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Analysis of Anti-Bacterial Activity of Ethanol Extract Fragrant Pandan Leaves (Pandanus amaryllifolius Roxb) Against the Growth of Disease Cause Pathogen Bacteria Using the Agar Diffusion Method

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Abstract: *Fragrant pandan (Pandanus amaryllifolius Roxb.) is a plant commonly used as fragrance and food coloring. Fragrant pandan contains many substances or bioactive compounds that have medicinal properties so they are also used as traditional medicine. One of the properties of fragrant pandan is as an antimicrobial (antibacterial and antifungal) which is thought to come from the content of alkaloids, flavonoids, phenolics, as well as steroids and terpenoids. This study aims to determine the inhibitory power of fragrant pandan extract (EPW) against the growth of pathogenic bacteria. The test method used is the agar diffusion method by looking at the temperature comparison. Time and Ph.*
Keywords: *anesthesia; mortality; children*

I. Introduction

The condition of illness or disease is one of the problems and problems in human life. Diversity of disease is unique which invites curiosity about the origin of the disease, the causes of the disease and how to treat it. Since ancient times until now, research on various diseases has always been carried out, and various methods have also been used to find drugs, either chemically or naturally.

One of the causes of diseases that can attack human health include bacteria from pathogenic types that can be found around the living environment and unwittingly enter the human body or other living things through food or drink that has been contaminated with bacterial rice, through the air inhaled through the respiratory tract or through the respiratory tract. direct skin contacts with objects that have been contaminated with pathogenic bacteria.

Preventive, curative, promotive and rehabilitative efforts have been carried out by various parties for the prevention and treatment of disease problems caused by bacteria, as well as efforts to break the chain of disease causes caused by bacterial contamination, various healing treatments are also carried out starting from medical and traditional treatment.

In this decade, people generally choose treatment and overcome their health problems by using herbal ingredients (natural ingredients from plants) and assume that using herbal ingredients is safer chemically, easy to find around the environment and has relatively small side effects and levels lower toxicity compared to the use of chemically produced drugs. However, the use of natural ingredients cannot be used just like that, because each element or substance contained in plants must first be researched by scientific research and consumed in doses that are in accordance with the needs of the body and the right benefits for the type of disease suffered, so that the benefits no doubt.

Based on this, a lot of research and development of knowledge has now been carried out to find drugs or antibacterials derived from natural ingredients from various types of plants that are considered important and can provide new contributions and hopes to find

antibacterials naturally through the process. which has been tested, and subsequently conducting various studies to motivate people to want to use traditional and natural treatment.

Antibacterial is one of the elements or substances that can interfere with the growth or kill bacteria by interfering with the metabolic system of harmful microbes. The mechanism of action of antibacterial compounds includes inhibiting cell wall synthesis, inhibiting the integrity of bacterial cell wall permeability, inhibiting enzyme work, and inhibiting nucleic acid and protein synthesis (Dwidjoseputro, 1980).

One of the widely used antibacterial substances are antibiotics. Antibiotics are typical chemical compounds produced or derived by living organisms including their synthetic analogue structures, which in low levels are capable of inhibiting important processes in the life of one or more species of microorganisms (Siswando and Soekardjo, 1995).

Various studies have found that about 40-62% of antibiotics are used inappropriately, among others, for diseases that do not actually require antibiotics. The relatively high intensity of antibiotic use causes various problems and is a global threat to health, especially bacterial resistance to antibiotic work activities (Kemenkes RI, 2011).

Combination of treatment using antibiotics can produce the opposite effect on bacterial defenses and antibiotics can be antagonistic. Although currently many pharmaceutical industries have produced a number of new antimicrobial drugs, resistance to these drugs is still increasing rapidly (Bueno, 2012).

The various plants that can be used as medicines, one of the herbal plants used and suspected of having an antimicrobial effect is the fragrant pandan leaf (Pandanus amaryllifolius Roxb) which is usually used as a green dye and flavoring food (Ambarwati, 2016). Fragrant pandan also has benefits such as overcoming rheumatism, aches and pains, increasing appetite, treating headaches, pain, antibacterial, reducing fever, overcoming dandruff and hair loss (Rosmawati, 1995). The chemical composition of pandan leaves includes alkaloids, saponins, 2-acetyl-1-pyrroline, flavonoids, steroids, tannins (Ariana, 2017).

Several studies have proven that fragrant pandan leaves can inhibit bacterial growth. As research that has been done by Dumoal et al, (2010) which proved that pandan leaf extract can inhibit the growth of Staphylococcus aureus bacteria at a concentration of 40% with an inhibitory power of 13 mm. In addition, Mardiyaningsih & Aini, (2014) also reported the results of their research and stated that the ethyl acetate extract of fragrant pandan leaves has the potential to inhibit the growth of Staphylococcus aureus bacteria by 15.7 mm and in Escherichia coli by 17.7 mm with a loading dose of 5 mg/disc. Furthermore, the research conducted by Hermanto describes the substances contained in fragrant pandan leaves that can function as medicines, antioxidants, antimicrobials and anti-bacterial. Substances contained in fragrant pandan leaves are:

Based on this background, in this study the researchers used one to two types of test bacteria, namely: Escherichia coli and Hellicobacter Pylori, to analyze the potential inhibitory power of the chemical compounds contained in the fragrant pandan leaf extract against bacteria by agar diffusion method and carried out with a temperature comparison. , Ph and time. The reason the researchers chose these two bacteria was based on several health problems that often occur in the community due to these bacteria and the tendency of people to be more interested in herbal medicines in the treatment of various disease problems.

