Antibacterial Activity of Lime Root Extract (Citrus Aurantifolia) Against Staphylococcus Aureus and Escherichia Coli Bacteria Using the Dilution Method

Uswatun Liza Najiya[1], Rohama[1], Ahmad Hidayat[2]

[1]Bachelor of Pharmacy Study Program, Faculty of Health, Universitas Sari Mulia,

[2]Information Systems Study Program, Faculty of Science and Technology, Universitas Sari Mulia,

Banjarmasin, South Kalimantan, Indonesia.

najiyaliza25@gmail.com

Keywords: (Citrus aurantifolia), E-coli, S.aureus, Dilution.

ABSTRACT

Empirically, lime is widely used by the people of Indonesia both as a cooking spice, cough medicine, shed phlegm, influenza, antibacterial treatment of acne, and as an ulcer medicine. The objective of this research is to determine the ability of lime (Citrus aurantifolia)root extract as an antibacterial against Staphylococcus aureus and Escherichia coli bacteria using the dilution method. Experimental research is used the maceration method with 96% ethanol solvent soaked 3x 24 hours, then thickened with a rotary evaporator, then antibacterial testing with S. aureus bacteria was carried out by the dilution method. Simplicia lime root as much as 200 grams using 96% ethanol obtained a thick extract of 3.5 grams. The secondary metabolites contain positive alkaloids, flavonoids, saponins and tannins. the minimum inhibitory concentration (MIC) of Staphylococcus aureus bacteria with Escherichia coli was present at a concentration of 50% while the Minimum Killing Concentration of Staphylococcus aureus bacteria with Escherichia coli was not found. The ethanolic extract of lime root (Citrus aurantifolia) has the ability as an antibacterial against Staphylococcus aureus bacteria with a MIC value of 50% concentration. contains positive secondary metabolite compounds, namely alkaloids, flavonoids, sapoons and tannins. The ethanolic extract of lime root (Citrus aurantifolia) did not have a Minimum Kill Concentration (KBM) value on Staphylococcus aureus and Escherichia coli.

INTRODUCTION

The benefits of biodiversity for humans are very diverse, such as medicines, cosmetics, fragrances, fresheners and dyes. Apart from being a producer of organic compounds of infinite types and numbers [1]. Traditional medicine is medicine that is processed traditionally, passed down from generation to generation, based on recipes from ancestors, customs, beliefs or local habits, both magical and traditional knowledge [2]. Plants in Indonesia have been widely used by the community as traditional medicine for generations to treat various diseases, including infectious diseases. However, there are not a few plants in Indonesia whose benefits are still not known to the public. One of the plants that can be used as a treatment but not many people know about it, the lime plant (Citrus aurantifolia) is empirically widely used by Indonesian people either as a cooking spice or empirically used as a medicine for coughs, shedding phlegm, influenza and acne [3]. Based on several studies conducted, one of them is lime extract which has antimicrobial activity against upper respiratory tract pathogenic bacteria [4]. The root of C. aurantifolia is used in traditional medicine for the treatment of fever [5].

Flavonoids, limonoids, and ascorbic acid are a group of citrus phytochemicals and micronutrients, which are responsible for anti-inflammatory activity, The purpose of this research was to determine the level of ability of lime root extract (Citrus aurantifolia) as an antibacterial against Staphylococcus aureus and Escherichia coli bacteria using the dilution method and to determine the Minimum Inhibitory

Concentration (MIC) and Minimum Killing Concentration (KBM) of lime root extract (Citrus aurantifolia) against Staphylococcus aureus and Escherichia coli bacteria using the dilution method.

MATERIALS AND METHODS

Tool

The tools used in this study consisted of measuring cups (Pyrex), test tubes (Pyrex), test tube racks, dropper pipettes, Erlenmeyer flasks (Pyrex), watch glass (Pyrex), blender, analytical scales (AciS AD-600i). , hot plate (Thermo Scientific-Cimarec), stir bar, stirrer, petri dish (Pyrex), rotary evaporator (Dragonlab RE 100 Pro), loop needle, tweezers, large jar for maceration, funnel (Pyrex), filter paper, aluminum foil , labels, scale rulers, spirits, incubators (ESCO Isotherm), (Biological Safety Cabinet (Thermo Scientific), and autoclaves (GEA YX-280D).

Ingredient

The materials used in this study consisted of lime root (Citrus aurantifolia) as the test material used as a sample, test bacteria (Staphylococcus aureus and Escherichia coli) obtained from the Microbiology Laboratory of Sari Mulia University, DMSO solution, sterile distilled water, 96% ethanol, ciprofloxacin as a comparison antibiotic, Nutrient Broth and Nutrient Agar (NA).

