

## Antibacterial Activity of Kaffir Lime Leaf (*Citrus hystrix* DC) Ethanol Extract against *Staphylococcus aureus* and *Escherichia coli*

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### Keywords:

Kaffir lime leaves  
*Escherichia coli*  
*Staphylococcus aureus*

Received: August 2024

Accepted: August 2024

Published: August 2024

### Abstract

The use of herbal medicine has been increasing, which encourages researchers to use herbal materials as an alternative antibacterial, one of which is the kaffir lime leaf plant. This study was to identify the group of secondary metabolite compounds and examine the antibacterial properties of ethanol extract of kaffir lime leaves (*Citrus hystrix* DC) against *Staphylococcus aureus* and *Escherichia coli* bacteria. This study began with the preparation of simplisia powder, extracting kaffir lime leaf with ethanol, and the antibacterial activity test of extracts. The results of the study showed that the secondary metabolites contained in kaffir lime leaves are alkaloids, flavonoids, saponins, tannins, steroids and glycosides. Antibacterial activity test on *Staphylococcus aureus* bacteria showed that the ethanol extract of kaffir lime leaves with the best concentration inhibited the bacterial inhibition zone at a concentration of 10% with an inhibition diameter of 22.21 mm with a very strong category and the best concentration is 10% with an inhibition diameter of 20.48 mm with a very strong category for *Escherichia coli* bacteria. Ethanol extract of Kaffir Lime leaves has been proven to inhibit *Staphylococcus aureus* and *Escherichia coli* bacteria.



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## Introduction

One type of disease many people in developing countries suffer is infectious disease. The causative factor of infectious diseases is bacteria (1). Bacteria are microorganisms that cannot be seen with the naked eye but can only be seen with the help of a microscope. The use of antibacterial drugs for infectious diseases caused by bacteria is now quite a lot. Still, the problem is the occurrence of side effects for users, as well as high maintenance costs. Therefore, it is necessary to conduct research to develop new antibacterials, especially from natural materials (2).

Overuse of antibiotics can lead to increased bacterial resistance to certain antibiotics. The high use of antibiotics is the biggest trigger for the

emergence of resistance. The development of drug resistance is one example of the process carried out by organisms to develop tolerance to new natural environmental conditions. So, the treatment of infectious diseases caused by bacteria that are resistant to antibiotics requires new products with high potential (3). Traditional medicines containing antibacterial qualities require research to identify new antibacterial solutions that can suppress or kill antibiotic-resistant bacteria at affordable prices (4,5).

The use of herbal medicine in the community has increased, and this has led many researchers to use herbal ingredients as an alternative antibacterial effect, one of which is the kaffir lime leaf plant (6). Kaffir lime leaves contain helpful chemical compounds, including flavonoids, alkaloids, saponins and tannins that

function as antibacterials. Flavonoids are antibacterial by inhibiting the function of the cytoplasmic membrane. Alkaloid-containing plants can inhibit the growth of gram-positive and gram-negative bacteria. Saponins act as antibacterials, damaging the permeability of bacterial cell walls. Tannins have the function of precipitating proteins to affect bacterial peptidoglycan (7).

Antibacterial substances are compounds used to control the growth of bacteria, which are intended to prevent the spread of disease and infection, eradicate microorganisms in infected hosts, and prevent spoilage and destruction of materials by microorganisms (8). Conduct an antibacterial test to determine a plant's antibacterial effect against certain bacteria.

Based on the description above, the researcher wants to investigate the antibacterial activity of ethanol extract from kaffir lime leaves against *Staphylococcus aureus* and *Escherichia coli* bacteria. The widespread use of chemical antibiotics as a therapy to kill bacteria often causes resistance. Therefore, research is needed on natural ingredients with antibacterial abilities that can reduce antibiotic resistance. To prove whether kaffir lime leaves containing active substances flavonoids, alkaloids, saponins and tannins, as mentioned above, are also able to inhibit *Staphylococcus aureus* and *Escherichia coli* bacteria, one of the bacteria that cause infection, using different concentrations.

## Methods

### Sample Preparation and Simplisia

The samples used were kaffir lime leaves taken in the village of Naga Beralih, Kampar Regency, North Kampar District, Riau. Purposive sampling is the sampling method.

Kaffir lime leaves (*Citrus hystrix* DC) as much as 5 kg have been collected, washed thoroughly with running water, then weighed wet weight, and then dried in the wind, kaffir lime leaves are considered dry when they can be broken, brittle and crushed, then weighed dry weight (9). Then,

it is pollinated using a blender stored in a dry container and protected from sunlight (10).