II. Review of Literatures

Pandan leaf is a type of herbaceous plant that has a height ranging from one or two meters, has a distinctive aroma in each leaf. Monocotyledonous plants from the Pandanaceae family can thrive in the tropics with an altitude of 500 meters above sea level. This plant is generally often used as a coloring agent and aroma enhancer in food. The distinctive aroma of fragrant pandan is thought to be due to the presence of a derivative of the amino acid phenyl alanine, namely 2-acetyl-1-pyrroline (Faras et al., 2014).

Figure 1. *Fragrant Pandan Leaf*

In addition to these uses, pandanus has also been reported to have antidiabetic activity in aqueous extracts, antioxidants in aqueous and methanolic extracts, anticancer activity in ethanol and methanol extracts, and antibacterial activity in ethanol and ethyl acetate extracts (Prameswari and Widjanarko, 2014; Ghasemzadeh and Jaafar, 2013; Chong et al., 2012; Muhardi et al., 2007).

This plant, which is thought to have antimicrobial properties, has long been used as a traditional medicine that can treat dandruff, nervous weakness accompanied by anxiety and can also be used as an antidiabetic, antioxidant, analgesic, antibacterial, compounds that are known to be contained in fragrant pandan are phenolic compounds, alkaloids. , flavonoids, saponins, tannins, essential oils and terpenoids, but the scientific data explaining the efficacy of the fragrant pandan plant is still few (Hermanto, 2014).

From various studies that have been carried out, and the results of these studies indicate that the choice of solvent used in the extraction of bioactive compounds derived from pandan leaves can be an important factor that can affect the therapeutic potential.

2.1 Classification of Pandan Plants

The scientific name of pandan leaves is Pandanus Amaryllifolius Roxb, according to Van Steenis (2008), the classification of fragrant pandan is:

Regnum : *Plantae*
Division : *Spermatophyta*
Classic : *Monocotyledonae*
Order : *Pandanales*
Family : *Pandanaceae*
Genus : *Pandanus*
Species : *Pandanus amaryllifolius, Roxb*
Synonym : *Pandanus Latifolius Hassk*. Pandanus Hasskarlii Merrill. Pandanus odorus Ridley (Van steenis, 2008)

2.2 General Characteristics and Habitat

Fragrant pandanus (Pandanus amaryllifolius Roxb.) or simply pandan is a type of monocotyledonous plant from the Pandanaceae family. The leaves are an important component in the culinary traditions of Indonesia and other Southeast Asian countries. Pandanus amaryllifolius Roxb. is the only species of Pandanus that has fragrant leaves. This plant is known for its distinctive fragrance, so it is called fragrant screw pine.

a. Small Fragrant Pandan

Stems of this type reach 1 – 1.6 m high, tapered, with a diameter of 2 – 5 cm. The leaves are long, fragrant, elongated oblong, with a length of 25-75 cm and a width of 2-5 cm. The leaves are pale green, thin and soft, and never flower or bear fruit.

b. Big Fragrant Pandan

This type of stem height reaches 2 - 4.5 m, diameter up to 15 cm, supported by a large taproot. The leaves are long longitudinal, with a length of 1.5 – 2.2 m and a width of 7 – 9 cm, with a dark green upper surface, generally not flowering. In Maluku, it is reported that only the males flower.

Fragrant pandan has two different growth forms. If growth is disturbed, then the tree grows into a small tree and is usually unbranched. The stems are palm-like (palm-like) and long (up to 2 m) leaves. If you continue to harvest leaves, you will get a low, bushier tree with smaller leaves (up to 75 cm) and an invisible trunk. This small growth form likes to grow in a tropical climate that is always wet, but can slowly revert to a large growth form if left undisturbed. These two quite different growth forms have been considered as two different pandanus species in the past (Hean Chooi 2008) and (Jacqueline 2011)

Fragrant pandanus has a round stem with leaves sitting, branching, creeping, and taproots out around the base of the stem and branches. Fragrant pandanus grows to a height of between 0.5 – 1 m, but can grow up to 2 m. Single leaf, sitting with the base hugging the stem, and arranged in three rows in a spiral line. The leaves are ribbon-shaped, thin, smooth, pointed tip, flat edge, parallel boned, 40-80 cm long, 3-5 cm wide, spiny attached to the lower surface of the leaf bone at the ends, and green. Stone fruit, spherical, hanging and orange, diameter 4 – 7.5 cm. Some varieties have serrated leaves. (Jacqueline 2011).

Figure 2. *Fragrant Pandanus*

2.3 Extract

Extract is a concentrated preparation obtained by extracting the active substance from vegetable simplicia or animal simplicia using a suitable solvent, then all or almost all of the solvent is evaporated and the remaining mass or powder is required in such a way that it meets the specified standard (Depkes RI, 2000).

The extract is based on 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 pandan plant contains several active substances whose properties depend on the type of solvent used to extract the leaves (Aisyah, 2015).

2.4 Extraction

Extraction is the activity of withdrawing soluble chemical substances so that they are separated from insoluble materials with liquid solvents. Extract manufacture has several stages: (Depkes RI, 2000).

