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Antibacterial activity test of *Etlingera elatior* (Jack) R.M. Sm leaf extract against bacteria *Staphylococcus aureus* and *Escherichia Coli* diffusion method

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Abstract
Patikala leaves are widely used by the community as a medicine for skin diseases and antimicrobials in food. The purpose of this study was to determine the antibacterial activity of patikala leaf extract from various types of solvents based on the level of polarity and the class of compounds that act as antibacterials. The extraction method used is maceration using several solvents, namely n-hexane, ethyl acetate, and ethanol. Then screened the compound class and tested its antibacterial activity using the diffusion method. The results of the research are ethanol, ethyl acetate and n-hexane extracts from patikala leaves can inhibit bacterial growth *S aureus*with diameters of inhibition zones namely 8.91 mm ethyl acetate, 8.17 mm ethanol and 8.03 mm n-hexane. on bacteria *E. coli*the ethanol and ethyl acetate extracts had an inhibition of 8.75 mm and 7.59 mm, while the n-hexane extract had no antibacterial inhibition, so the antibacterial activity of the patikala leaf extract was smaller when compared to the (+) tetracycline control. So it can be concluded that n-hexane extract, ethyl acetate extract and ethanol extract have activity against bacteria *Staphylococcus aureus* whereas in bacteria *Escherichia coli* only ethanol and ethyl acetate extracts that have an antibacterial effect.).

Keywords: *Etlingera elatior* (Jack) R.M. Sm., antibacterial, *Staphylococcus aureus*, *Escherichia coli*

Introduction

Infectious disease is a disease that is a major problem in developing countries such as Indonesia, infectious diseases caused by gram-positive and gram-negative bacteria are still a problem that is difficult to overcome because it involves public awareness of cleanliness and health (Tjay and Rahardja, 2002) [10]. One of the bacteria that can cause infection is *Staphylococcus aureus* and *Escherichia coli*.

Staphylococcus aureus and *Escherichia coli* is a normal flora on the skin and human digestive tract which in certain circumstances can cause diarrhea and become a pathogen (Tjay and Rahardja, 2002) [10]. Therefore it takes antibacterial to cure infections caused by bacteria. Antibacterials are compounds that can be used to treat infections caused by bacteria (Wardhani, 2012) [11].

Several studies have stated that these two bacterial species are resistant to several types of antibiotics. This shows that the treatment of infectious diseases by administering antibiotics has adverse side effects, so that exploration of medicinal plants needs to be done to overcome this problem (Ami, 2016).

One of the plants that has potential as an antibacterial is patikala, this is in accordance with the empirical experience of the community, this plant can be used as food and also used in medicine. Patikala flowers can be used as a medicine for diseases related to the skin, pseudostems and leaf sheaths can be used as natural soap and have antimicrobial properties against pathogenic microbes and food spoilage (Handayani *et al*, 2014) [2]. Patikala plant has three compounds that can function as antimicrobials namely saponins, flavonoids and tannins. Saponin compounds are compounds that can have an antimicrobial effect by disrupting the stability of bacterial cell membranes, causing bacterial cell lysis, flavonoid compounds also have an antimicrobial effect through their ability to form complexes with extracellular proteins and bacterial cell walls and tannin compounds function as antimicrobials by coagulating and agglomerating microbial cells so that microbes die (Kusumawati, 2015) [5].

The antibacterial potential of patikala leaves has also been proven by several previous studies. In the study of Ningtyas (2010) [7] it was found that the water extract of Patikala leaves could inhibit bacteria *E. coli* with a MIC value of 90% and *Staphylococcus aureus* 15% using the diffusion method. In Kusumawati's study (2016) [6] tested the antibacterial activity of the ethanol extract of patikala leaves against gram-positive bacteria (*Bacillus cereus*) and negative bacteria (*Escherichia coli*) using the well diffusion method, it was found that the ethanol extract of Patikala leaves had antibacterial activity where an increase in the concentration of the extract resulted in a greater diameter of inhibition. In Kusumawati's research (2015) [5] Test the antibacterial activity of the ethanol extract of patikala leaves (*Etlingera elatior* (Jack) R.M.Sm against *Salmonella thypi* produces an inhibition zone of 9.28 mm which is included in the category of moderate acting antibacterial.

It can be seen from these studies that the antibacterial activity can be different due to the solvent used. The choice of solvent based on the level of polarity is very useful for obtaining extracts with a greater yield and is also intended so that the class of antibacterial compounds that have the highest activity can be extracted. Extraction of antibacterial compounds from plants generally uses solvents with different polarity levels, such as ethanol, ethyl acetate, and n-hexane (Susana *et al*, 2018). Based on the description above, a study was carried out to test the activity of Patikala leaf extract based on the level of solvent polarity using the diffusion method.

