



Antibacterial activity test of 70% ethanol extract on leaves katuk (*Sauropus androgynous* (L.) Merr.) against *Klebsiella pneumoniae* bacteria

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ABSTRACT

Bacteria *Klebsiella pneumoniae* known as reason infection channel breathing lower I with symptoms of cough and difficulty breathing. *Klebsiella pneumoniae* is a gram-negative bacterium from the Enterobacteriaceae family, non-motile, short rod-shaped and facultative anaerobic which can result in infection channel urine, infection breathing and bacteremia especially in individual whose immune system is weak. One of the herbal plants with active ingredients that have antibacterial properties is katuk leaves. Katuk leaves have the ability to inhibit growth bacteria Because contain compound saponins, flavonoid and tannin. The study aims to determine the antibacterial activity and the best concentration of 70% ethanol extract of katuk leaves against *Klebsiella pneumoniae* bacteria. The cup-plate or well method was used with concentrations of 100%, 70%, 40%, 10%, ciprofloxacin as K+ and aquadest as K-. It was found that 70% ethanol extract of katuk leaves has antibacterial activity against *Klebsiella pneumoniae* bacteria. The conclusion of this study was that the most optimum antibacterial activity at a concentration of 100% with an inhibition zone diameter of 7.69 mm was classified as a moderate growth inhibition response category.

Keywords: Antibacterial, Leaf Katuk, Extract, *Klebsiella pneumoniae*

ABSTRAK

Bakteri *Klebsiella pneumoniae* dikenal sebagai penyebab infeksi saluran pernapasan bawah akut dengan gejala batuk dan kesulitan bernafas. *Klebsiella pneumoniae* adalah bakteri gram negatif yang berasal dari family Enterobacteriaceae, non-motil, berbentuk batang pendek dan bersifat fakultif anaerob yang bisa mengakibatkan infeksi saluran kemih, infeksi pernafasan dan bacteremia terutama di individu yang daya tahan tubuhnya lemah. Salah satu tanaman herbal dengan kandungan zat aktif yang memiliki kemampuan sebagai antibakteri yaitu daun katuk. Daun katuk mempunyai kemampuan menghambat pertumbuhan bakteri karena mengandung senyawa saponin, flavonoid dan tanin. Penelitian bertujuan untuk mengetahui aktivitas antibakteri dan konsentrasi yang paling baik dari ekstrak etanol 70% daun katuk terhadap bakteri *Klebsiella pneumoniae*. Digunakan metode cup-plate atau sumuran dengan konsentrasi 100%, 70%, 40%, 10%, ciprofloxacin sebagai K+ dan akuades sebagai K-. Diharapkan bahwa ekstrak etanol 70% daun katuk memiliki aktivitas antibakteri terhadap bakteri *Klebsiella pneumoniae*. Kesimpulan penelitian ini didapatkan bahwa aktivitas antibakteri yang paling optimum pada konsentrasi 100% dengan diameter zona hambat 7,69 mm tergolong kategori respon hambat pertumbuhan sedang.

Kata Kunci: Antibakteri, Daun Katuk, Ekstrak, *Klebsiella pneumoniae*

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INTRODUCTION

Infectious diseases are a major health problem in developed and developing countries. These diseases are caused by the proliferation of microorganisms, a large group of microscopic organisms consisting of bacteria, fungi, parasites and viruses. Infection occurs due to interaction with microorganisms that cause damage to the human body which causes various clinical signs and symptoms. Symptoms can cause several infections including urinary tract and respiratory tract. Respiratory tract infections in the form of coughs and pneumonia are caused by the bacteria *Klebsiella pneumoniae* (Islawati, 2022).

Based on WHO data for 2019, there are 740.180 child Which die due to pneumonia. The incidence of pneumonia continues to worsen due to increasing antibiotic resistance (Khasanah, 2019). Resistance antibiotics causing increased medical costs, length of hospital stay, and increased number death. Bacteria The most frequently reported resistance is *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Sariyanto et al., 2022).

