a small portion of them can be usable as drugs, including streptomycin via injection, Tetracycline capsules, Kanamicin capsules, Erythromicin capsules, Colistin tablets, Cefadroxil tablets and Rifampicin capsules (Djide, 2003).

The activity of antibiotics was discovered for the first time by a British scholar, dr. Alexander Flemming in 1928 (penicillin). This discovery was only developed and used in therapy in 1941 by Dr. Florey (Oxford), then many other substances with antibiotic properties were isolated by investigators around the world, but due to their toxic properties only a few can be usable as drugs (Djide, 2003).

Antibiotics are usable to kill microbes that cause infection. Symptoms of infection occur due to direct interference by microbes and various toxic substances produced by microbes. Body's defense system can overcome an infection, but sometimes this system needs support by the use of antibiotics. Antibiotics used to eradicate microbes that cause infection in humans must have selective toxicity properties (Ganiswarna, 1995).

Types of Antibiotics

According to Misra (2012), antibiotics can be classifiable into several types and different functions, including:

1. Penicillin

Penicillin is widely usable to treat certain infections such as skin infections, strep throat, chest infections and urinary tract infections. Some types of widely used penicillin include: Antibiotics Amoxicillin and Flucloxacillin. About 1 in 15 people will experience an allergic reaction after using penicillin drugs and a small number of people will have a severe allergic reaction to antibiotics (anaphylaxis).

2. Cephalosporin Antibiotics

Cephalosporins are broad-spectrum antibiotics. It means that they are effective in treating a wide variety of infections, including more serious infections, such as: Septicemia - infection of the blood, Pneumonia, and Meningitis - infection of the outer protective layer of the brain and spinal cord. Examples of Cephalosporins include the drugs Cefalexin and Cefixime.

3. Aminoglycosides

Aminoglycosides are a type of antibiotic drug that was usable widely prescribed until it was discovered that Aminoglycosides could cause damage to both hearing and kidneys.

Because of this, Aminoglycosides tend to now be usable only to treat very serious illnesses such as meningitis. Aminoglycosides dissolve quickly in the digestive system so that they must be given using injection or drops.

4. Tetracycline drugs

Tetracycline is another type of broad-spectrum antibiotic that can be usable to treat a wide variety of infections. Tetracycline is also commonly an antibiotic used to treat severe acne and a condition called rosacea, which causes redness of the skin and spots.

5. Macrolides

Benefits of antibiotics Macrolides are a type of antibiotic that is useful in treating lung and chest infections. Macrolides can also be a useful alternative treatment for people having penicillin allergies or may prevent bacteria that are resistant to penicillin drugs. Examples of macrolide antibiotics are Erythromycin and Spiramycin.

6. Fluoroquinolone

Fluoroquinolone is the newest type of antibiotic. Fluoroquinolone is broad-spectrum antibiotics that can be usable to treat a wide variety of infections. Examples of fluoroquinolones are drugs of Ciprofloxacin and Norfloxacin.

CHAPTER III

METHOD

3.1. Location and Time

This study started from February to March 2017 at the Microbiology Laboratory of the Industrial Chemical Technology Polytechnic of Medan and the Biology Laboratory of the University of Medan Area.

3.2. Materials and Tools

The tools used in this study were petri dishes, beaker glass, spatula, measuring cup, analytical balance, knife, napkin, lighter, filter, mortar, test tube, water bath, blankdisch, tweezers, Bunsen, label paper, camera. The main materials used in this study were handeuleum leaves obtained from residents' yards and parks in the Tanjung Rejo area, Medan Sungggal sub-district. The chemicals used in this study were aquadest, amoxicillin, ethyl acetate ($C_4H_8O_2$) (technical), H_2SO_4 (concentrated), acetic acid (CH_3COOH) (concentrated), hydrochloric acid (HCl), n-hexane (C_6H_{14}) (technical) , Ferric trichloride ($FeCl_3$), iodine (I), magnesium powder (Mg). The test media used in this study were Sodium Agar (NA) and Mueller Hinthon Agar (MHA). The test microbes used in this study were *Escherichia coli* and *Pseudomonas aeruginosa* obtained from subcultures at the Microbiology Laboratory, University of North Sumatra.

3.3. Methods

This study is experimental using qualitative and quantitative methods. The concentration of the extract in five treatments by extracting

the leaves of handeuleum used was 30%, 45%, 60%, 75% and the comparison antibiotic was Amoxicillin 500 mg with five replicates, the bacteria used were *Escherichia coli* and *Pseudomonas aeruginosa*. The parameter observed was the diameter of the inhibition zone caused by each extract concentration.