Research Methods

Tools and materials

The tool used is an autoclave maceration vessel*(When®)*, chamber, petri dish, Erlenmeyer*(Pyrex®)*, Incubator *(Memmert®)*, spirit lamp, UV lamp 254 nm and 365 nm *(Spectroline®)*, round ose, oven *(Falc®)*, tweezers, capillary tube, 1 ml syringe, 10 ml syringe, test tube*(Pyrex®)*, and analytical balance*(Mettler toledo®).*

The materials used in this study were aluminum foil, Aqua Pro Injection, DMSO (*dimetil sulfoxide*), ethanol, ethyl acetate, n-hexan, 0.9% NaCl, cotton, tetracycline disc paper, blank disc paper, pure bacterial culture *Staphylococcus aureus* and *Escherichia coli*, medium *Nutrient Agar* (MERCK®)

Sample collection and processing

Patikala leaves are collected and then wet sorted, washed with running water to remove soil and other impurities that are still attached. The chopped leaves are then dried to obtain dried simplicia. Then it is sorted dry and then the simplicia is crushed.

Patikala Leaf Extraction

Patikala leaf simplicia powder was macerated using each solvent with a different polarity level of ethanol, ethyl acetate and n-hexane). 500 g of each simplicia was put into the maceration container, moistened with solvent and then allowed to stand for ± 15 minutes. After that, the filter is added until all the simplicia is completely submerged. Then it was left in a place protected from sunlight for 3 x 24 hours and occasional stirring was carried out, then remaceration was carried out using the same solvent. The maceration results were filtered using a filter cloth. The filtrate obtained was then concentrated using a rotary evaporator.

Uji Fitokimia

1. Alkaloid Test

The extract was weighed as much as 0.5 gram and then put into a test tube, added 5 ml of 2 N HCl and heated in a water bath, after cold filtered and the filtrate was dripped with Dragondrop reagent (potassium iodide solution), red precipitate to orange indicating positive for containing alkaloid compounds, yellowish white precipitate with Mayer's reagent and brown precipitate with Wagner's reagent.

2. Saponin Test

The extract was weighed as much as 0.5 gram and then put into a test tube then added 10 ml of hot water, shaken vigorously for 10 seconds. If froth or foam is formed for approximately 10 minutes as high as 1 cm to 10 cm, and when added with 2 N hydrochloric acid as much as 1 drop and the foam does not disappear, it means that it contains positive saponins.

3. Tanin Test

The extract was weighed as much as 0.5 gram and then put into a test tube then added with 10 ml of warm water, then filtered and the filtrate was added 2-3 drops of FeCl3 1% if dark blue or blackish green is formed, then the extract indicates a tannin group compound.

4. Flavonoid Test

The extract was weighed as much as 0.5 gram put into a test tube and then added with 70% ethanol, then added 0.5 mg of magnesium powder and 5-6 drops of concentrated HCI, if formed a red color indicates a flavonoid compound, a dark red color indicates a compound flavonols and flavonones, if an orange color is formed it indicates a flavone compound and if a green color is formed it indicates an aglycone or glycoside compound.

5. Triterpenoids and steroids Test

± 1 ml of extract was mixed with 3 ml of chloroform or 70% ethanol and added 2 ml of concentrated sulfuric acid and 2 ml of anhydrous acetic acid. The color change from purple – red contains terpenoids and green – blue indicates the presence of steroid compounds (Harborne, 1996) [3].

Tool Sterilization

Glass tools (test tubes, glass beakers, petri dishes) are washed, then dried, wrapped in paper and sterilized in an oven at 160º-180ºC for 1-2 hours and materials to be used such as media, aquadest, sterilized by autoclaving at 121°C for 15 minutes. Ose is sterilized by heating it.

Media Creation *Nutrient Agar* (NA)

5 grams of NA medium was weighed into an Erlenmeyer, then dissolved with 250 mL of distilled water and heated to boiling while stirring, then sterilized in an autoclave for 15 minutes at 121°C.

Bacterial Rejuvenation Test

7 mL of NA medium that had been sterilized was taken into a sterile test tube and stored at room temperature for approximately 30 minutes until the media solidified at a slope of 30°. Pure culture of bacteria *Staphylococcus aureus* and *Escherichia coli* 1 ose of each was taken, then inoculated by streaking on slanted NA medium, then incubated at 37°C for 1 x 24 hours.

