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TEST OF ANTIBACTERIAL ACTIVITY OF THE ETHANOL EXTRACT OF PULUTAN LEAVES (*Urena lobata* L.) AGAINST *Escherichia coli*

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ABSTRACT

This study aims to determine the content of secondary metabolites, antibacterial activity test of pulutan leaf ethanol extract (*Urena lobata* L.). Phytochemical screening tests are carried out with several appropriate reagents. Antibacterial activity test using disc diffusion method against *Escherichia coli* and *Staphylococcus aureus bacteria*. The results of phytochemical screening of ethanol extract of pulutan leaves contain; flavonoids, saponins, tannins, and steroids/terpenoids. The results of antibacterial activity tests with the disc diffusion method of pulutan leaf ethanol extract 15%, 25%, 35%, 45%, 55% and 65% against *Escherichia coli* bacteria were 4.0625 mm, 4.2 mm, 4.85 mm, 3.075 mm, 4.5875 mm, and 4.3 mm respectively. The results showed that pulutan leaf ethanol extract had antibacterial activity against *Escherhicia coli* bacteria in the weak category.

Introduction

Infectious diseases are one of the diseases due to the presence of microscopic organisms consisting of one or many cells. One infection that is widely disputed is diarrhea. Diarrhea is a major trigger of mortality in young children in developing countries. Diarrhea is the discharge of feces that are continuously soft to liquid texture with the frequency of feces released three or more times in one day (Arda *et al*., 2020). Data from the Ministry of Health of the

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Republic of Indonesia 2019 states that the problem of diarrhea in Indonesian society reached 7,265,013 cases or 270/1000. Attacks of infections that cause diarrheal diseases can be prevented with the use of antibiotics.

Antibiotics are compounds produced by microorganisms that are toxic to other microorganisms, so antibiotics are used as drugs to prevent bacterial infections. High use of antibiotics triggers problems in the use of antibiotics. One of them is antibiotic resistance (Anggraini *et al*., 2020). Antibiotic resistance occurs when bacteria are unable to respond to a drug to kill it. As a result, the activity of bacteria in the human and animal bodies cannot be disturbed by antibiotics. This is a complex challenge of global public health where no simple strategy will successfully solve the emergence of the spread of infection-causing organisms that become resistant to existing antibiotics (Yunita *et al.*, 2021).

Another solution that can be done to overcome this resistance is to use plants. One plant that has the potential to inhibit bacterial growth is pulutan plants (*U. lobata*). Pulutan plants are herbal plants belonging to the Malvaceae family and are still not widely used by the community as antidiarrheals. U. *lobata leaves* are used as a remedy for tonsil inflammation, influenza, malaria, cough, rheumatism, vaginal discharge, blood wounds, swelling, and ulcers (Fadillah *et al*., 2020). According to Shealer *et al*., (2017) pulutan plants can be useful as antidiarrheal, rheumatism, colic, toothache, wound healer, and wound, and malaria. The part that is most often used as medicine is the leaf because the leaves are thought to accumulate a lot of secondary metabolite compounds that are useful as drugs compared to stems or roots. In addition, the leaf part is a part that is very easy to find and always available, and a place of accumulation of photosynthetes that are thought to contain elements (organic substances) that have disease-curing properties (Suarsini, 2011).

According to research by Fadillah *et al.*, (2020) pulutan has benefits as cough medicine, influenza medicine, malaria, inflammatory drugs on the tonsils, ulcers, bleeding wounds, fractures, rheumatism, vaginal discharge, swelling, and medicine for snake bites. Pulutan leaf extract (*U. lobata*) has potential as bioactivity including as antibacterial, antioxidant, as antifungal and inhibits the growth of cancer cells. Pulutan (*U. lobata*) is used as a traditional medicine because it has secondary metabolite compounds and bioactivity. Methanol extract of pulutan leaves contains chemicals in the form of tannins, terpenoids, saponins, cardioglycosides, and alkaloids that have the potential to prevent the growth of bacteria Enterococcus species *Pseudomonas aeruginosa* and *Staphylococcus aureus,* Klebsiella *sp,* Escherichia coli *(Fagbohun* et al., *2012).*