### Characteristics of Simplisia

Microscopic and macroscopic examination, determination of water content, determination of water-soluble juice content, determination of ethanol soluble juice content, determination of total ash content, and determination of acid-insoluble ash content are all part of the characterization examination of Simplisia (11).

### Preparation of Kaffir Lime Leaf Ethanol Extract

Preparation of ethanol extract of kaffir lime leaves was carried out by maceration using 96% ethanol solvent. 10 parts (500 g) of simplisia powder was put into a vessel, then 75 parts (3750 mL) of ethanol distillation liquid was closed while stirring occasionally and left for 5 days protected from sunlight. After 5 days, the pulp mixture is squeezed out. Wash the pulp with enough ethanol distillation liquid for 100 macerate parts (5 litres). The macerate was then transferred into a closed vessel, left in a cool place, protected from light for 2 days, and filtered (12). The macerate was then concentrated with a rotary evaporator and weighed (13).

### Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolite chemical compounds in simplified kaffir lime leaves, and ethanol extracts of kaffir lime leaves, including alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides (14).

### Antibacterial Test of Kaffir Lime Leaf Ethanol Extract

Prepare Mueller Hilton Agar (MHA) media made in a petri dish. Homogenize a suspension of bacterial culture that is by the McFarland 0.5 standard. Take a 0.1 mL bacterial culture suspension using a micropipette and flatten it on hardened agar media. Apply a sterile cotton swab to all parts of the media to evenly distribute the inoculum. Place discs that have been soaked with kaffir lime leaf extract test solution with concentrations of 2, 4, 6, 8, and 10% on the

surface of the media. Position the dish upside down and incubate at 37 °C for 1 x 24 hours. After that, the zone of inhibition of bacterial growth was measured using a calliper (15).

## Result and Discussion

### Yield of Simplisia

The sample used in this study was kaffir lime leaves. The wet weight of kaffir lime leaves obtained was 5000 g, then the weight of the sample after drying was 16500 g, and the simplisia obtained was 500 g of simplisia powder. The extraction method used was maceration using 96% ethanol solvent so that a thick blackish-green extract weighing 143 g was obtained, with a yield of 28.6%.

### Characteristics of Kaffir Lime Leaf Simplisia

The results of the characterization examination of Kaffir lime leaf powder can be seen in Table 1.

**Table 1.** Examination of the Characteristics of Kaffir Lime Leaf Simplisia Powder

No	Inspection parameters	Results (%)	MMI Edition VI (%)
1	Water content	8	≤ 10
2	Water soluble essence content	37,9	≥ 5
3	Ethanol-soluble juice content	19	≥ 4
4	Total ash content	7,65	≤ 9
5	Acid insoluble ash content	0,6	≤ 1

Based on the results in Table 1 shows that the determination of water content of kaffir lime leaf simplisia aims to determine the nature of the sample used. The water content of the sample obtained is 8%. Water content is one of the important things to note because if the water content does not meet the requirements, it is feared that the extract that will be used will be easily damaged and easily become a medium for microbial growth. The determination of water-soluble juice content is 37.9%, while the determination of ethanol-soluble juice content is

19%; the results of determining the water-soluble juice content are greater than the ethanol-soluble juice content; this shows that the simplisia contains many polar compounds. Determination of water-soluble juice content and ethanol-soluble juice content aims to summarize the ingredients that can be distilled by water and ethanol (16).

### Phytochemical Screening

Phytochemical screening was carried out to determine the class of secondary metabolite compounds contained in simplisia and kaffir lime leaf extract. Phytochemical screening includes examination of alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids and glycosides. The results of simplisia screening and kaffir lime leaf extract can be seen in Table 2.

**Table 2.** Phytochemical Screening of Kaffir lime leaf powder and extracts

No	Chemical secondary metabolite	Simplisia	Extract
1	Alkaloids	(+)	(+)
2	Flavonoids	(+)	(+)
3	Saponins	(+)	(+)
4	Tannins	(+)	(+)
5	Steroids/triterpenoids	(+)	(+)
6	Glycosides	(+)	(+)

Information:

+ : contains the substance being examined

- : does not contain the substance being examined

Based on the results obtained in Table 2, it shows that the simplisia and extracts of kaffir lime leaves positively contain alkaloid compounds, flavonoids, saponins, tannins, steroids, and glycosides. Phytochemical screening of ethanol extracts of kaffir lime leaves (*Citrus hystrix* DC) from the district of Klaten shows the results of phytochemical screening tests of ethanol extracts of kaffir lime leaves positively contain flavonoid compounds, alkaloids, saponins, tannins, triterpenoids, alkaloids and glycosides (17).