Method Raw Material Collection

Collection of raw materials from lime root plants (Citrus aurantifolia) which are taken directly in the Central Kalimantan region and take their roots as samples.

Simplicia Management

The simplicia management is processed starting from the collection of raw materials, namely all the roots of lime leaves, which are then carried out by wet sorting, washing, slicing or changing shape, then drying using an oven. After drying, simplicia is made into powder.

Extraction

Lime root extraction, which starts from processing lime root simplicia into lime root extract by using the maceration method. Maceration was carried out by soaking 200 grams of lime root simplicia powder in 96% ethanol solvent. Maceration is carried out until a clear solution is obtained, namely the condition in which all extracts are considered to have been dissolved by the solvent by changing the solvent every 1x24 hours. Solvent replacement in the extraction process is carried out for 3x24 hours for clear solvents. The collected maserate was concentrated using a rotary evaporator to obtain a thick extract from lime roots. The extract was weighed and stored in a closed glass container before being used for testing.

Phytochemical Screen Test

In the phytochemical screening test, the color reaction test was carried out, namely, Alkaloids, Flavonoids, Terpenoids, Steroids, Saponins, Tannins.

Antibacterial Activity Test

Antibacterial activity test of lime root extract (Citrus aurantifolia) in this study was carried out using the dilution method. The dilution method is a method of testing antibacterial activity based on observations at the lowest concentration which inhibits the growth of microorganisms with liquid media or solid media which is diluted after being mixed with antimicrobial substances. With the stages of making negative control solutions, making positive controls, making test solutions, making media, preparing inoculums, making bacterial suspensions, making standard McCarland 0.5 solutions, testing the antibacterial activity of lime root (Citrus aurantifolia).

RESULTS

The dried simplicia of lime root (Citrus aurantifolia) obtained is 200 grams.

% Yield = Extract weight obtained (grams) / Simplicia weight before extraction (grams) X 100% = 13.5 grams X 100% / 200 grams = 6.75% Extract Yield Value

Phytochemical Screening Test

Phytochemical screening tests include tests on Alkaloids, Flavonoids, Terpenoids, Steroids, Saponins, and Tannins, as well as phytochemical screening tests for the ethanol extract of lime root (Citrus aurantifolia).

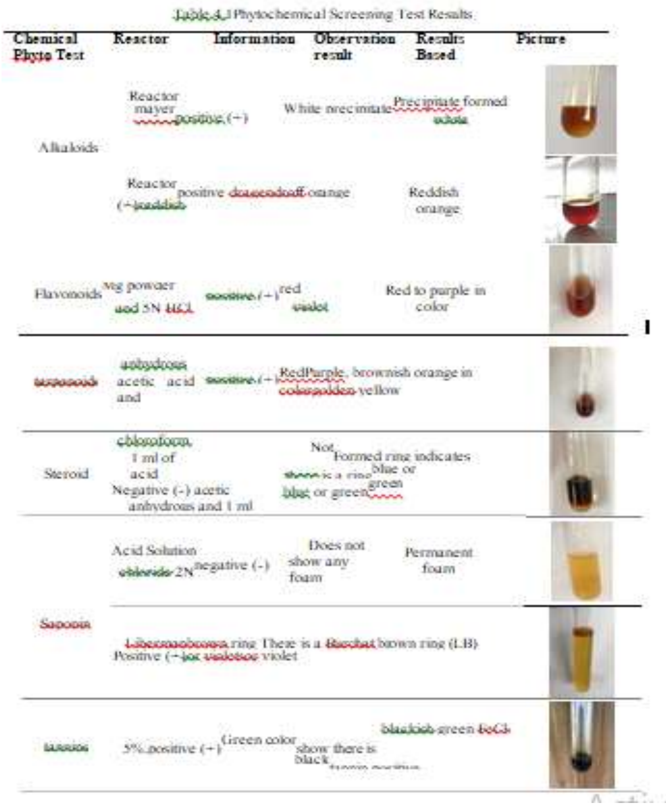
Table 4.1 Phytochemical Screening Test Results

Chemical Phyto Test	Reactor	Information	Observation result	Results Based	Picture
Alkaloids	Reactor mayer	positive (+)	White precipitate	Precipitate formed white	
	Reactor [illegible]	positive (+)	reddish orange	Reddish orange	
Flavonoids	Mg powder and 5N HCl	positive (+)	red violet	Red to purple in color	
[illegible]	anhydrous acetic acid and	positive (+)	RedPurple, brownish orange in color golden yellow		
Steroid	chloroform, 1 ml of acid acetic anhydrous and 1 ml	Negative (-)	Not shown is a ring blue or green	Formed ring indicates blue or green	
Saponin	Acid Solution chloride 2N	negative (-)	Does not show any foam	Permanent foam	
	Liebermann-Burchard	Positive (+)	There is a brown ring (LB) violet		
Tannins	5%	positive (+)	Green color show there is black	blackish green FeCl3	