Bacteria that need to be inhibited are *E. coli* bacteria and *S. aureus* as pathogenic bacteria against diarrheal infections. *E. coli* is a gram-negative bacteria that causes diarrhea. In addition to the main cause of diarrhea, *E. coli* can also cause symptoms of diseases such as cholera, gastroenteritis and several other gastrointestinal diseases (Hutasoit, 2022). Based on Rasyid *et al*., (2020) that Ubud tourists suffering from diarrheal infections contain *E. coli bacteria as* many as 20 out of 30 fecal samples. *E. coli* is the cause of digestive disorders characterized by pain in the stomach, liquid feces, and increased bowel movements. *E. coli* will become pathogenic if the amount increases in the digestive tract.

Pulutan leaves (*U. lobata) have the ability to prevent the growth* of E. coli bacteria *in terms of overcoming antibiotic resistance by using ethanol extract of* U. lobata leaves. The solvent to be used in extraction is ethanol p.a. Research Wendersteyt *et al*., (2021), states that ethanol is used as a solvent because ethanol is universal, good absorption, quickly obtained, selective, non-toxic, and high irritability so that it can extract compounds that are polar, semipolar, and non-polar.

Based on the background that has been described that diarrheal diseases and antibiotic resistance are problems that require special attention and need to be evaluated

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against antibiotics. Therefore, further tests are needed on the antibacterial activity of pulutan leaf ethanol extract (*U. lobata*) in order to be a problem solver regarding diarrheal diseases caused by *E. coli bacteria*.

Materials And Methods

Location and Time of Research

This research was carried out at the Microbiology Laboratory of FMIPA Medan State University, Jl. Williem Iskandar Pasar V Medan Estate. The research time is from February 2023- June 2023.

Tools and Materials

The tools used in this study were catering knives, ovens, analytical scales, blenders, 120 mesh sieves, glass containers, stirring rods, glass funnels, erlenmeyer, measuring cups, test tubes, test tube racks, petri dishes, pingsets, ose needles, calipers, isopads, vortexes, glass beakers, *rotary vacuum evaporators*, sample bottles, autoclaves, laminar air flow, injections, digital cameras, and stationery.

The materials used in this study were pulutan leaves (*U. lobata*), ethanol solvent p.a, alcohol 70%, aquades, BaCl2, H2SO4, physiological NaCl 0.9%, pure culture of *Escherichia coli* bacteria, Nutrient Agar (NA) media, *Mueller Hinton Agar* (MHA) media, Nutrient Broth *(NB) media, spirits, chloramphenicol antibiotics 250 mg (positive control), ethanol (negative control), Mc farland turbidity standard.*

Sampling

The sample used in this study was pulutan leaves (*U. lobata*) obtained from from vacant land on Jl. Pendidikan, Siborutorop Village, Paranginan District, Humbang Hasundutan Regency, North Sumatra Province.

Sterilization Tools

Sterilization is carried out by chemical sterilization method and moist heat method. Sterilization by moist heat method is used for heat-resistant tools using an autoclave for 15 minutes at a temperature of 121^0 C with a pressure of 1 atm. While sterilization by smearing and soaking using 70% alcohol within 2 minutes is used for tools that are not heat resistant (Biological) *et al.*, 2022). Ose wire is sterilized by flamming in the bunsen flame (Utomo *et al*., 2018).

Simplisia Creation

Pulutan leaves (*U. lobata*) that have been collected are washed with running water until clean and then dried in a room that is not exposed to direct sunlight for 5 days. Then the pulutan leaves (±*U. lobata*) are dried using an oven at 500C for 2 hours to maximize the drying process. The sample is then mashed using a blender until it is dry leaf powder. The pulutan leaf powder is then filtered using a 120 mesh sieve. The simplisia of pulutan leaves that have been obtained is stored in a jar for use in the next process.

Extraction

Using the solvent ethanol pro analysis (p.a), maceration methods were used to make pulutan leaf extract. A total of 300 grams was soaked in 900 ml of P.A ethanol solvent with a solvent ratio of 1: 3 (w/v). In the maceration process, 3 repetitions are carried out. At the first maceration soaked for 5 days and stirred every 2x24 hours for 5 minutes. After 5 days, the marinade is filtered using filter paper So that filtrate and residue are obtained and accommodated in different containers. The residue in the first maceration process is macerated again with a soaking time of 3 days in a ratio of 1: 2. After obtaining the filtrate from the second maceration, it is then combined in a sample bottle of extract from the first maceration. The residue from the second maceration is macerated again in a ratio of 1:1 with the same treatment. After that the results of the filtrate obtained are combined in a container, then concentrated using a tool *rotary vacuum evaporator* at a temperature of 500 C so that a viscous extract is obtained.