### Antibacterial Test Results

The average results of the anti-bacterial test inhibition zone on kaffir lime leaf ethanol extract

samples can be seen in Table 3. Based on the results of the antibacterial activity test of kaffir lime leaf extract against *Staphylococcus aureus* bacteria, the results of inhibition power are obtained, namely at a concentration of 2% has no inhibition zone diameter, concentration 4% inhibition diameter is 11.35 mm with a strong category, concentration 6% inhibition diameter is 15.2 mm with a strong category, concentration 8% inhibition diameter is 17.95 mm with a strong category, and at a concentration of 10% inhibition

diameter 22.21 mm with a very strong category. While the *Escherichia coli* bacteria obtained the results of the diameter of inhibition, namely at a concentration of 2% has no diameter of inhibition, a concentration of 4% diameter of inhibition is 10.71 mm with a moderate category, a concentration of 6% diameter of inhibition is 15.3 mm with a strong category, a concentration of 8% diameter of inhibition is 16.87 mm with a strong category, and a concentration of 10% diameter of inhibition is 20.48 mm with a very strong category.

**Table 3.** Inhibition Test Results of Kaffir lime leaf extract against *Staphylococcus aureus* and *Escherichia coli*

Test Bacteria	Extract Concentration (%)	Zone of Inhibition (mm)			Average (mm)	Category
		I	II	III		
<i>Staphylococcus aureus</i>	2	0	0	0	0	Weak
	4	10,55	11,7	11,85	11,35	Strong
	6	15,8	15,4	14,4	15,2	Strong
	8	17,85	16,4	19,6	17,95	Strong
	10	24,2	18,95	23,5	22,21	Very Strong
	Positive Control (Kloramfenikol)	37,7	37,05	37,45	37,4	Very Strong
	Negative Control	0	0	0	0	Weak
<i>Escherichia coli</i>	2	0	0	0	0	Weak
	4	9,5	12	10,65	10,71	Medium
	6	13,1	15,65	17,15	15,3	Strong
	8	16,55	16,75	17,3	16,87	Strong
	10	20,3	19,35	21,8	20,48	Very Strong
Positive Control (Kloramfenikol)	36,45	36	36,35	36,2	Very Strong	
Negative Control	0	0	0	0	Weak	

Based on Table 3 shows the antibacterial sensitivity of kaffir lime leaf extract in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria; the smallest inhibition zone is located at a concentration of 4%, while the largest inhibition zone is at a concentration of 10%. This shows that the greater the concentration of kaffir lime leaf extract given, the greater the inhibition against the growth of a bacterium.

The phytochemical content of the kaffir lime leaf plant, which functions as an antibacterial, is tannin, alkaloid, flavonoid, and saponin. Tannins have a mechanism of action by inhibiting reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells cannot be formed. The mechanism of alkaloid compounds as

antibacterial is disrupting the components that make up peptidoglycan in bacterial cells. The antibacterial mechanism of alkaloids inhibits dihydrofolate reductase activity, thus inhibiting nucleic acid synthesis. Flavonoids, as antibacterial, inhibit bacterial growth by inhibiting inflammation in 2 ways, namely releasing the release of arachidonic acid and secretion of lysosomal enzymes from neutrophil cells and endothelial cells and inhibiting the proliferation phase and exudation phase of the inflammatory process. The mechanism of action of saponins as an antibacterial is by damaging the permeability of the cell wall so that it can cause cell death [18].

## Conclusion

Based on the results of this study, secondary metabolites contained in kaffir lime leaves are alkaloids, flavonoids, saponins, tannins, steroids and glycosides. The results of the antibacterial activity test on *Staphylococcus aureus* bacteria showed that the ethanol extract of kaffir lime leaves with the best concentration inhibited the bacterial inhibition zone at a concentration of 10% with a very strong category (22.21 mm). For *Escherichia coli* bacteria, the best concentration is 10%, which is a very strong category (20.48 mm).