According to Mardiyaningsih, extraction is an attempt to extract chemical substances/compounds from plant parts/organs (simplicia). Extraction of chemical content in plants is carried out with the aim of attracting chemical substances contained in simplicia. The fragrant pandan plant contains several active substances whose properties depend on the type of solvent used to extract the leaves. Pandan fragrance has antibacterial activity in ethanol and ethyl acetate extracts. Ethanol can dissolve alkaloids, flavonoids, diglycosides, flavonoids, and some essential oils. Meanwhile, ethyl acetate can dissolve alkaloids, aglycones, monoglycosides, terpenoids, and steroids (Mardiyaningsih, 2014).

a. Simplicia Powder Manufacture

Simplicia is formed into powder so that the wetting process can be evenly distributed and the diffusion of the active substance is increased.

b. Solvent Liquid

Solvents are used to separate active substances. Pharmacopoeia states that ethanol is a good solvent for universal use. The solvent is selected selectively depending on the desired active substance. Ethanol can dissolve substances from plants without damaging the parts of the plant.

c. Separation and Purification

The stages of separating the expected active substances so as to get a pure extract.

d. Extract Drying

Extract drying aims to remove the solvent from the material so as to produce a brittle dry mass.

e. Yield

Yield is the ratio between the extract obtained and the initial simplicia.

Maceration extraction method is a method of separating active substances by stirring and filtering. Maceration method is used to make plant extracts. The liquid solvent enters the cell creating a concentration difference between the solution inside and outside the cell. Low concentration solutions are inside the cells while high concentration solutions are pushed out of the cells (Depkes RI, 2000).

The solvent used in the extraction process is 96% ethanol. The reason for using 96% ethanol solvent is to produce a thick extract (pure) so that it is easier for the identification

process. In addition, 96% ethanol is a semi-polar solvent so that it is able to dissolve both polar and non-polar compounds.

2.5 Pandan Leaf Chemical Compounds and Benefits

Fragrant pandanus has secondary metabolic compounds which are chemical defense compounds produced by plants in their plant tissues, these compounds are toxic and function as a means of self-protection from competitors' interference (Mardalena, 2009).

Fragrant pandan leaf extract contains alkaloids, saponins, flavonoids and tannins (Dalimartha, 2009). alkaloids, saponins, flavonoids, tannins, polyphenols, and dyes (Margaretta S et al, 2011) The results of the phytochemical screening of the fragrant pandan extract that have been carried out by the researchers can be seen in the table below.

Table 1. Results of Phytochemical Screening of Fragrant Pandan Leaf Extract

No.	Secondary Metabolites	Results
1.	Flavonoids	+
2.	Tannins	+
3.	Saponins	+
4.	Terpenoids	+
5.	Glycoside	+
6.	Alkaloids	+

From the table above shows that the pandan fragrant leaf extract contains secondary metabolite compounds such as flavonoids, tannins, triterpenoids/steroids, alkaloids, and saponins. According to Robinson (1995), secondary metabolites such as flavonoids, saponins and steroids/triterpenoids are chemical compounds that have potential as antibacterial and antiviral agents.

The efficacy of fragrant pandan, especially in the leaves. Fragrant pandan leaf is an important component in the culinary tradition of Indonesia and other Southeast Asian countries, which is used as a food fragrance because of the aroma it produces. Apart from being a food fragrance, pandan leaves are also used as a source of green color for food, as a component of food presentation decorations, and also as part of flower arrangements at weddings to scent the room. Because of the aroma it produces, fragrant pandan is used as a raw material for making perfume. The alkaloid 2-acetyl-1-pyrroline is a substance that gives it a fragrant taste.

In the leaves, there are essential oils consisting of acetylpyrroline, linalool, pandamarilactone, and hydrocarbon sesquitants. In the roots, there is 4-hydrobenzoic acid, (Hean Chooi, 2008).

2.6 Bacteria

Bacteria are prokaryotic cells with circular genomes and plasmids. Besides being known as disease-causing agents, bacteria also have great benefits for human life such as the use of bacteria in the manufacture of yogurt and antibiotics. In the human body, bacteria provide many benefits in defense against infection, play a role in the immune system, source nutrients and stimulate epithelial turnover. Bacteria that inhabit the human body are called normal flora microbes. Inhabit the skin and mucous membranes of healthy

and normal individuals, most bacteria are anaerobic and facultative anaerobes.

Normal flora microbes are divided into two major groups, namely (1) permanent resident flora found in certain areas, disappears when disturbance occurs and returns to normal. (2) transit flora; pathogenic & non-pathogenic microbes on mucous membranes and mucosa temporarily. The proliferation of normal flora microbes is influenced by factors of temperature, humidity, the presence or absence of food, and inhibitory materials.