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Phytochemical Screening

Alkaloid Test

A total of 0.5 grams of pulutan leaf simplisia mixed with 9 ml of aquadest and 1ml of HCl 2N heated on a bath for 2 minutes cooled and filtered. After cooling, it is filtered and the filtrate is used for further testing.

(1). Phytrate is taken as much as 3 drops then added 2 drops of mayer reagent solution, if it contains a positive white precipitate containing alkaloids.

(2). The filtrate is taken 3 drops and then 2 drops of dragondroff reagent solution are added, if it contains a red or orange precipitate it positively contains alkaloids.

(3). The filtrate is taken 3 drops then 2 drops of bauchardat reagent solution are added, if it contains a positive dark brown precipitate containing alkaloids (Sari *et al*., 2019).

Flavonoid Test

A total of 1 gr of simplisia powder is added 10 ml of hot aquades and then simmered for 5 minutes, filtered in a still hot state. The filtrate obtained is taken 5 ml and then added 0.1 gr of magnesium powder, 1 ml of HCl and 2 ml of alcohol, then shaken and left to separate. Powder contains flavonoids when there is a red yellow color change in the filtrate or red orange color (Sari *et al*., 2019) .

Saponin Test

A total of 0.5 gr of simplisia powder is put into a test tube and then added 10 ml of hot aquades, cool for a while after cold shaken strongly for 15 minutes, if foam forms for 10 minutes and foam as high as 1-10 cm and when dripped 1 drop of hydrochloric acid 2 N foam is still there, the powder contains saponin compounds (Sari *et al*., 2019).

Tannin Test

A total of 1 gr of simplisia powder is boiled for 3 minutes in 10 ml of aquades and then cooled and filtered. The filtrate is diluted to almost colorless, then 1-2 drops of iron(III) chloride reagent are added, if a blue-black or blackish-green color indicates the presence of tannins (Sari *et al*., 2019) .

Steriod/Triterpenoid Test

A total of 1 gr of simplisia powder was macerated for 2 hours with 20 ml of n-hexane, then filtered. The filtrate is evaporated in an evaporating dish. On the rest are added a few drops of the Liebermann-Burchad reagent. The appearance of blue or blue green indicates the presence of steroids, while red, pink or purple indicates the presence of triterpenoids (Sari *et al*., 2019).

Rejuvenation of Test Bacteria

A total of 2 gr of NA media is dissolved with 100 ml of aquades in the enlenmeyer and covered using aluminum foil. The media is heated in idoped to boil and thoroughly mixed then put into a test tube. Each test tube is filled with 6 ml of NA media. The tube that has contained the media is then autoclave at 1210C for 15 minutes. Next, the substrate is left at room temperature for 2x24 hours in an inclined position. The culture of the test bacteria is taken one ose and then inoculated on the media *Nutrient Agar* (NA) is aseptically tilted by continuous stroke method (*zigzag*) and when scratching bacteria, the tube is brought closer to the Bunsen fire, then cover the tube with cotton and incubated at 37oC for 1x24 hours (Panaungi, 2022).

Test Bacteria Suspension

The pure culture inoculum of the test bacteria was taken as much as 1 ose and suspended into a tube containing 10 ml of 0.9% physiological NaCl. Furthermore, homogenized using vortex until turbidity is obtained in accordance with the standard 0.5 Mc Farland or comparable to the number of bacteria 108 (CFU / ml) (Nor, 2018).