*** Note:**(+) Contains Chemical Substances
(-) Does Not Contain Chemical Substances

Minimum Inhibitory Concentration (MIC)

The results of observing the Minimum Inhibitory Concentration (MIC) value of the ethanol extract of lime root (Citrus aurantifolia) against Staphylococcus aureus and Escherichia coli bacteria where it was seen that at concentrations of 50% and 100% there was no bacterial growth marked as clear, it was

concluded that the MIC value at concentration 50% for Staphyllococcus aureus bacteria while for Eschericia colli bacteria it can be seen that at all concentrations there is cloudy growth of bacteria.

Table 4.2Results of Bacterial MIC*Staphylococcus Aureus*

Treatment	Replicati on I	Replicati on II	Replicati on III
Concentration 6.25 %	+	+	+
Concentration 12.5 %	+	+	+
Concentration 25%	+	+	+
Concentration 50%	-	-	-
Concentration 100%	-	-	-
Control (+)	-	-	-
Control (-)	+	+	+

* Information:
Control (+) : Ciprofloxacin Control (-) : DMSO
Sign (+) : there is growth of bacteria (turbid) Sign (-) : no growth of bacteria (clear)

Table 4.3Results of Bacterial MIC*Escherichia Colli*

Treatment	**Replicati on I**	**Replicatio n II**	**Replicati on III**
Concentration 6.25 %	+	+	+
Concentration 12.5 %	+	+	+
Concentration 25%	+	+	+
Concentration 50%	+	+	+
Concentration 100%	+	+	+
Control (+)	-	-	-
Control (-)	+	+	+

* Information:
Control (+): Ciprofloxacin
Control (-) : DMSO
Sign (+): there is growth of bacteria (turbid)
Sign (-): no bacterial growth (clear)

Minimum Kill Concentration (KBM)

It can be seen that at all concentrations there was growth of bacteria which showed no minimum kill in all replications.

Table 4.4Results (KBM) Bacteria*Staphylococcus Aureus*

Treatment	**Replicati on I**	**Replicati on II**	**Replicati on III**
Concentration 50%	+	+	+
Concentration 100%	+	+	+
Control (+)	-	-	-
Control (-)	+	+	+

*Information:
Control (+): Ciprofloxacin
Control (-) : DMSO
Sign (+): there is growth of bacteria (turbid)
Sign (-): no bacterial growth (clear)

DISCUSSION

The raw material (sample) used in this study was lime root (Citrus aurantifolia) obtained from the province of Central Kalimantan, Murung Raya district. The sample is processed into lime root simplicia (Citrus aurantifolia) which is washed using running water until it is clean to remove dirt that is still attached. Clean lime roots are dried in a room that is protected from direct sunlight. The purpose of drying is to reduce the water content in the roots so that microorganisms such as bacteria or fungi are not easy to grow.

The dried root is obtained as much as 200 grams. Simplisia that has been dried is sorted again from the impurities that are still left behind. Sorted simplicia is mashed using a blender to reduce the particle size and expand the surface of the sample so that the solvent can more easily enter the cell and pull the soluble active substance out of the cell. The simplicia powder obtained is stored in a dry and airtight container and protected from direct sunlight to prevent damage to the simplicia. Extraction of lime root simplicia powder using 200 grams of simplicia powder. Extraction was carried out to extract secondary metabolites found in lime root simplicia. Extraction was carried out using the maceration method with 96% ethanol solvent which was replaced every 1 x 24 hours for 3 days. The solvent used is 96% ethanol, because ethanol can attract almost all chemical compounds contained in plants, This is due to the presence of a polar OH group and an ethyl group (CH3CH2-) which is nonpolar. The concentration of 96% ethanol will increase the ability to withdraw compounds and also accelerate the solvent evaporation process. The maceration method was chosen because this method is easy to do, fast, and can extract metabolite compounds in extracts without damaging these compounds. The maserate that has been collected is concentrated using a rotary evaporator with a temperature of 50°C. Concentration results obtained dark brown viscous extract as much as 13.5 grams with a yield of 6.75%. Yield is a parameter of extract quality by comparing the extract obtained with the initial simplicia.