Bacteria *Klebsiella pneumoniae* known as the cause of acute lower respiratory tract infection with symptoms of cough and difficulty breathing. *Klebsiella pneumoniae* is bacteria grams negative which comes from the Enterobacteriaceae family, is non-motile, has a short rod shape and has facultative anaerobic properties which can cause respiratory infections, urinary tract infections and bacteremia in individuals with weak immune systems (Islawati, 2022).

One of the herbal plants with active ingredients that have antibacterial properties is katuk leaves. Katuk leaves have the benefit of inhibiting bacterial growth because they contain saponin, flavonoid and tannin compounds. Specifically, the content of 2 flavonoids in katuk leaves plays a role in disrupting the integrity of bacterial cell membrane components. In addition That, flavonoid Which There is on leaf Katuk plays a role in forming complex compounds in extracellular proteins in bacterial cell membranes (Wandira et al., 2022). Katuk leaves can also be used as a weight loss, overcome constipation, increase breast milk, antihypertensive, constipation, antihyperlipidemia, and can treat infections caused by bacteria. (Zukhri et al., 2018).

RESEARCH METHOD

Tool

Scales, blender, glass bottles closed, oven, rotary evaporator, 65 mesh sieve, funnel, test tube, beaker, test tube rack, Erlenmeyer flask, measuring cup, spatula, test tube, stirring rod, aluminum foil, wooden clamp, label, Ose needle, autoclave, laminary air flow, petri dish, incubator, bunsen burner, micropipette, vernier caliper, mortar and pestle, porcelain cup, cotton, filter paper, string, scissors.

Material

Katuk leaves (*Sauropus androgynus* (L.) Merr.), 70% ethanol, distilled water, 0.9% NaCl, Mayer's reagent, FeCl solution, acetic acid, concentrated HCl, ammonia, *Klebsiella Pneumoniae* bacteria, nutrient agar (NA), Mueller Hinton Agar (MHA) media, ciprofloxacin, H₂SO₄ 1%, BaCl₂.2H₂O 1%, DMSO.

Determination Plant

The first stage of this research was to determine the katuk leaves (*Sauropus androgynus* (L.) Merr.), plant determination is done by showing the katuk leaves and determining its existence in accordance characteristic features morphology. Plant determination was carried out at the Biology Learning Laboratory of Ahmad Dahlan University, Jl. South Ringroad, Tamanan, Banguntapan, Bantul.

Sampling of Katuk Leaves (*Sauropus androgynus* (L.) Merr.)

This katuk leaf sample was taken from Jalan Kampung Cipurut, Cirebonhas District, Sukabumi Regency, West Java 43193. The leaves taken were leaves with a diameter of 1-10 cm.

Making Simple Katuk Leaves (*Sauropus androgynus* (L.) Merr.)

Samples of katuk leaves (*Sauropus androgynus* (L.) Merr.) which have been collected Then cleaned up moreover formerly, washed until clean and there is no dirt and foreign objects in the katuk leaves. Furthermore, the drying process is separated between the stems and leaves, then approximately 10 kg of katuk leaves are prepared, then chopped using a knife and dried in an oven at a temperature of $\leq 50^{\circ}\text{C}$ until dry. The simplicia is weighed as a dry weight, then the simplicia is powdered using a blender and sieved with a mesh of 40, the results obtained are weighed first. Then stored in a tightly closed container, protected from heat and sunlight (Hartanto, 2018). Testing parameter specific simplisia, including identity testing simplicia and organoleptic (form (solid, powder, thick, liquid) color, odor and taste). While testing parameter non specific simple, including levels water, ash content and drying loss (Ministry of Health, 1985).

Making extract ethanol 70% leaf cat (*Sauropus androgynus* (L.) Merr.)

Katuk leaf extract is made using the maceration method, namely by soaking katuk leaf simple powder in a ratio of 1:10, as much as 500 g of katuk leaves with 5 liters of ethanol. 70%, process extraction done in a way maceration during 5 x 24 hours at room temperature and cover with a black cloth to avoid sunlight, while stirring once every 24 hours for 3-5 days, then filtering is carried out using filter paper so that the filtrate is evaporated using a rotary evaporator at a temperature of 50 °C until a thick extract is obtained, then thickened using a water bath. Testing parameter specific extracts, including identity testing simplicia and organoleptic (form (solid, powder, thick, liquid) color, odor and taste). While testing parameter non specific extract, including levels water, and ash content (Ministry of Health, 2000).

