

Bioautographic Analysis And Antibacterial Activity Test Of Curry Leaf (*Murraya Koenigii* (L) Spreng) Ethanol Extract On The Bacteria *Propionibacterium Acnes*

Muhammad Wahyu Ikhsan¹, Haris Munandar Nasution^{2*}, Yayuk Putri Rahayu³, M.Pandapotan Nasution⁴

^{1,2,3,3}Department of Pharmacy, Universitas Muslim Nusantara Al Washliyah., Jalan Garu II Medan Amplas 20147, North Sumatera, Indonesia

*Corresponding author:

Email : harismunandar@umnaw.ac.id

Abstract.

Curry leaf (*Murraya koenigii* (L) Spreng) is one of the plants known to have antibacterial compounds. Curry leaves are also known as temurui leaves and are useful as a natural food flavoring. Curry leaves contain flavonoid compounds, alkaloids, calcium, zinc, riboflavin, folic acid. This study aimed to analyze the bioautography and antibacterial activity of ethanol extract of curry leaves against *Propionibacterium acnes* bacteria. The research method was carried out experimentally. Curry leaf simplicia extract was made with 96% ethanol as solvent. Curry leaf ethanol extract was made in various concentrations, namely 30%, 40% and 50%. The positive control used was Tetracycline HCl and the negative control was DMSO. The tests carried out on curry leaf simplicia included phytochemical screening, macroscopic examination, microscopic examination, water content examination, examination of water soluble extract content, examination of ethanol soluble extract content, examination of ash content and examination of acid insoluble ash content, bioautographic analysis the results of phytochemical screening showed that curry leaves contain secondary metabolites, namely flavonoids, alkaloids, tannins, saponins, steroids/triterpenoids, and glycosides. The results of the antibacterial activity showed that curry leaves could be used as antibacterial because they had strong inhibition at concentrations of 30%, 40% and 50%, namely 10.3 mm, 11.7 mm and 13.6 mm against *P. acnes* bacteria. The results of bioautography showed that the Rf value in the clear zone was 0.17 with a ratio of chloroform:n-Hexane (8:2) on the chromatogram spots by spraying 10% FeCl₃ positive for flavonoid compounds which were marked in black.

Keywords: Curry leaves (*Murraya koenigii* (L) Spreng), curry leaf extract, antibacterial activity, *Propionibacterium acnes* and bioautography test

I. INTRODUCTION

Curry leaf (*Murraya koenigii*) is one of the most familiar leaves and is found in almost all parts of Indonesia and especially in Aceh Province, curry leaves can generally be used as a cooking spice because it will provide a distinctive taste and have a strong aroma. Curry leaves are also known as “temurui leaves” and are useful as a natural flavoring agent. This leaf is a leaf of a typical dish originating from India. Even though the curry leaves have been dried, they have a sharp, bitter and sour taste. Curry leaves are also used in traditional medicine such as wound healing and maintaining eye health because curry leaves have vitamin A which is good for the eyes (Das, 2011). Based on a study by Murugesh (2005), curry leaves are rich in alkaloids, flavonoid compounds, terpenoids, and steroids. One alternative that can be used is the use of antimicrobial active substances contained in medicinal plants. One of the medicinal plants that is widely known by the Indonesian people as a source of medicinal ingredients is curry leaves (*Murraya koenigii* (L) Spreng).

The benefits of this medicinal plant are found in several chemically active substances that affect the activity of certain physiological processes in the human body. The most important active substances in this medicinal plant are alkaloids, flavonoids, and phenolics. Curry leaves have many benefits as a cure for diseases. The leaves, roots, and bark of the curry leaf plant can be made as a tonic to stimulate digestion and stomach upset or as an antiemetic. Boiled curry leaves will be bitter in taste and can reduce fever, curry leaf root juice can also be used as stomach pain, leaves and roots can also be given as analgesics, ambient medicine. Raw curry leaves can be used as a cure for dysentery (Balakrishnan, 2020). *Propionibacterium acnes* is one of the bacteria that causes acne. *Propionibacterium acnes* is an anaerobic

gram-positive bacterium that is tolerant to air, this bacterium is also a normal flora of bacteria. *Propionibacterium acnes* bacteria can produce various biological molecules and enzymes that can act as acne inflammatory agents (Pothitirat, 2010).

II. METHODS

2.1 Simplified Characterization Examination

Examination of simplicia characterization includes macroscopic, microscopic examination, determination of water content by the azeotropic method, determination of water-soluble extract content, determination of ethanol-soluble extract content, determination of total ash content, and determination of acid-insoluble ash content (Depkes RI, 1995).