Antibacterial Activity Test of Pulutan Leaf Ethanol Extract

The antibacterial activity test was carried out by disc diffusion method, using 6 mm diameter disc paper on MHA media. MHA media is poured into sterile petri dishes ±10 ml and allowed to solidify, *Escherichia coli* bacteria suspension and grown on MHA media using cotton swabs. Sterile disc paper is placed on the surface of the media that has

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been planted with test bacteria after dripping 0.5 ml pulutan leaf ethanol extract that has been diluted at the specified concentration. Disc paper dipped in chloramphenicol served as a positive control and disc paper dipped in ethanol p.a served as a negative control. After that, it was incubated for 1x24 hours at a temperature of 37ºC, after a 24-hour incubation period the area of the inhibitory zone formed was measured with a caliper to determine its antibacterial activity.

Data Analysis

The data obtained from the results of the study were analyzed descriptively. The first stage is qualitative extraction of pulutan leaves. The second stage of observation on antibacterial activity tests is characterized by clear zones formed and presented in the form of figures and tables. According to Panaungi &; sakka (2022), the activity of the inhibitory zone of an antimicrobial drug is grouped into four parts, namely: (1). > 20-30 mm (very strong), (2). 10-20 mm (strong), (3).5-10 mm (medium) and (4) 5 mm (weak).

Results And Discussion

Phytochemical assays with color reactions are used to identify active compounds and secondary metabolites in pulutan leaf simplisia (*Urena lobata* L.). Pulutan leaf simplisia contains saponins, flavonoids, tannins and triterpenoids. Flavonoid testing on pulutan leaf simplisia showed positive results indicated by the change in color to orange. Flavonoids are a class of phenol compounds that are polar in almost every plant. Flavonoids will generally be dissolved by solvents with the same polarity properties e.g. ethanol or methanol. Flavonoid testing uses magnesium powder added to pulutan leaf simplistics. The addition of magnesium powder can cause flavonoid compounds to be reduced resulting in a change in the color of the extract solution to a brick red color (Simaremare, 2014).

Testing of saponin compounds on pulutan leaf simplisia showed positive results characterized by the formation of foam on the tube for no less than 10 minutes. Saponins have two groups with different properties, namely a hydrophilic group and a hydrophobic group. The addition of HCl to saponin testing causes an increase in the polarity of saponin compounds so that there is a change in the location of the constituent groups. In this state, polar (hydrophilic) groups will face outward and non-polar (hydrophobic) groups face inward and form structures called micellar structures (Simaremare, 2014).

Testing of steroid compounds on pulutan leaf simplisia showed positive results characterized by the formation of blue-green color while triterpenoid tests showed positive results characterized by a change in color to red or purple with the addition of *Lierberman-Bouchard reagent*. The formation of green color in the experiment proved that the sample was positive for steroids. A number of color reactions occur because steroid compounds will destabilize with the addition of strong acids and form salts.

Tannin test is done by adding 1-2 drops of 1% FeCl3 to 2 ml of pulutan leaf simplisia. Testing of pulutan leaf simplisia tannin compounds showed positive results characterized by a change in color to blackish-green in pulutan leaf simplisia. The color change occurs due to a reaction that occurs between the tannin compound group and the 1% FeCl3 reagent. Simaremare (2014) suggests that the hydroxyl group in tannin compounds will react with 1% FeCl3 reagent so that the color of the extract can change to blackish green.

Alkaloid testing does not occur precipitate. Alkaloids are basic compounds, HCl is added to form salts when testing alkaloid compounds to separate alkaloids from other components. Using reagents from Mayerr, Dragendroff, and Wagner, alkaloid salts can be identified White precipitate is a positive indication Results on Mayer's reagent, brown precipitate shows positive results on Wagner's reagent, and orange or orange precipitate shows positive results on Dragendroff's reagent (Muawanah *et al*., 2019). Using Mayer, Dragendroff, and Bouchardat reagents, alkaloid tests on pulutan

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leaf simplisia gave negative results. When the extract is treated with Mayer reagent a white precipitate is formed, Bouchardat's reagent produces a red precipitate, and Dragendroff's reagent produces an orange color or orange precipitate.

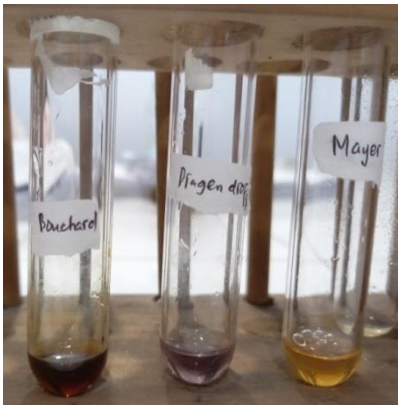
Alkaloid compounds work by preventing peptidoglycan, a component that forms cell walls, resulting in unhealthy cell lining and bacterial cell death. In addition, nitrogen in the alkaloid base group has the potential to alter bacterial DNA, change the structure and composition of amino acids, and cause lysis of bacterial cells. (Maftunah *et al*., 2015).