2.7 Bacterial Morphology

Bacterial species can be distinguished based on morphology (shape), chemical composition (usually detected by biochemical reactions), nutritional requirements, biochemical activity and energy sources (sunlight or chemicals). There are several basic shapes of bacteria, namely round (singular: coccus, plural: cocci), rods or cylinders (singular: bacillus, plural: bacilli), and spirals, which are curved or circular rods (Sylvia T. Partiwi 2008)

2.8 Gram Positive Bacteria and Gram-Negative Bacteria

Bacteria are divided into two groups based on cell wall composition and staining properties, namely gram-positive bacteria and gram-negative bacteria. In addition to differences in staining properties, gram-positive and gram-negative bacteria differ in their sensitivity to mechanical/physical damage, to enzymes, disinfectants and antibiotics. The relative differences in the characteristics of gram-positive and gram-negative bacteria can be seen as follows:

Table.1 Difference Between Gram Positive Bacteria and Gram Negative Bacteria

No	Characteristic	Relative Difference	
		Gram bacteria Positive	Gram bacteria Negative
1.	Cell wall composition	Lipid content low (1-4%)	Lipid Content high (11-22%)
2.	Resistance to Penicillin	more sensitive	More durable
3.	Inhibition by dye regular (e.g. crystal violet)	More inhibited	Less inhibited
4.	Nutrient Needs	On generally species relatively complex	Relatively simple
5.	Resistance to physical treatment	More resistant	Less resistant (more sensitive)

Source: Fardiaz, 1992

2.9 Diseases caused by bacteria

As a pathogen, bacteria can infect human infections and are still a cause of high morbidity and mortality in various parts of the world and especially in developing countries such as Indonesia. Types of diseases that can be caused by gram-positive and negative bacteria are:

Table 2. Diseases of gram-positive and negative bacteria

Gram bacteria	**Genus**	**Disease**
Gram Positive	Staphylococcus	Food poisoning, Bronchitis
	Streptococcus	Pneumonia, dental caries
	Enterococcus	*Enteritis*
	Listeria	*Listeriosis*
	Bacillus	*anthrax*
	Clostridium	*Theta*nus
	Mycobacterium	*Diphtheria*
	Propionibacterium	*Pubercolosis*
	Mycoplasma	Acne, pneumonia
Gram Negative	*Salmonella*	*Salmonellosis*
	Escherichia	Inflammation of the gastrointestinal tract
	Shigella	*Dysentery*
	Neisseria	*Gonorrhea*
	Bordetella	Whooping cough
	Legionella	Legionnaires' disease
	Pseudomonas	Burn infection
	Vibrio	*Cholera*
	Campylobacter	*Gastroenteritis*
	Helicobacter	Stomach ulcer
	Haemophilus	*Bronchitis*
	Treponema	*Syphilis*
	Chlamydia	*Pneumonia, urethritis, trachoma*

2.10 *Escherichia coli*

E. coli bacteria are Gram-negative rod-shaped bacteria, have a size of 2.4 micro 0.4 to 0.7 micro, mobile, not spore, positive on indole, glucose, lactose, sucrose tests (Greenwood et al., 2007). Escherichia coli bacteria is one of the normal flora in the human colon (Ramadhianto, 2019).

In general, the bacteria discovered by Theodor Escherichini can be found in the human colon. E. Coli is generally harmless, but some species, such as E. Colitype O157:H7, can cause serious food poisoning in humans, and harmless E Coli can benefit humans by producing vitamin K2, or by preventing other bacteria in the gut. Commonly used as a vector to insert certain genes that are desired to be developed. E coli constitute both the principal bacteria responsible for septicemia and the osteomyelitis (Ngunde, 2019).

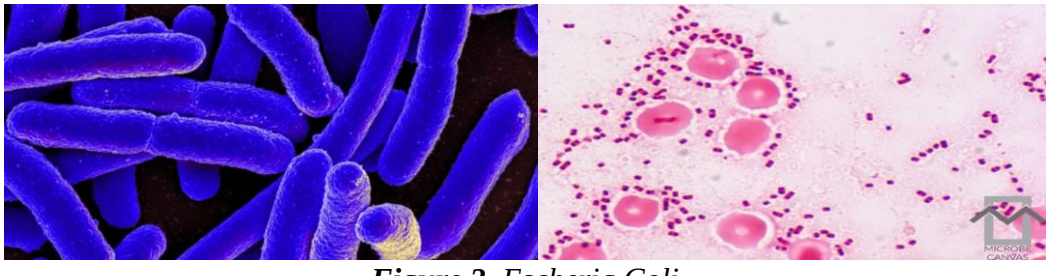


Figure 2. *Escheria Coli*

Domain : Bacteria
phylum : Proteobacteria
Class : Gamma Proteobacteria
Order : Enterobacteriales
Family : Enterobacteriaceae
Genus : Escherichia
Species : E.coli

Escherichia coli (E. coli) is also a member of a large group of bacteria that inhabit the digestive tract of humans and other warm-blooded animals (mammals, birds). Newborns have a sterile digestive tract, which within two days becomes colonized with E.coli. More than 700 E.coli serotypes have been identified, and their "H" "O" antigens and flagella differentiate different E. coli erotifes, respectively. The E.coli serotypes responsible for reports of various outbreaks traced to consumption of contaminated food and drink are those that produce Shiga toxin (STX), so named because the toxin is nearly identical to that produced by another bacterium known as the dysentery strain Shigella1 (which also causes bloody diarrhea and hemolytic uremic syndrome (HUS) in developing countries such as Bangladesh), the most famous is the one that produces STX, namely E.coli O157: H7. Shiga toxin is one of the most potent toxins, while HUS is a syndrome defined by a trilogy of hemolytic anemia (destruction of red blood cells), thrombocytopenia (low platelet count) and acute renal failure.