Preparation of Test Bacterial Suspensions

Rejuvenation test bacteria were taken as much as 1 ose, suspended with 0.9% NaCl ± 3 mL in a sterile test tube, then homogenized and equated with turbidity standard *Mc Farland.*

Antibacterial Activity Testing with the Diffusion Method

10 ml of NA medium was put into the petri dish and allowed to solidify. After the medium has solidified, the bacteria are taken using a sterile swab, then scratched evenly on the surface of the medium, then each extract is made in a concentration of 1% dissolved in 10% DMSO, each paper disc is inserted into the n-hexane extract, ethyl acetate and ethanol aseptically using sterile tweezers. Paper disc containing extract, control (+) tetracycline, and control (-) 10% DMSO was inserted into the surface of the medium. Then incubated at 37ºC for 1 x 24 hours. The clear zone formed was observed and the diameter of the inhibition area was measured with a caliper, this treatment was carried out 2 times.

Results and Discussion

In this study, testing the antibacterial activity of n-hexane, ethyl acetate and ethanol extracts from patikala leaf extract (*Etlingera elatior* (Jack) R.M. Smith) against gram-positive

bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli.*

Table 1: Percentage of Yield of Patikala Leaf Extract

Solvent	Heavy simplicity (g)	Heavy Extract (g)	Yield (%)
Ethanol	500	50,81 g	10,162 %
Ethyl acetate	500	39,242 g	7,8484 %
n-Hexane	500	26,729 g	5,3458 %

500 g of patikala leaf simplicia was mashed using a blender, then extracted based on the level of solvent polarity by maceration method using 70% ethanol, 15 liters of ethyl acetate and n-hexane. The patikala leaf extract was then concentrated using*rotary evaporator*, from the extraction process, 50.81 g of viscous ethanol extract was obtained, 39.242 g of ethyl extract and 26.729 g of n-hexan extract, with the respective yields of 10.162% ethanol extract, 7.848% ethyl acetate extract and 5 n-hexane extract, 3458 %. Ethanol is a polar solvent so that it can attract polar, semi-polar compounds and a few non-polar compounds so that the yield of the extract is greater than that of other extracts, while a semi-polar solvent (ethyl acetate) can attract semi-polar and slightly non-polar compounds. and non-polar solvents (n-hexane) will only attract non-polar compounds.

After the viscous extract was obtained, a phytochemical test was carried out to determine the content of chemical compounds contained in the extract, namely tests for alkaloids, tannins, flavonoids, saponins, steroids, and terpenoids which can be seen in table 2.

Table 2: Phytochemical Test Results of Patikala Leaf Extract

Compound class	Solvent Type		
	Ethanol	Ethyl Acetat	n-Hexane
Flavonoid	+	+	-
Saponin	+	-	-
Polifenol/tannin	+	-	-
Alkaloid	+	-	+
Steroid	+	+	-
terpenoids	-	-	+

The results of the analysis in table 2 show that the sample with ethanol solvent showed a positive reaction in the test for the class of flavonoids, saponins, tannins, alkaloids and steroids which was marked by the appearance of a dark red color for the flavonoid compound test, foam formed for the saponin compound test, green color formed blackish for tannin compounds, green color for steroids and for alkaloid compounds formed white precipitate for Mayer reagent and brown precipitate for wagner reagent.

For ethyl acetate solvent, it showed a positive reaction in the flavonoid and steroid compound test which was indicated by the presence of an orange color in the flavonoid compound test and a green color in the steroid compound test. While the solvent*n*-hexane showed a positive reaction for the terpenoid and alkaloid compounds test which was indicated by the appearance of a brown precipitate on the terpenoid compound test and for alkaloid compounds a white precipitate was formed for Mayer's reagent and brown precipitate for Wagner's reagent.

The compound profile of the sample extract can change with the difference in the polarity of the solvent used. Differences in the level of solvent polarity certainly affect the type of secondary metabolites extracted. It is known that the ethanol, ethyl acetate and n-hexane extracts of patikala leaves contain bioactive compounds such as polyphenols, alkaloids, flavonoids, steroids, saponins and essential oils which have potential as antioxidants capable of capturing free radicals (Handayani *et al*, 2014) [2].

Table 3: The results of the antibacterial activity test of patikala leaf extract using the diffusion method

S aureus	**Inhibition Zone Diameter(mm)**				
	Ethyl acetate	**Ethanol**	**n-hexane**	**Positive Control**	**Negative Control**
I	8.53	8.17	8.03	30.27	-
II	9.29	-	-	30.81	-
Total	17.82	8.17	8.03	61.08	-
Rate-rate	8.91	8,17	8.03	30.54	-
SD	0.537401154	-	-	0.381837662	-
One Resistor (mm)	8.91 ± 0,537	8.17	8.03	30.54 ± 0.381	-
E. coli	**Ethyl acetate**	**Ethanol**	**n-hexane**	**Positive Control**	**Negative Control**
I	8.24	7.71	-	22.49	-
II	9.27	7.48	-	22.64	-
Total	17.51	15.19	-	45.13	-
Rate-rate	8.75	7.59	-	22.56	-
SD	0.728319985	0.16263456	-	0.106066017	-
One Resistor (mm)	8.755 ± 0,728	7.595 ± 0,162	-	22.565 ± 0,106	-