According to research by Gea *et al.*, (2021) screening the content of secondary metabolites contained in acetone extract, pulutan leaves are known to contain secondary metabolites of alkaloid groups, flavonoids, steroids, tannins, and saponins. While in the study of Wulandari *et al.*, (2009) pulutan leaf ethanol extract cultivated in the Pasir lor area, Banyumas only contains alkaloids and polyphenols. Differences in the presence of a class of secondary metabolites in the same plant can be caused by growing conditions such as the environment in which they grow and differences in climate (Frengki *et al*., 2014) and can be influenced by different solvents for extraction used.

Table 1. Phytochemical Screening Results

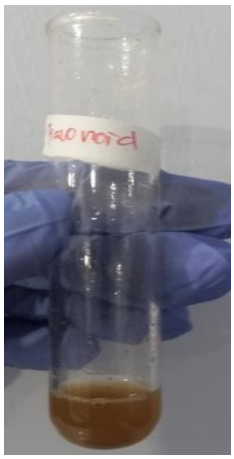
Phytochemical Test	Reacting	Reaction Results	Information
Alkaloids	Mayer	-	No white or yellow deposits are formed
	Dragendroff	-	No red or orange deposits form
	Bauchardat	-	No dark brown deposits are formed
Flavonoid	Concentrated Mg+ HCl	+	No orange color formed
Saponin	Air +HCl 2N	+	Formed permanent foam
Tannin	FeCl3	+	Not formed blackish-green
Steroids/Triterpenoids	Lieberman Bauchard	+	Formed red color indicates the presence of triterpenoids

Alkaloid Test



Bouchardat Dragendroff Mayer

Flavonoid Test



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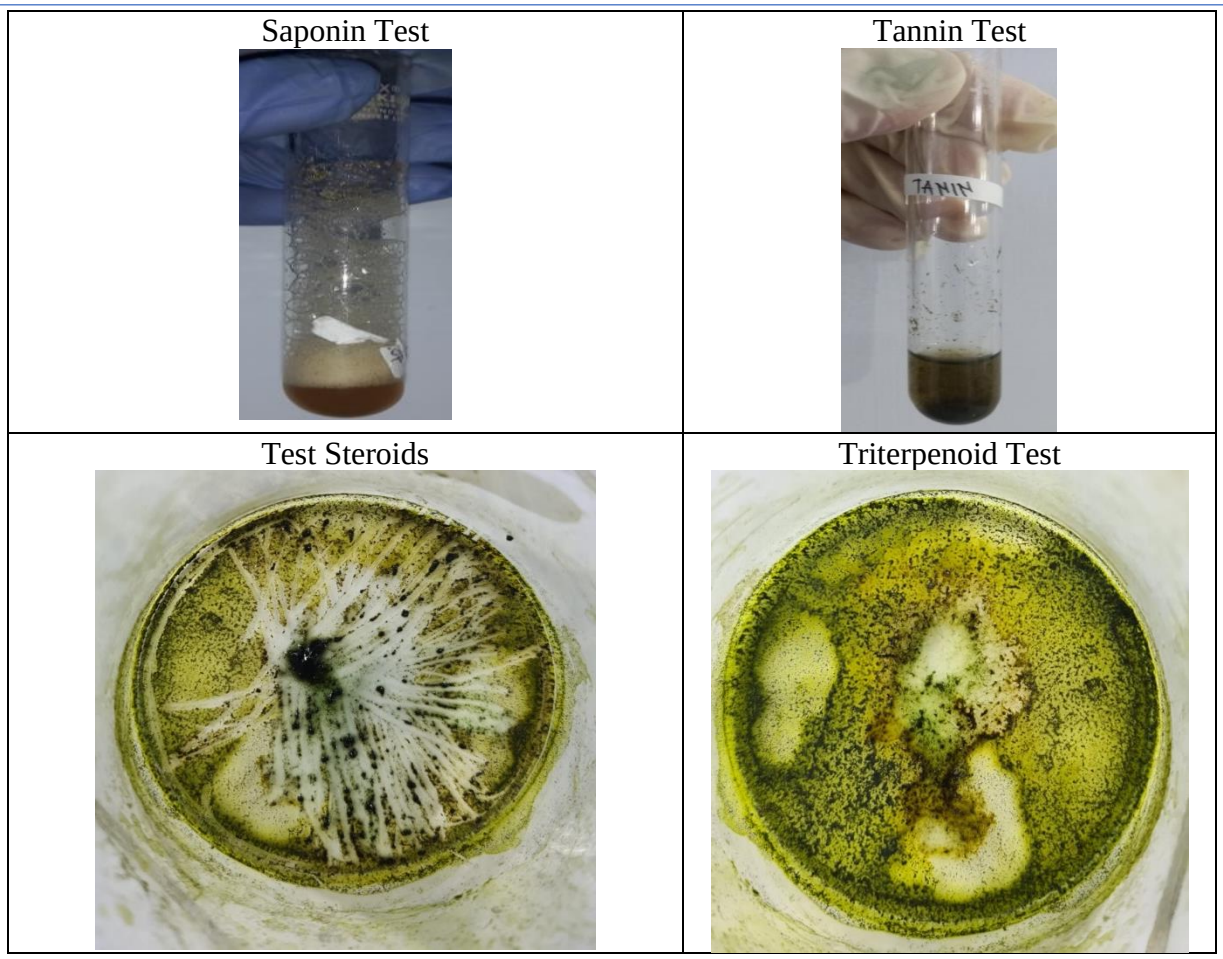