Phytochemical Screening

Phytochemical screening aims to provide an overview of the compounds contained in plants. One way to find out the content of compounds contained in plants is by using color reagents. The results of the phytochemical screening of concentrated lime root extract (Citrus aurantifolia) showed the presence of secondary metabolites, namely alkaloids, flavonoids, saponins, and tannins. Terpenoid compounds are indicated by reacting anhydrous acetic acid and H_2 $S0_4$ The dark color shows a purple-orange golden yellow color. Alkaloid compounds are indicated by the presence of a white precipitate with mayer reagent or with reddish orange dragendroff reagent. The presence of flavonoid compounds when the solution has a violet red color with Mg powder reagent and 5N HCL. Saponin compounds are indicated by the presence of brown or violet rings with Liberman Burchat (LB) reagent. Tannin compounds are indicated by the appearance of a green-black color with 5% FeCL3 reagent.

Antibacterial Activity Test of Lime Root Ethanol Extract (Citrus aurantifolia)

Antibacterial activity test was carried out using ethanol extract of lime root (Citrus aurantifolia) with various concentrations namely 62.5%, 12.5%, 25%, 50%, 100% concentration was prepared by dissolving 0.02 gram of ethanol extract of lime root (Citrus aurantifolia) with 10 ml DMSO solution. This test was carried out on Staphylococcus aureus bacteria as a representative of Gram-positive bacteria and Escherichia coli bacteria as a representative of Gram-negative bacteria. This test was carried out using the dilution method, where this method is a method of testing antibacterial activity based on observations at the lowest concentration which inhibits the growth of microorganisms with liquid media or solid media which is diluted after being mixed with antimicrobial substances. The dilution method was used in this study because this method is the most appropriate method for determining the value of MIC (Minimum Inhibitory Concentration) and Minimum Killing Concentration (KBM) values. This method can estimate the concentration of the antimicrobial substance being tested in agar media (Dilution Agar) or in broth media (macrodilution or microdilution) [6]. The negative control used was DMSO solution. The substance used as a negative control is a solvent used as a diluent of the compound to be tested. In this study the solvent used to dissolve the sample was DMSO solution. The aim is as a comparison that the solvent used as a diluent does not affect the results of the antibacterial test of the sample to be tested. The positive control used was Ciprofloxacin which is a bacteriostatic antibiotic agent and has a broad spectrum against Gram-positive and Gram-negative aerobic and anaerobic organisms. Antibacterial activity is closely related to secondary metabolites which in this study contained alkaloids, flavonoids, saponins, tannins and steroids. Flavonoids act as antibacterial by causing damage to the permeability of bacterial cell walls, microsomes and lysosomes as an interaction between flavonoids and bacterial DNA. Tannins can shrink cell walls or cell membranes thereby interfering with the permeability of the cell which results in the cell not being able to

carry out life activities so that its growth is stunted. Saponins act as antibacterial by reducing surface tension resulting in increased permeability or cell leakage and causing intracellular compounds to come out. Steroid compounds inhibit bacterial growth by inhibiting protein synthesis because they cause changes in the constituent components of the bacterial cell itself. Alkaloids work as an antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not completely formed and causes cell death [7]. Steroid compounds inhibit bacterial growth by inhibiting protein synthesis because they cause changes in the constituent components of the bacterial cell itself. Alkaloids work as an antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not completely formed and causes cell death [7]. Steroid compounds inhibit bacterial growth by inhibiting protein synthesis because they cause changes in the constituent components of the bacterial cell itself. Alkaloids work as an antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not completely formed and causes cell death [7]

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the concentration of an antibacterial substance that inhibits bacterial growth in test tubes containing liquid media. The results of the Minimum Inhibitory Concentration (MIC) in the ethanol extract of lime root (Citrus aurantifolia) were obtained by looking at the clarity (no bacterial growth) and turbidity (no bacterial growth) that occurred in the tube after incubation for 24 hours at 37°C. The MIC results of the ethanol extract of lime root (Citrus aurantifolia) against Staphylococcus aureus bacteria can be seen in table 4.2 and against Escherichia coli bacteria it can be seen in table 4.2 it can be seen that at concentrations of 50% and 100% there was no bacterial growth at all but based on the data it was seen that at all concentrations and there was bacterial growth. The Minimum Inhibitory Concentration (MIC) value is determined by looking at the smallest concentration that is still clear or does not show bacterial growth. The MIC value of the ethanol extract of lime root against Staphylococcus aureus and Escherichia coli obtained from the antibacterial activity of a plant extract will be classified as strong if the MIC value is > 100 mg/mL, moderate if 100 ≥ MIC ≤ 500 mg/mL and weak if the MIC value is MIC < 500 µg/mL. Based on this statement it can be concluded from the results of the study that the ethanol extract of lime root (Citrus aurantifolia) can inhibit Staphylacocus aureus bacteria at a concentration of 50%, and this is included in the strong category where the MIC value results are in the standard value provisions > 100 mg/mL i.e. 50%. Meanwhile, for Eschericia colli bacteria, the ethanol extract of lime root (Citrus aurantifolia) could not inhibit at all at the concentrations used by practitioners based on the results.