Phytochemical screening of katuk leaf (*Sauropus androgynus* (L.) Merr.)

Phytochemical screening in this study was carried out to determine the secondary metabolites contained in the extract of katuk leaves (*Sauropus androgynus* (L.) Merr., including tests for flavonoids, saponins, alkaloids, and tannins (Harbone, 1996).

Antibacterial Activity Test of Katuk Leaf Extract Against *Klebsiella pneumonia* Bacteria

The method used for this antibacterial activity test is the method diffusion with method well. Media Mueller Hinton So that Which has been densely perforated using a blue tip with a diameter of 6 mm. Then extract the ethanol of katuk leaves with K1 10%, K2 40%, K3 70%, K4 100%. Each entered the hole as much as 20µl each concentration and repeated three times (triplicate). The Mueller Hinton Agar media that had undergone the treatment was incubated for 18-24 hours at 37°C without being turned over. In this study, K+ used Ciprofloxacin 500mg as much as 20 µl, while as K- used Aquades 1 ml. Results activity antibacterial extract ethanol leaf cat observed in a way visual with measure diameter of the inhibition zone around the well using a vernier caliper (Wandira, 2022).

Data collection technique

The data obtained was by measuring the clear zone formed at each concentration of 70% ethanol extract of katuk leaves using a ruler. Then compared with positive control and negative control.

Data Analysis Techniques

The data obtained in this study were analyzed using the SPSS One Way ANOVA application to determine the differences between each treatment group.

RESULT AND DISCUSSION

Determination Plant

The sample used was the extract of katuk leaves (*Sauropus androgynus* (L.) Merr.) obtained from Jalan Kampung Cipurut, Cireunghas District, Sukabumi Regency, West Java 43193. Determination was carried out in the Biology Learning Laboratory. Faculty Science and Applied Technology Ahmad Dahlan University Jl. South Ringroad, Tamanan, Banguntapan, Bantul. The determination results show the Latin name of the katuk leaf plant (*Sauropus androgynus* (L.) Merr.) with the name of the Euphorbiaceae tribe.

Making Simple Leaf Katuk

The results of the study showed that katuk leaf plants (*Sauropus androgynus*) were needed (L.) Merr.) wet as much as 10 kg and produces powdered simple katuk leaf plant totaling 2,510 grams.

Testing Parameter Specific

Testing of quality parameters of simple drugs includes tests parameter specific that is identity simple and organoleptic.

Table 1. Parameter results specific namely identity simple and organoleptic.

Specific	Characteristics	Results
Specific	Organoleptic	
	1. Smell	Green Special
	2. Color	Simple powder
	3. Form	

Non-Specific Parameter Testing

Testing of quality parameters of simple drugs includes tests parameter non specific that is level water, ash content and drying shrinkage.

Table 2. Specific parameter results of simplex

Type study	Results	Condition
Water content	3.95%	≤10%
Level ash	7.93%	≤8.3%
Drying shrinkage	4.8%	≤10%

Results inspection Which got as big as 3.95%. Matter This in accordance with the water content requirements of no more than 10% (Ministry of Health of the Republic of Indonesia, 1997). The examination results obtained were 7.93%. This is in accordance with the ash content requirements of no more than 8.3%. The examination results obtained were 4.8%. This is in accordance with the drying shrinkage requirements of no more than 10% (Ministry of Health, 2017).

Making 70% Ethanol Extract of Katuk Leaves

Making extract katuk leaves using the maceration method with a ratio of 1:10 (Ministry of Health, 2000). A total of 500 grams of katuk leaf simplicia powder was soaked using 5000 ml of 70% ethanol solvent and then macerated for 5x24 O'clock And done stirring for 1x24 hours after the maceration process is carried out then filtered. The filtrate is then concentrated using a Rotary evaporator at a temperature of 50°C at a speed of 4 rpm. Concentrated again using a water bath until it becomes a thick extract then weighed and obtained a result of 131.6613 grams.

Testing Parameter Specific

Testing of extract quality parameters includes tests parameter specific that is identity simple and organoleptic.