Figure 1. Phytochemical Screening Results of Pulutan Leaf Simpilisia (*Urena lobata*)

Table 2. Antibacterial Activity Test Results of Pulutan Leaf Ethanol Extract

Pulutan leaf extract concentration	Inhibitory Zone Diameter (mm) Deuteronomy				Average	Category (Panaungi &; sakka, 2022)
	I	II	III	IV		
15 %	3,8	2,7	3,85	5,9	4,0625	Weak
25 %	4	3,1	3,9	5,9	4,2	Weak
35 %	4,7	4,5	4,65	5,95	4.85	Weak
45 %	3,4	2,2	4,2	2,5	3.075	Weak
55 %	4	4,3	6,2	3,85	4.5875	Weak
65 %	4,2	5	3,75	4,25	4,3	Weak
Chloramphenicol					19,9	Strong
Ethanol p.a					0,00	Ineffective

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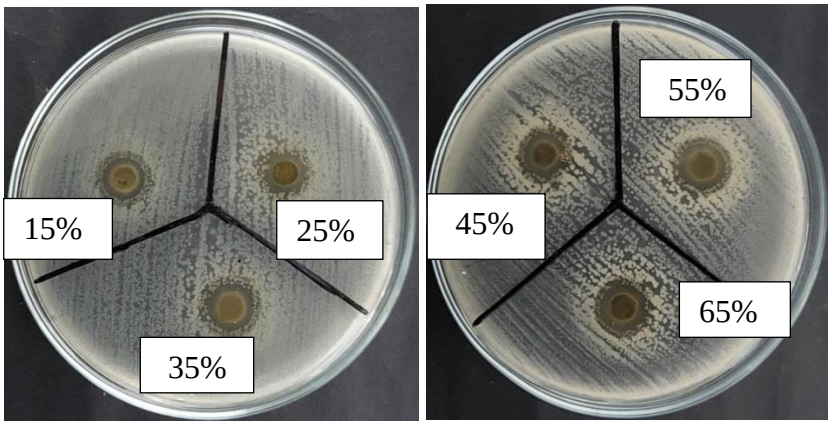
The results revealed that pulutan leaf ethanol extract (*Urena lobata*) able to stop the growth of bacteria *Escherichia coli* on antibacterial activity test. Based on the results of inhibition zone measurements, it shows that pulutan leaf ethanol extract has a weak inhibitory power against bacteria *E. coli*. Concentrations of 15%, 25%, 35%, 45%, 55%, and 65% have weak inhibitory. The determination of this criterion is based on Panaungi & sakka (2022) saying that the strength of bacterial inhibitory power based on the size of the diameter of the inhibitory zone, with an inhibitory zone diameter of 5 mm in the weak category, an inhibitory zone diameter of 5-10 mm in the medium category, an inhibitory zone diameter of 10-20 mm in the strong category, and an inhibitory zone diameter of 20 mm is a very strong category.< >