Minimum Kill Concentration (KBM)

Minimum Kill Concentration (KBM) is the lowest concentration of an antibacterial substance that kills bacterial growth in solid media. The results of the Minimum Kill Concentration (KBM) in the ethanol extract of lime root (Citrus aurantifolia) were obtained by observing the growth of bacteria on solid media after 24 hours of incubation at 37°C. Study results of ethanol extract of lime root (Citrus aurantifolia) against Staphylococcus aureus bacteria. The Minimum Inhibitory Concentration (MBC) value is determined by scratching the Minimum Inhibitory Concentration (MIC) test solution on solid media which is indicated by the presence or absence of bacterial growth on the solid media. It is known that the ethanol extract of lime root (Citrus aurantifolia) does not have a MBC value against Staphylococcus aureus and Escherichia coli bacteria, due to the discovery of bacterial growth in solid media at various extract concentrations. Pictures of the results of determining the value of the KBM can be seen in the attachment. Based on these data, it can be seen that at the highest concentration the extract still did not show a MBC value, but at this concentration the number of bacteria that grew was the least compared to other concentrations. Based on the data in the appendix, it can also be seen by naked eye the growth of Staphylococcus aureus and Escherichia coli bacteria on the solid media, although there is no KBM value for each bacteria. The growth of Escherichia coli bacteria is more than Staphylococcus aureus bacteria. This is due to differences in the structure of the cell walls of gram-positive and gram-negative bacteria. The cell wall of gram-positive bacteria (Staphylococcus aureus) has a simpler structure than the more complex cell wall structure of gram-

negative bacteria (Escherichia coli), which makes it more difficult for antibacterial substances to penetrate. Based on the results of testing the antibacterial activity, it can be said that this study is in accordance with the hypothesis made, namely the ethanol extract of lime root (Citrus aurantifolia) has the ability as an antibacterial. Marked by the presence of MIC value which means it can inhibit the growth of bacteria.

CONCLUSION

The ethanol extract of lime root (Citrus aurantifolia) contains secondary metabolites, namely alkaloids, flavonoids, saponins, and tannins. lime root (Citrus aurantifolia) has the ability as an antibacterial against Staphylococcus aureus bacteria with a MIC value (Minimum Inhibitory Concentration) at a concentration of 50%, while Escherichia coli bacteria has no bacterial growth at all and does not have a Minimum Kill Concentration (KBM) value.

REFERENCES

[1.] Arisandi, & Andriani, General Standard Parameters of Medicinal Plant Extracts, Jakarta, Ministry of Health of the Republic of Indonesia, (2009).

[2.] Indonesian Institute of Sciences, Definition of Traditional Medicine, Jakarta, LIPI Indonesia, (2016).

[3.] Lauma, SW, Test the effectiveness of lime juice (Citrus aurantifolia s) against the growth of staphylococcus aureus in vitro, Pharmacon, 4(4), (2014).

[4.] Adeyemo, DA, & Adeleye, AT, Emotional Intelligence , Religiosity and Self Efficacy as Predictors of Psychological Well - Being Among Secondary School Adol ... Emotional Intelligence , Religiosity and Self Efficacy as. Europe's Journal of Psychology, 4, 1. http://ejop.psychopen.eu/rt/printerFriendly/423/html, (2008).

[5.] Gharagozloo, M., Karimi, M., & Amirghofran, Z, Double-faced cell-mediated immunity in $β$-thalassemia major, stimulated phenotype versus suppressed activity, Annals of Hematology, 88(1), 21–27, (2009).

[6.] Balouiri, M., Sadiki, M., & Ibnsouda, SK, Methods for in vitro evaluating antimicrobial activity, A review, Journal of Pharmaceutical Analysis, 6(2), 71–79, https://doi.org/10.1016/j.jpha.2015.11.005, (2016).

[7.] Anuzar, CH, Hazar, S., & Suwendar, Antibacterial Activity Test of Ethanol Extract of Cayenne Pepper (Capsicum frustescens L.) Leaves on the Growth of Acne-causing Bacteria Propionibacterium acnes in vitro, Journal of Pharmacy, 3(2), 457–464, (2017).