## References

1. Rani Z, Nasution HM, Kaban VE, Nasri N, Karo NB. Antibacterial Activity of Freshwater Lobster (*Cherax quadricarinatus*) Shell Chitosan Gel Preparation against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Applied Pharmaceutical Science*. 2023;13(2):146-53.
2. Nasution HM, Rani Z, Fauzi ZPA, Ridho AR. Antimicrobial Activity Test of Ethanol Extract of Senggani Leaves (*Melastoma malabaticum* L) Against *Propionibacterium Acnes* and *Staphylococcus Epidermidis*. *Jurnal Sains dan Kesehatan*. 2024;6(2):292-8.
3. Pratiwi RH. Mekanisme pertahanan bakteri patogen terhadap antibiotik. *Jurnal pro-life*. 2017;4(3):418-29.
4. Indarto I, Narulita W, Anggoro BS, Novitasari A. Aktivitas antibakteri ekstrak daun binahong terhadap *propionibacterium acnes*. *Biosfer: Jurnal Tadris Biologi*. 2019;10(1):67-78.
5. Rafita RY, Nasution HM, Rani Z, Fahmi F. The Buas Buas Leaf Utilization of Buas Buas Leaf (*Premna pubescens* Blume) Ethanol Extract as Liquid Soap with Anti-Bacteria Activity. *International Journal of Science, Technology & Management*. 2022;3(3):733-43.
6. Adrianto H, Yotopranoto S, Hamidah H. Efektivitas Ekstrak Daun Jeruk Purut (*Citrus hystrix*), Jeruk Limau (*Citrus amblycarpa*), dan Jeruk Bali (*Citrus maxima*) terhadap Larva *Aedes aegypti*. 2014;
7. Ifandari EN. Aktivitas Antibakteri Ekstrak Etanolik Daun Jeruk (*Citrus nobilis*, *Citrus sinensis*, dan *Citrus maxima*) Terhadap Bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa*. [cited 2024 May 24]; Available from: <http://jurnal.ut.ac.id/index.php/manilkara/article/view/3168>
8. Yuza M, Ridwanto R, Rani Z. Determination Of Total Flavonoid Content Of Yellow Wood (*Arcangelisia Flava* (L.) Merr) Extract And Antibacterial Activity Against *Staphylococcus aureus*. *Journal of Agromedicine and Medical Sciences*. 2023;9(3):140-5.
9. Rani Z, Ridwanto R, Miswanda D, Yuniarti R, Sutiani A, Syahputra RA, et al. Cytotoxicity Test of Cocoa Leaf Ethanol Extract (*Theobroma Cacao* L.) With Brine Shrimp Lethality Test (BSLT) Method. *Indonesian Journal of Chemical Science and Technology (IJCST)*. 2022;5(2):80-7.
10. Syahputra RA, Sutiani A, Silitonga PM, Rani Z, Kudadiri A. Extraction and phytochemical screening of ethanol extract and simplicia of moringa leaf (*Moringa oleifera* Lam.) from sidikalang, north sumatera. *International Journal of Science, Technology & Management*. 2021;2(6):2072-6.
11. Pulungan AF, Ridwanto R, Dalimunthe GI, Rani Z, Dona R, Syahputra RA, et al. Phytochemical Screening and Antioxidant Activity Testing of Porang (*Amorphophallus Muelleri* Blume) Leaf Ethanol Extract from Kuta Buluh Region, North Sumatera. *International Journal of Health and Pharmaceutical (IJHP)*. 2022;3(1):1-7.
12. Nurmazela V, Ridwanto R, Rani Z. Antioxidant Activity Test of Barangan Banana Hump's Ethanol Extract (*Musa Paradisiaca* (L.)) with DPPH (1, 1 Diphenyl-2-Picrylhydrazyl)

- Method. International Journal of Science, Technology & Management. 2022;3(5):1478-83.
13. Robiatun RR, Pangondian A, Paramitha R, Rani Z, Gultom ED. Formulation and Evaluation of Hand Sanitizer Gel from Clove Flower Extract (*Eugenia aromatica* L.). International Journal of Science, Technology & Management. 2022 Mar 26;3(2):484-91.
  14. Alviana L, Ridwanto R, Daulay AS, Rani Z. Characterization and Phytochemical Screening of Tampala Bajakah Wood (*Spatholobus littoralis* Hassk) Extract with Methanol and Ethyl Acetate Solvents. Indonesian Journal of Science and Pharmacy. 2024;1(3):80-5.
  15. Nasution HM, Yuniarti R, Rani Z, Nursyafira A. Phytochemical Screening and Antibacterial Activity Test of Ethanol Extract Of Jengkol Leaves (*Archidendron Pauciflorum* Benth.) IC Nielsen Against *Staphylococcus Epidermidis* and *Propionibacterium Acnes*. International Journal of Science, Technology & Management. 2022;3(3):647-53.
  16. Ridwanto R, Trizaldi A, Rani Z, Daulay AS, Nasution HM, Miswanda D. Antioxidant Activity Test of Methanol Extract of Gaharu (*Aquilaria Malaccensis* Lam.) Bark with Dpph (1, 1 Diphenyl-2-Picrylhydrazyl) Method. International Journal of Health and Pharmaceutical (IJHP). 2023;3(2):232-40.
  17. Mayasari U, Laoli MT. Karakterisasi Simplisia dan Skrining Fitokimia Daun Jeruk Lemon (*Citrus limon* (L.) burm. f.). KLOROFIL: Jurnal Ilmu Biologi dan Terapan. 2018;2(1):7-13.