On the table 2 It was seen that the diameter of the inhibitory zone was greatest in bacteria *E.coli* found at a concentration of 35% of 4.85 mm and the smallest concentration of 45% of 3.075 mm this shows that at concentrations of 35%, 55% and 65% decreased, positive control (+) in bacteria *Escherichia coli* has a strong inhibitory category of 19.9 mm and negative control (-) has no inhibitory or ineffective category.

In this study, it was known that the high and low concentration of pulutan leaf ethanol extract was not directly proportional to the diameter of the inhibitory zone produced. The test results of pulutan leaf ethanol extract showed that the greater concentration did not provide a greater inhibitory effect but had a smaller inhibitory ability than other concentrations (Zeniusa *et al*., 2019).

There are several possibilities that can cause this to happen, such as the lack of diffusion power of the extract into the medium. The diffusion process of the extract can be affected by dilution factors. The higher the concentration of the extract, the lower the solubility (thickens like *gel*), so that this can slow down the diffusion of the active ingredients of the extract into the medium and can eventually reduce the ability of extracts with high concentrations to inhibit growth *E. coli* (Dewi, 2010).

Another factor that supports the difference in the diameter of the inhibitory zone in this study is the type of bacteria used. In this study, the bacteria used were *E. coli*. Bacteria *E. coli* is a gram-negative bacterium. Gram-negative bacteria have a very complex cell wall structure, containing three polymer layers located outside the peptidoglycan layer namely lipoproteins, the outer membrane consists of phospholipids and lipopolysaccharides. This condition can cause the ability of antibacterial substances to enter bacterial cells to decrease, so that it only slightly affects the life cycle of bacteria.



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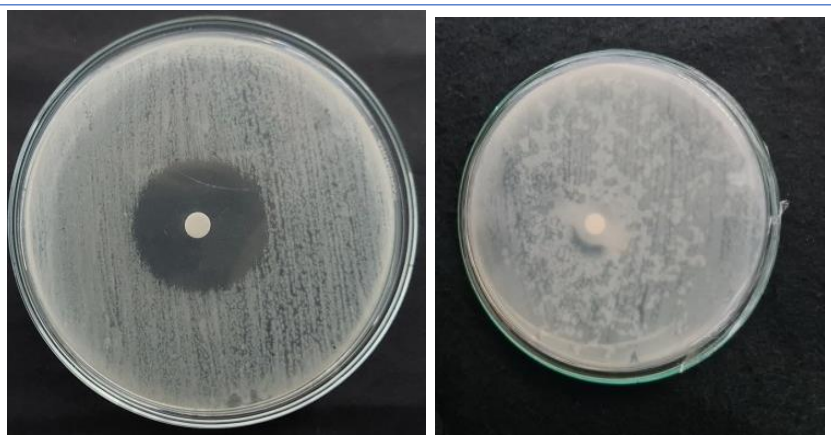

Figure 2. Antibacterial Aactivity Test of pulutan leaves and control on *Escherichia coli bacteria*

Conclusion

Based on the results of pulutan leaf ethanol extract research (*Urena lobata* L.) against bacteria *Escherichia coli*, then the conclusion can be drawn of pulutan leaf ethanol extract (*Urena lobata* L.) contains secondary metabolite compounds including flavonoids, saponins, steroid tannins and triterpenoids. Pulutan leaf ethanol extract bark extract (*Urena lobata* L.) has antibacterial activity against bacteria *Escherichia coli* with an inhibitory zone of 4.3 mm weak category at a concentration of 66%, an inhibitory zone of 4.5875 mm a weak category at a concentration of 55%, an inhibitory zone of 3.075 mm a weak category at a concentration of 45%, an inhibitory zone of 4.85 mm a weak category at a concentration of 25%, and an inhibitory zone of 4.0625 mm a weak category at a concentration of 15%.

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