2.2 Phytochemical Screening Test

2.2.1 Alkaloid Examination

Ethanol extract of Kari Leaves was weighed 0.5 g added 1 ml HCl 2 N added 9 ml distilled water, then heated on a water bath for 2 minutes, cooled and filtered the filtrate was used for the examination of alkaloids:

1. 3 drops of filtrate are added with 2 drops of Mayer reagent, a white or yellow lumpy residue will be formed.
2. 3 drops of filtrate are added with 2 drops of Bouchardat reagent, a brown to black residue will be formed.
3. 3 drops of filtrate are added with 2 drops of Dragendroff reagent to form brown or orange.

If there is a residue or turbidity in at least 2 test tubes in the above experiment, the alkaloid is positive (Ditjen POM, 1979).

2.2.2 Flavonoid Examination

As much as 10 g of ethanol extract of kari leaves was weighed and then added 100 ml of hot distilled water, boiled for 5 minutes, and filtered in a hot state, into 5 ml of the filtrate added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the presence of a red, yellow, or orange color on the amyl alcohol layer (Ditjen POM, 1979).

2.2.3 Saponin Examination

As much as 0.5 g of ethanol extract of kari leaves was put into a test tube, then added 10 ml of hot distilled water and cooled, then shaken vigorously for 10 minutes. If the foam is formed with a height of 1-10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl, it indicates the presence of saponins (Ditjen POM, 1979).

2.2.4 Tannin Examination

As much as 1 g of ethanol extract of kari leaves with 10 ml of distilled water and then filtered the filtrate was diluted with distilled water until it was colorless. 2 ml of the solution was taken and 1-2 drops of 1% iron (III) chloride reagent were added. If a blue-black or green-black color occurs, it indicates the presence of tannins (Ditjen POM, 1979).

2.2.5 Glycoside Examination

A total of 1 gram of simplicia powder and kari leaf extract was extracted with 30 ml of a mixture of 96% ethanol - distilled water (7:3), then added 10 ml of 2N HCl, refluxed for 10 minutes, then cooled and filtered. 20 ml of the filtrate was taken plus 25 ml of distilled water and 25 ml of 0.4 M lead (II) acetate, shaken, allowed to stand for 5 minutes, and then filtered. The filtrate was extracted 3 times, each time with 20 ml of the chloroform-isopropanol mixture (3:2). In the collection of juices added anhydrous sodium to taste. Filtered and evaporated at a temperature of not more than 50°C. The remainder was dissolved with 2 ml of ethanol, then 0.1 ml of the experimental solution was taken into a test tube, evaporated over a water bath. 2 ml of water and 5 drops of Molisch reagent were added to the residue, 2 ml of concentrated sulfuric acid was added carefully, a purple ring was formed at the boundary of the two liquids indicating the presence of glycosides (Depkes RI, 1995).

2.2.6 Steroid/Triterpenoid Examination

As much as 1 g of kari leaves. Simplicia powder and ethanol extract were each macerated with 20 mL of n-hexane for 2 hours and then filtered. The filtrate is evaporated in a vaporizer cup. To the remainder, a few drops of Liebermann-Buchard reagent were added. The appearance of blue or green-blue indicates the presence of steroids, red, pink, or purple colors indicate the presence of triterpenoids (Depkes RI, 1995).

2.3 Antibacterial Activity Test

2.3.1 Equipment Sterilization

The tools used in this antibacterial activity test were sterilized before being used. Glass utensils are sterilized in the oven at 170°C for 1-2 hours. Bacterial growth media were sterilized in an autoclave at 121°C for 15 minutes. While the ose needles are sterilized by burning them in a spirit lamp until they glow.

2.3.2 Making Tilt Media MHA

As much of 38 grams of MHA media was dissolved into sterile distilled water little by little. Then the volume is made up to 1 L and heated until completely dissolved. The media is sterilized in an autoclave at a temperature of 121°C for 15 minutes (Nani, 2014)

2.3.3 NaCl solution 0.9%

Weighed as much as 0.9 grams of sodium chloride then dissolved in sterile distilled water little by little in a 100 mL volumetric flask until completely dissolved. Sterile distilled water was added up to the marked line, put in a sterile Erlenmeyer with a lid, and then sterilized in an autoclave at 121°C pressure at 1 atm for 15 minutes.

2.3.4 Suspension Standard McFarland

9.95 ml of 1% sulfuric acid solution was mixed with 0.05 ml of BaCl₂ solution in an Erlenmeyer. Then shaken until the solution is cloudy. If the turbidity of the tested bacterial suspension is the same as the turbidity of the standard Mc Farland 0.5 solution, the concentration of the bacterial suspension is 1.5x10⁸ CFU/ml (Borges, 2004).