2.11 *Hellicobacteria Pylori*

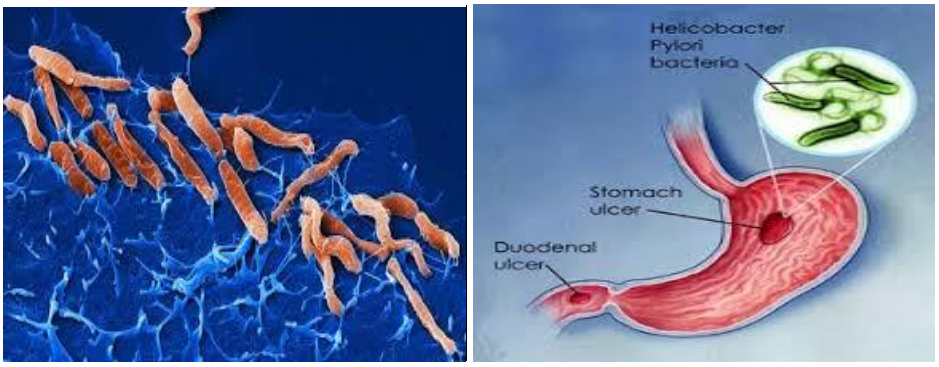


Figure 3. *Hellicobacteria*

Phylum : Proteobacteria
Class : EpsilonProteobacteria
Order : Campylobacteriales
Family : Helicobacteriaceae
Genus : Helicobacter
Species : H. Pylori

Helicobacter pylori (H) is the main cause of gastritis in humans and is an etiologic factor for gastric ulcer, duodenal ulcer, gastric carcinoma and primary gastric B-cell lymphoma. H. pylori infection is often found in some cases of nonulcer dyspepsia and is a common prevalence in the world. Chronic infection can occur for several years and can cause changes in the gastric mucosa which can eventually lead to ulcers and even gastric cancer. The habitat of H. pylori is limited to gastric mucosal cells, especially the antrum

area and is set most often in the deepest layer of the mucosa that lines the epithelial cells and will not be visible if the mucosa still covers the epithelial cells (Graham and Graham, 2002; Liska, 2004; Gatta et al., 2013).

Several endoscopic studies evaluating the prevalence of H. pylori infection in patients with nonulcer dyspepsia have shown that these two phenomena are frequently associated, with an average proportion of about 50–60% of patients with nonulcer dyspepsia exhibiting H. pylori in the stomach. In the prevalence study, it was also said that H. pylori infection in asymptomatic individuals (asymptomatic) showed a lower prevalence than the prevalence in patients with non-ulcer dyspepsia symptoms. Several clinical trial studies have also shown a causal relationship between the treatment of H. pylori infection and the score reduction in dyspepsia symptoms. However, this is still a contradiction (Liska, 2004).

The overall prevalence of H. pylori infection is 40%. Sero-epidemiological studies of several cities in Indonesia found the frequency of positive anti-Hp IgG, namely Malang, Solo and Medan (34 - 37%), Mataram 54%, Denpasar 35%, Surabaya 36%, Jakarta 50-67%, Jakarta 52.3%, and Trenggalek 45.6% (Agung et al., 2011). An interim report from an epidemiological study in Jakarta by the Gastroenterology Division showed that the prevalence of H. pylori infection was 52.3% of 310 patients, while in Yogyakarta in 2009 it was 22.2% of the total patients who underwent gastroscopy at Dr. Sardjito (Utia et al., 2010; Triwikatmani, 2014).

2.12 Antibacterial

Antibacterial substances in plants are active substances in plants that have the potential as antibacterial. The active substances in fragrant pandan that have the potential as antibacterial are alkaloids, flavonoids, saponins, phenolics, steroids, tannins and terpenoids (Margaretta S.2011)

III. Research Method

The method used is the agar diffusion method. This method was used to determine the activity of antimicrobial agents. 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. A clear area on the surface of the media in order to identify the presence of inhibition of the growth of microorganisms by antimicrobial agents (Pratiwi, 2008).

IV. Discussion

4.1 Antibacterial Activity of Fragrant Pandan Leaf Ethanol Extract Based on Temperature

The criteria for soaking the ethanolic extract of fragrant pandan leaves at 1 day, 3 days and 5 days, the results of measuring the diameter of the inhibition zone of the ethanolic extract of fragrant pandan leaves against E. coli and H. pylori bacteria can be seen in the table.

Table 2. Measurement of the Diameter of the Inhibition Area (mm)* the Average Bacterial Growth of the Ethanolic Extract of Fragrant Pandan Leaves Based on the Length of Maceration

No.	Immersion Time	Test Bacteria	Concentration (%)	Average Inhibitory zone diameter (mm) ± SD
1.	1 day	*E. coli*	50	5.70±0.1
			100	6.67±0.15
2.	3 days		50	8.07±0.12
			100	9.70±0.17
3.	5 days		50	10.67±0.1
			100	12.77 ± 0.15
4	1 day	*H. pylori*	50	5.27±0.15
			100	6.53±0.06
5	3 days		50	8.20±0.1
			100	9.30±0.1
6	5 days		50	10.53±0.40
			100	12.73 ± 0.31
7	Negative Control			0.00 ± 0.00

From the table above, it can be seen that the average diameter of the bacterial inhibition zone on E. coli bacteria is:

1. In 1 day immersion with a concentration of 50% has an average value of the inhibition zone diameter of 5.70± 0.1 while for H. pylori it was 5.27 ± 0.15. At 100% concentration for E.coli bakteri bacteriathe average value of the diameter of the inhibition zone of pandan leaf ethanol extract was 6.67 ± 0.15 and for H.pylory bacteria was 6.53±0.06. Ethanol extract of fragrant pandan leaves with long soaking
2. 3 days of immersion and a concentration of 50% on E.coli bacteria had an average inhibition zone diameter of 8.07± 0.12 while in H. pylori it was 8.20 ± 0.1. At a concentration of 100% the average diameter of the inhibition zone for E. coli bacteria was 9.70 ± 0.17 and H. pylori was 9.30 ± 0.1.
3. For soaking ethanol extract of fragrant pandan leaves for 5 days with a concentration of 50% the average value of the inhibition zone diameter of E. coli bacteria was 10.67 ± 0.15 and H. pylori was 10.53. Meanwhile, at 100% concentration the average value of the inhibition zone diameter for E.coli was 12.77 ± 0.15 and for H. pylori it was 12.73 ± 0.31.

Matter This shows the average value of the diameter of the inhibition zone on bacteria e.coli and H.pylori pThe highest concentration was found in the ethanol extract with a soaking time of 5 days and a concentration of 100% with an average value of 12.77 ± 0.15. Likewise, what is seen in the average diameter of the bacterial inhibition zone on H. pylori bacteria, where the ethanolic extract of pandan fragrant leaves which has the

highest average value of the inhibition zone is found in immersion for 5 days and a concentration of 100% with a value of 12.73 ± 0.31. The results from the table above can also be seen from figure 4 and 5 below.

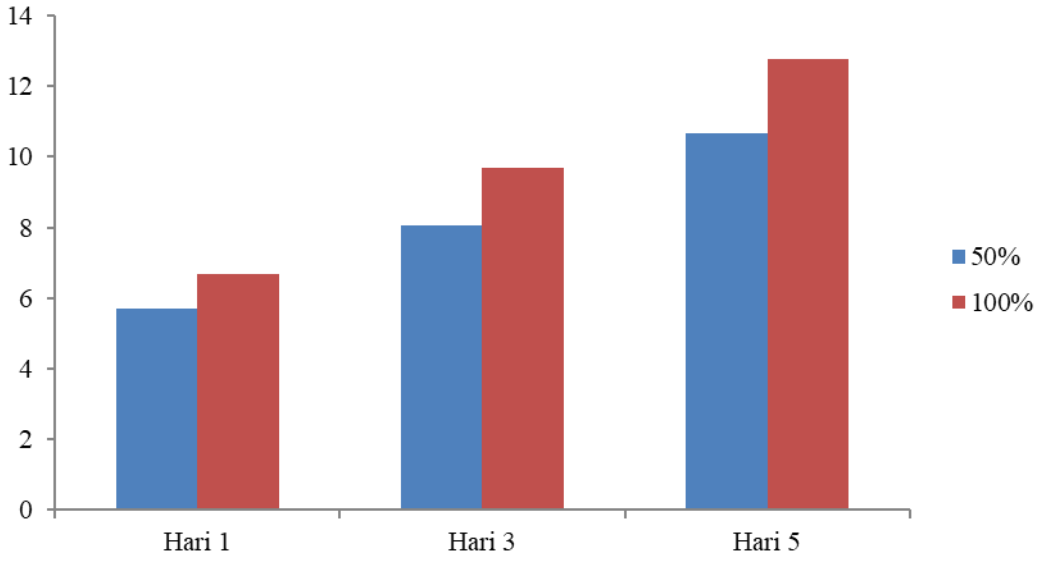
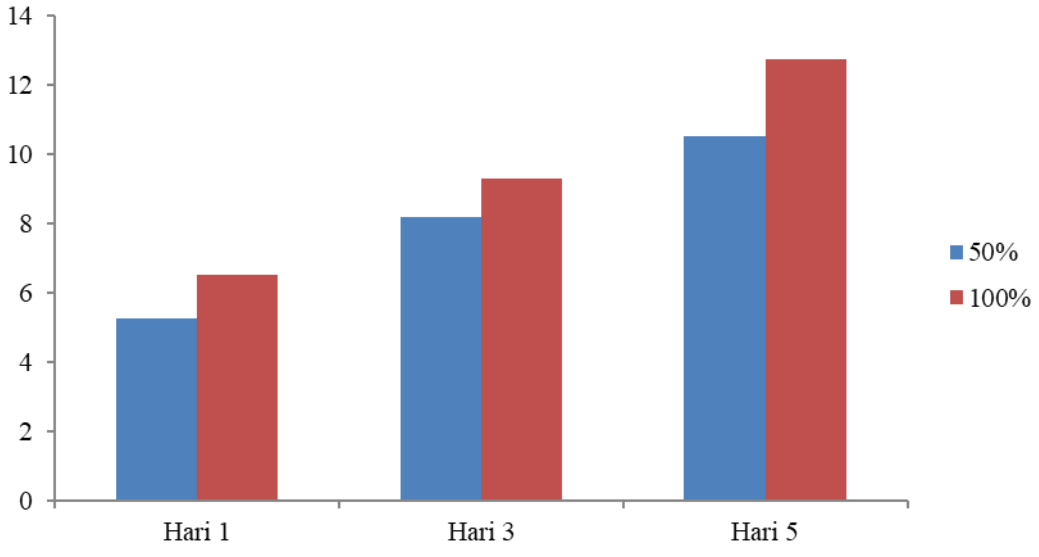