3.4. Procedure

This study carried out provision of reagents, sample preparation, extraction of active compounds from handeuleum leaves, phytochemical screening tests and antimicrobial tests.

Provision of Reagents

The reagents used in this study include 2N HCl, 1% FeCl3, Wagner and *Lieberman-Burchad*.

1. 2N.HCl solution

A total of 16.7 ml of 2N HCl was put into a 100 ml volumetric flask added with the distilled water up to the mark and homogenized.

2. 1% $FeCl_3$ solution

A total of 1 gram of $FeCl_3$ was put into a 100 ml volumetric flask, added with distilled water up to the mark and homogenized.

3. Wagner's reagent

A total of 2 grams of KI was put into a 100 ml volumetric flask, added with distilled water and stirred until the KI dissolved, then 1.27 grams of Iodine (I2) was added and aquadest was added up to the mark and homogenized.

4. *Lieberman-Burchard* reagent

A total of 20 ml of acetic acid p.a plus 10 ml of H_2SO_4 (p.a) was then dissolved in 50 ml of chloroform.

Sample Preparation

The samples used in this study were fresh handeuleum leaves. Fresh handeuleum leaves were cleaned, cut into small pieces, and dried in the sun, in moisture content of up to 10%. The handeuleum leaf samples were then mashed, filtered using a 40 mesh sieve and weighed as much as 200 grams.

Extraction of Underneath Leaf Active Compounds

Extraction was carried out by maceration method using ethylacetate and n-hexane solvent on 200 grams of handeuleum leaf simplicia in 600 mL of ethylacetate and n-hexane solvent for 3 x 24 hours and solvent replacement every 24 hours. The maceration results were filtered using a *Buchner* funnel, then the extract was concentrated using a *water bath* at 70°C to evaporate the solvent to obtain concentrated extracts of ethylacetate and n-hexane from handeuleum leaves, then the two extracts were tested for phytochemical screening.

Phytochemical Screening Test

To determine the class of secondary metabolites from handeuleum leaves, a phytochemical screening was carried out, consisting of alkaloids, flavonoids, saponins and tannins. Phytochemical screening was carried out using specific reagents for these compounds.

1. Flavonoid Compound Test

In the flavonoid compound test, 1 gram of the sample was put into a test tube. Then, 0.1 Mg powder and 1 ml HCl (p.a) and 0.4 mL amyl alcohol were added (a mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and 4 mL of alcohol was shaken and observed whether there was a red/purple, yellow/orange color change on the amyl alcohol layer indicating the presence of flavonoids.

2. Alkaloid Compound Test

In the test for alkaloid compounds, 1 gram of the sample was put into a test tube, 10 mL of 0.2N HCl was added and heated for 10 minutes at a temperature of 100 ^{o}C, then cooled and filtered. Then, 2 drops of iodine solution were added into 0.5 mL of the filtrate. If there was turbidity, until a light brown/red precipitate formed.

3. Tannin Compound Test

The tannin compound test was carried out using 10% $FeCl_3$ reagent. The appearance of black, blue, yellow/green precipitates indicates a positive tannin compound.

4. Saponin Compound Test

The saponin compound test was carried out using aquadest in a test tube and shaking vigorously for 30 seconds and permanent foam was formed for more than 10 minutes with the addition of 2 drops of 2N HCl, then it showed positive test for saponins.

5. Steroid Compound Test

The steroid compound test was carried out using 1 drop of H_2SO_4 (concentrated) reagent and 1 drop of acetic acid (concentrated) was added. The appearance of a blue/green precipitate indicates a positive steroid compound.

Test Suspension Making

Preparation of the test suspension was to take pure colonies of *Escherichia coli* and *Pseudomonas aeruginosa*, which had been cultured purely on *Nutrient Agar* (NA) media. Each of cultured bacteria was suspended in a test tube, added with 10 ml of distilled water in a turbidity level of 10^8 CFU.

Antimicrobial Test

To test the antimicrobial activity, handeuleum leaf extract was made by making a solution in concentrations of 30%, 45%, 60%, 75%, and amoxicillin with dimethylsulfuoxide (DMSO) and sterile distilled water was used for dilution. The bacteria were taken by using a sterile cotton bath. They were rubbed evenly on *Mueller Hinthon Agar* (MHA) media, which had been poured into a petri dish, using 4 blank discs in each petri dish and each of them had been soaked with extract according to the concentration, by pressing the blank disc that already contained handeueum leaf extract to stick well.