Then tested the antibacterial activity to see the antibacterial activity of the ethanol, n-hexan and ethyl acetate extracts using the agar diffusion method. The bacteria used are *Staphylococcus aureus* and *Escherichia coli* to represent gram positive and gram negative bacteria, using*paper disc* tetracycline as control (+), 10% DMSO as control (-) and using growth media *Nutrient Agar* (NA). Antibacterial activity testing was carried out in duplicate using the same concentration, namely 1% each.

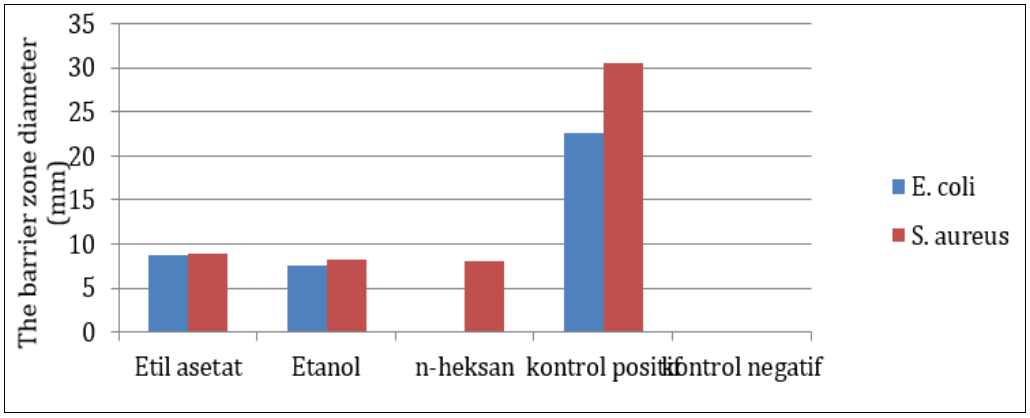

Fig 1: The results of the patikala leaf extract activity test against bacteria *Staphylococcus aureus*and *Escherichia coli*

Based on the results of observations that test the activity of patikala leaf extract against bacteria *staphylococcus aureus* i.e. 8.91 mm of ethyl acetate extract, 8.17 mm of ethanol extract and 8.03 mm of n-hexane extract. Meanwhile, the activity test of patikala leaf extract against bacteria *Escherichia coli* ethyl acetate which is equal to 8.75 mm, ethanol extract 7.59 mm and n-hexane extract have no activity against bacteria *Escherichia coli*. The results of measuring the inhibition of each patikala leaf extract can be categorized as having a moderate response in inhibiting bacterial growth because it has an appropriate inhibition zone diameter of 5-10 mm (Ratina *et al*, 2015).

As for the control (+) tetracycline, the diameter of the inhibition zone for bacteria was obtained *Saureus*is 30,54 mm and*E. coli*22.56mm. This result was higher when compared to the damer of the inhibition zone on the ethanol, ethyl acetate and n-hexane extracts, indicating that the antibacterial activity of the patikala leaf extract was lower when compared to the (+) tetracycline control. whereas in the control (-) 10% DMSO did not give an inhibition zone.

Inhibition zone test results using bacteria *Staphylococcus aureus* and *Escherichia coli* shows that the resulting inhibition zone on testing with bacteria *Staphylococcus aureus* bigger than the test with bacteria *Escherichia coli*. The difference between gram positive bacteria and gram bacteria The negative effect is on the composition and structure of the cell wall. The cell wall structure possessed by gram-positive bacteria is simpler, namely single-coated with content lower lipids so that it is easier for bioactive substances to enter the cells.

Meanwhile, the structure of the cell wall of gram-negative bacteria is more complex bioactives are difficult to enter into cells (Pelczar and Chan, 2008) [8].

Conclusion

The ethanol, ethyl acetate and n-hexane extracts of patikala leaves have antibacterial activity which can inhibit bacterial growth *S aureus* with diameters of inhibition zones namely 8.91 mm ethyl acetate, 8.17 mm ethanol and 8.03 mm n-hexane. on bacteria *E. coli* the ethanol and ethyl acetate extracts had an inhibition of 8.75 mm and 7.59 mm, while the n-hexane extract had no antibacterial inhibition, so the antibacterial activity of the patikala leaf extract was smaller when compared to the (+) tetracycline control.

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