Table 3. Parameter results specific

Specific	Characteristics	Results
Specific	Organoleptic	
	1. Smell	Typical Blackish Green
	2. Color	Thick extract
	3. Form	

Non-Specific Parameter Testing

Testing parameter quality extract covers the test parameter non specific that is level water, ash content.

Table 4. Specific parameter results extract

Type study	Results	Condition
Level water	4,466%	≤10%
Level ash	7.97%	≤8.3%

Screening Phytochemicals

Based on the results of the phytochemical screening that has been carried out, the secondary metabolite compounds detected in the 70% ethanol extract of katuk leaves are as follows:

Table 5. Results screening phytochemicals 70% ethanol extract of katuk leaves

No	Phytochemical Screening	Results	Information
1	Test Flavonoid Test	+	Formation of red orange foam stable ± 30 seconds
2	Saponin Test Alkaloid Test	+	Presence of sediment
3	Tannin Test	+	The existence of color green black
4		+	

Screening phytochemicals extract ethanol 70% leaf cat (*Sauropus androgynous* (L.) Merr.) show results positive to compound metabolit secondary flavonoid, saponin, alkaloid, tannin groups. According to the results of research conducted

by Anggarito et al., where katuk leaf extract contains flavonoid, saponin, alkaloid and tannin compounds. The results of phytochemical screening of 70% ethanol extract of katuk leaves.

Antibacterial Activity Test of Klebsiella Pneumoniae

Antibacterial activity test of 70% ethanol extract of katuk leaves using the Cup plate Technique (well) diffusion method. The working principle is to make a well in the media then insert the test concentration solution, positive control and negative control into the media that has been perforated, the clear zone around the well indicates that the test control has an active compound that is antibacterial. The more big results clear zone so the more sensitive a antimicrobial compounds.

Table 6. Activities Klebsiella Antibacterial pneumoniae

Name bacteria	Concentration	Observation inhibition zone growth bacteria (mm)			Average inhibition zone (mm) ± SD	Information
		I	II	III		
Klebsiella pneumoniae	Control positive	7.73	7.73	7.55	7.67 ±0.08	Currently
	Control Negative	0.00	0.00	0.00	0.00 ±0.00	Weak
	100%	7.78	7.65	7.63	7.69 ±0.07	Currently
	70%	7.58	7.61	7.65	7.58 ±0.02	Currently
	40%	6.60	6.74	6.65	6.66 ±0.06	Currently
	10%	4.60	4.93	4.84	4.79 ±0.14	Currently

Information :

- K (+) : Control Positive Ciprofloxacin 500 mg
- K (-) : Negative Control Aquadest
- K1 : Concentration 1 100%
- K2 : Concentration 2 70%
- K3 : Concentration 3 40%
- K4 : Concentration 4 10%

Based on Table 6. 70% ethanol extract of katuk leaves can inhibit Klebsiella pneumoniae bacteria in the moderate category. This is because 70% ethanol extract of katuk leaves has secondary metabolite compounds such as alkaloids, flavonoids, tannin And saponins. According to Wandira et al., (2022) In general special, The flavonoid content in katuk leaves can play a role in disrupting the integrity of bacterial cell membrane components. Flavonoids contained in katuk leaves play a role in forming complex compounds in extracellular proteins in bacterial cell membranes. The greater the inhibition zone obtained at a concentration, the greater the active substance component contained in it, so that the inhibition zone formed will also be different at each concentration.

CONCLUSIONS AND SUGGESTIONS

Based on the research results it is concluded that 70% ethanol extract of katuk leaf plant (Sauropus androgynus (L.) Merr.) showed antibacterial activity. In addition, 70% ethanol extract of katuk leaf plant leaf cat (Sauropus androgynous (L.) Merr.) showed the most optimum concentration, namely 100%.

SUGGESTION

To increase the utilization of katuk plants, wider education is needed for the community about its benefits, especially in increasing breast milk production and maintaining health. In addition, the development of processed products based on katuk, such as herbal tea or supplements, can be a business opportunity with high economic value. Support for local farmers through training and mentoring is also important to ensure the quality and availability of this plant on the market. With further research and more scientific publications, public awareness of the benefits of katuk can increase, so that its use can be more optimal in everyday life.

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