Figure 4. *The Average Value of the Diameter of the Bacterial Inhibition Zone of the Ethanolic Extract of Pandan Leaves with Different Soaking Times for E.coli Bacteria*



Figure

Figure 5. *The Average Value of the Diameter of the Bacterial Inhibition Zone of the Ethanolic Extract of Pandan Fragrant Leaves with the Difference in Soaking Time in H.pylori Bacteria*

From the graph above, it can also be seen that the longer the immersion time and the higher the concentration of the ethanolic extract of pandan fragrant leaves, the larger the diameter of the bacterial inhibition zone or in other words the concentration and duration of immersion are directly proportional to the diameter of the bacterial inhibition zone. The length of immersion time can affect the amount of secondary metabolites that are attracted.

At the beginning of the immersion, when the pandan leaves first come into contact with the ethanol solvent, the part of the pandan leaf extracted is the outer surface (Gustone, 2004). At a high concentration of 100%, the antibacterial activity of fragrant pandan leaves also increased compared to a concentration of 50%. This shows that the higher the concentration, the antibacterial activity also increases.

The active compounds that play a role in inhibiting the growth of bacteria produced by fragrant pandan leaf extract are terpenoids, phenolic compounds, steroids (Cowan, 1999; Palczar and Chan, 1988); Madduluri et al., 2013). Terpenoids have a mechanism of inhibiting bacterial growth by reacting with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall and forming strong polymeric bonds which can cause damage to the porin and the bacterial cell will lack nutrients thereby inhibiting bacterial growth (Cowan, 1999).

Phenolic compounds bind to form hydrogen bonds that are formed between phenols and proteins resulting in the cytoplasmic membrane causing an imbalance of macromolecules and ions in the cell which results in cell lysis (Palczar and Chan, 1988). Steroids as antibacterials are related to lipid membranes and sensitivity to steroid components that cause leakage in liposomes. Steroids can interact with cell phospholipid membranes which are permeable to lipophilic compounds, causing decreased membrane integrity and cell membrane morphology to change which causes cells to become brittle and lyse (Madduluri et al., 2013).

4.2 Antibacterial Activity of Fragrant Pandan Leaf Ethanol Extract Based on Temperature

The extraction temperature greatly affects the bioactive compounds contained in the resulting extract. used in the manufacture of ethanol extract of fragrant pandan leaves are 35ºC and 50ºC. The temperature will affect the content of compounds contained in the extract. Temperatures that are too high can cause damage to secondary metabolite components that cannot withstand excessive heating.

Table 3. Measurement of the Diameter of the Inhibition Area (mm)* Average Bacterial Growth of Ethanol Extract of Fragrant Pandan Leaves Based on Temperature

No.	Temperature	Test Bacteria	Concentration (%)	Average Inhibitory zone diameter (mm) ± SD
1.	35ºC	*E. coli*	50	7.53±0.25
			100	9.37±0.15
2.	50ºC		50	10.97 ± 0.13
			100	12.53±0.12
3	35ºC	*H. pylori*	50	7.47 ± 0.12
			100	9.13 ± 0.21
4	50ºC		50	10.67 ± 0.12
			100	12.67±0.06
5	Negative Control			0.00 ± 0.00

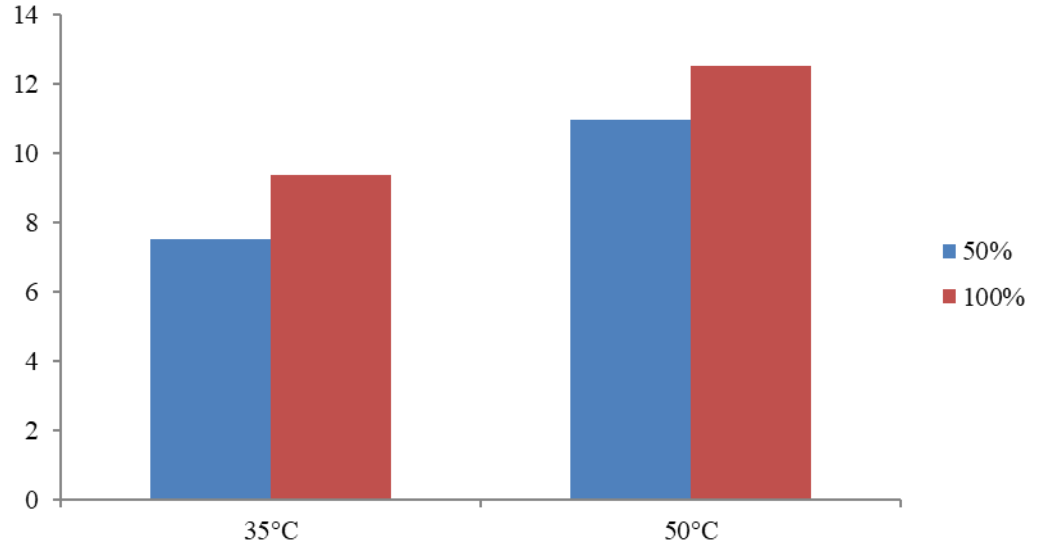
From In the table above, it can be seen that the average diameter of the bacterial inhibition zone of the ethanolic extract of pandan leaves on E. coli bacteria is:

1. With a heating temperature of 35ºC extract with a concentration of 50% has an average inhibition zone diameter of 7.53± 0.25 while for H. pylori it was 7.47 ± 0.12. At 100% concentration for E.coli bakteri bacteriathe average value of the

diameter of the inhibition zone of pandan leaf ethanol extract was 9.37 ± 0.15 and for H.pylory bacteria was 9.13±0.21.