The cup that has been given bladisch and has been divided into four based on the concentration was incubated at 37^oC for 1 x 24 hours.

Observations were made by looking at the clear zone around the handeuleum leaf extract. This showed that the handeuleum leaf extract had the potential as an antimicrobial agent. A caliper could measure the diameter of the formed inhibition zone according to the *Kirby-Baurier of Susceptibility Test* method (Cappucino et al., 1999).

3.5. Data analysis

The data obtained from the results of the study were the diameters of the inhibition zone of each concentration of handeuleum leaf extract against *Escherichia coli* and *Pseudomonas aeruginosa* bacteria using five replications.

The data measured were diameters of the inhibition zone starting from the center point to the outermost area not overgrown with bacteria.

This study used a *completely randomized design* (CRD) for each type of bacteria consisting of 5 (five) treatment concentrations of handeuleum leaf extract, namely:

C_0 = control (Amoxicillin 500 mg)

C_1 = 30% concentration

C_2 = 45% concentration

C_3 = 60% concentration

C_4 = 75% concentration

The treatment was repeated 5 times

The data were analyzed by analysis of variance using a linear model as follows:

$$Yij = \mu + \alpha i + \varepsilon ij$$

$$i = 1,2,3,4,5$$

Where:

Yij : clear zone (mm) on the i-th treatment and the j-th repetition

μ : Median value (general average value)

αi : the influence of the i-th treatment

εij : the effect of error on the i-th treatment in the j-th repetition.

The data having a significant effect were continued by the mean difference test based on the *Duncan's Multiple Distance Test* (DMRT) at the 5% level.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1. Concussion

The results of the phytochemical and antimicrobial screening tests conducted, the phytochemical screening test showed the presence of secondary metabolites, namely alkaloids, flavonoids, triterpenoids, tannins, and saponins using ethyl acetate solvent and antimicrobial tests, namely handeuleum leaf extract. The results showed that the handeuleum leaf extract could inhibit the growth of *Escherichia coli* bacteria at the optimum concentration or the best concentration, namely, C3 with an mean inhibition zone of 1.82 cm and C4 with mean inhibition zone of 2.06 cm. While for *Pseudomonas aeruginosa,* the optimum concentration or the best concentration is C3 with an average inhibition zone of 1.99 cm and C4 with an average inhibition zone of 2.12 cm.

5.2. Recommendation

This study recommends the next authors to continue studies by finding the most effective solvent to extract the active compounds contained in the handeuleum leaf plants spread in Indonesia and to isolate, purify and identify active compounds contained in these plants of. handeuleum leaves spread in Indonesia.

PROOFREADING

1.	diversity	:	a diversity
2.	Medicinal	:	Restorative
3.	is a plant planted mostly	:	is planted mostly
4.	and was able to inhibit the growth of	:	and inhibit the growth
5.	Question used	:	The question used
6.	this study was to extract	:	this study were to extract
7.	found having medicinal	:	found to have medicinal
8.	believe that these have medicinal	:	believe have medicinal
9.	even to plants and marine	:	even plants and marine
10.	traditional medicine is difficult	:	traditional medicine are difficult
11.	It is shrub or small tree	:	It is a shrub or small tree
12.	obtuse angled	:	obtuse-angled
13.	purplish red	:	purplish-red
14.	While flavonoids function to produce yellow	:	At the same time, flavonoids produce yellow
15.	included in the group of secondary metabolites	:	included in the secondary metabolites
16.	simple and fast	:	fast and straightforward
17.	spices and food	:	spices food
18.	taste so that they can resist	:	taste to resist
19.	presence of substances that are termite and fungal	:	presence of termite and fungal substances
20.	contain a lot of phenol	:	contain much phenol
21.	substances from parts of medicinal plants	:	substances from medicinal plants
22.	different as well as their thickness	:	different and their thickness
23.	executable with the aim at attracting chemical	:	executable to attract chemical
24.	material so as to produce	:	material to produce
25.	difficult	:	challenging
26.	certain	:	specific
27.	little	:	minor
28.	a very important adjustment period	:	a significant adjustment period
29.	routine	:	normal
30.	solid	:	reliable
31.	known	:	available
32.	major	:	significant
33.	diarrhea are very common throughout the world	:	diarrhea are prevalent throughout the world