2. With an extract heating temperature of 50ºC and a concentration of 50% on E.coli bacteria, the average inhibition zone diameter was 10.97.± 0.13 while in H. pylori it was 10.67 ± 0.12. At a concentration of 100% the average diameter of the inhibition zone for E. coli bacteria was 12.53 ± 0.12 and H. pylori was 12.67 ± 0.06.

Results This can also be seen in graphs 6 and 7 below.



Graph

Figure 6. The Average Value of the Diameter of the Bacterial Inhibition Zone of the Ethanolic Extract of Pandan Leaves with Temperature Differences in E.coli Bacteria

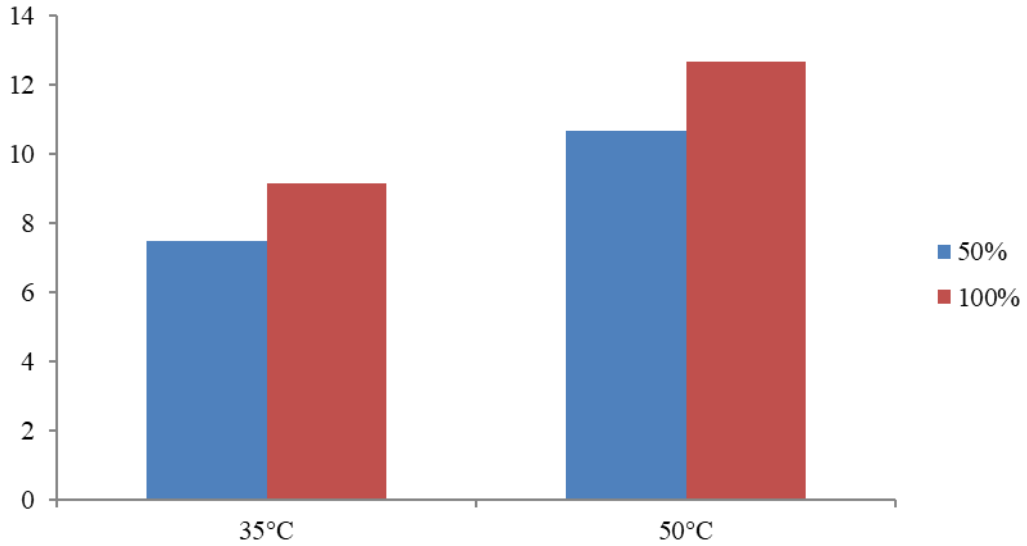


Figure 7. *The Average Value of the Diameter of the Bacterial Inhibition Zone of the Ethanolic Extract of Pandan Leaves with Temperature Differences in H.pylori Bacteria*

The graph above shows that the ethanol extract of fragrant pandan leaves with a temperature of 50ºC and a concentration of 100% has an average diameter of the inhibition zone of E.coli bacteria which is greater than the temperature of 35°C and a concentration

of 50%. Likewise, the average value of the diameter of the inhibition zone of H.pylori bacteria, where the greatest value was found in the ethanol extract of fragrant pandan leaves with a temperature of 50ºC and a concentration of 100%.

Low heating temperature can cause the ability of antibacterial activity to be reduced. This is because the extract takes too long to dry. To obtain a thick extract, the extract will undergo a heating process to evaporate the solvent. At low temperatures the solvent will be difficult to evaporate so it takes a long time to obtain a thick extract. At that time, the extract will be exposed to various contaminants that can damage the components in the extract. According to Harbone (1987) the extract obtained will depend on several factors, including the natural condition of the compound, the extraction method used, the sample particle size, storage conditions and time, the length of the extraction time and the ratio of the amount of solvent to the number of samples.

The inhibition of the growth of bacterial colonies was caused by damage to the structural components of the bacterial cell membrane. Terpenoid compounds can bind to proteins and lipids found in cell membranes and can even cause cell lysis (Nursal, 2006). Damage to the bacterial cell membrane will interfere with the nutrient transport process, so that the cell will experience a lack of nutrients needed in the growth process.

V. Conclusion

1. The ethanolic extract of fragrant pandan leaves with variations in soaking time and concentration had the best antibacterial activity at a time of immersion of 5 days with a concentration of 100% with an average value of the inhibition zone of E. Coli bacteria of 12.77 ± 0.15 mm and an average value of the diameter of the inhibition zone of H. Pylory bacteria was 12.73 ± 0.31 mm.
2. The ethanolic extract of fragrant pandan leaves with variations in temperature and concentration has good antibacterial activity at a temperature of 50ºC and a concentration of 100% with an average value of the inhibition zone diameter of E. Coli bacteria of 12.53, ± 0.12 ± 0.15 mm and the average value – the average diameter of the inhibition zone of H. Pylory bacteria is 12.67 and ± 0.06.

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