2.3.5 Preparation of Bacterial Suspension

From the stock culture of *Propionibacterium acnes* that has been overgrown, a sterile ose needle is taken and then suspended in a tube containing 10 ml of 0.9% sodium chloride solution until the turbidity of the bacterial suspension is the same as the turbidity of Mc.Farland's standard solution, the concentration of bacteria is 1.5x10⁸ CFU/ ml. After that, the dilution was carried out by pipetting 0.1 ml of the bacterial suspension, put into a sterile tube and added 9.9 ml of 0.9% sodium chloride and shaken homogeneously. From here obtained a bacterial suspension of 1.5x10⁸ CFU/ml (Silaban, 2009)

2.3.6 Antibacterial Activity Testing

The antibacterial activity test was carried out by the diffusion method consisting of 5 treatments, namely 3 extract concentrations (30%, 40% and 50%) and negative control (DMSO), positive control (Tetracycline) 1 ml of bacterial suspension was pipetted and then put into a petri dish, added 20 ml of MHA media which had been sterilized and then put into a petri dish and homogenized and allowed to solidify. The disc paper was dipped in each extract concentration and then placed on the agar surface. Then all petri dishes that have been treated are incubated in an incubator at 37°C for 18-24 hours, then the zone of inhibition was measured using a caliper (Silviana, 2020), (Nasution, 2022).

2.3.7 Bioautography Test

MHA media that has been sterilized as much as 20 ml is put into a petri dish which already contains 1 ml of bacterial suspension, homogenized and then allowed to solidify. The chromatograms resulting from the separation of compounds by TLC were placed on the solidified medium. Let stand for 30 minutes in the refrigerator, the plate is removed and removed from the medium. Furthermore, it is incubated for 18-24 hours at a temperature of 37°C, sprayed with 10% FeCl₃ if it produces a black color indicating a positive flavonoid (Widya, 2019).

III. RESULT AND DISCUSSION

Tabel 1. Results of Simplicia Characterization Examination

No	Inspection	Earning Rate %
1	Determination of water content	7 %
2	Determination of water-soluble extract content	28%
3	Determination of ethanol-soluble extract content	15,3%
4	Determination of total ash content	8,3%
5	Determination of acid-insoluble ash content	1%

Description : \geq : No more than

\leq : Not less than

Table 2. Results of Phytochemical Screening of Powder and Ethanol Extract of Kari Leaves

Description:

No.	Group of Chemical Compounds	Identification
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Glycoside	+
6.	Steroids/Triterpenoids	+

(+) = contains the substance being examined

(-) = does not contain the substance examined

Table 3. The results of testing the antibacterial activity of ethanol extract of Kari leaves against *Propionibacterium acnes*

Test Sample	Concen- tration (%)	Inhibiti on zone (mm)			Average inhibition zone (mm)
		Replikasi			
		1	2	3	
EEJL (Ethanol Extract of Kari Leaves)	30%	11,8mm	10,0 mm	9,3 mm	10,3 mm
	40%	12,9 mm	11,8 mm	10,4 mm	11,7 mm
	50%	14,7 mm	14,2 mm	12,0 mm	13,6 mm
Positive Control Tetracycline HCl		17,1 mm	-	-	17,1 mm
Negative Control DMSO 1%		-	-	-	-

Discussion

The table shows that the ethanol extract of curry leaves with concentrations of 30%, 40% and 50%, respectively, has an inhibitory zone against the bacteria *Propionibacterium acnes*. The diameter of the clear inhibition zone formed around the disc at each concentration of 30%, 40% and 50% was different. At 30% concentration, the average clear inhibition zone was (10.7 mm), and at 40% concentration, the average clear inhibition zone was (11.7 mm) and at 50% concentration, the average clear inhibition zone was (13, 6 mm). At a concentration of 30%, 40% and 50% concentrations gave a strong response. The largest inhibition zone was at a concentration of 50% , while in the clear zone a positive control was formed on the bacteria *Propionibacterium acnes*, a clear zone was formed (17.1 mm). Each positive control gave a strong positive response. Curry Leaf Ethanol Extract with various different concentrations contains different antimicrobial substances, the higher the concentration of curry leaf extract means the more concentrated the solution and the more antimicrobial substances it contains.

The positive control used was tetracycline. This shows that tetracycline contains a very strong antibacterial because it has bacteriostatic and broad-spectrum antibacterial properties so that tetracycline is able to inhibit the growth of gram-positive and gram-negative bacteria. The mechanism of action of

tetracycline is by binding to the 30s rRibosome subunit so that it will inhibit the aminoacyl-tRNA bond on the A side of the rRibosome so that it will disrupt the peptide bond (Yetty, 2015).

The negative control used was DMSO solution, because DMSO did not provide antibacterial activity against bacteria. The purpose of using DMSO is as a comparison that the solvent used as a diluent does not affect the antibacterial activity. DMSO (Dimethyl Sulfoxide) is an organosulfur compound, which can dissolve both polar and nonpolar compounds and is soluble in various water-capable organic solvents, besides that DMSO is also not toxic so it will not interfere with inhibition (Retno, 2010).

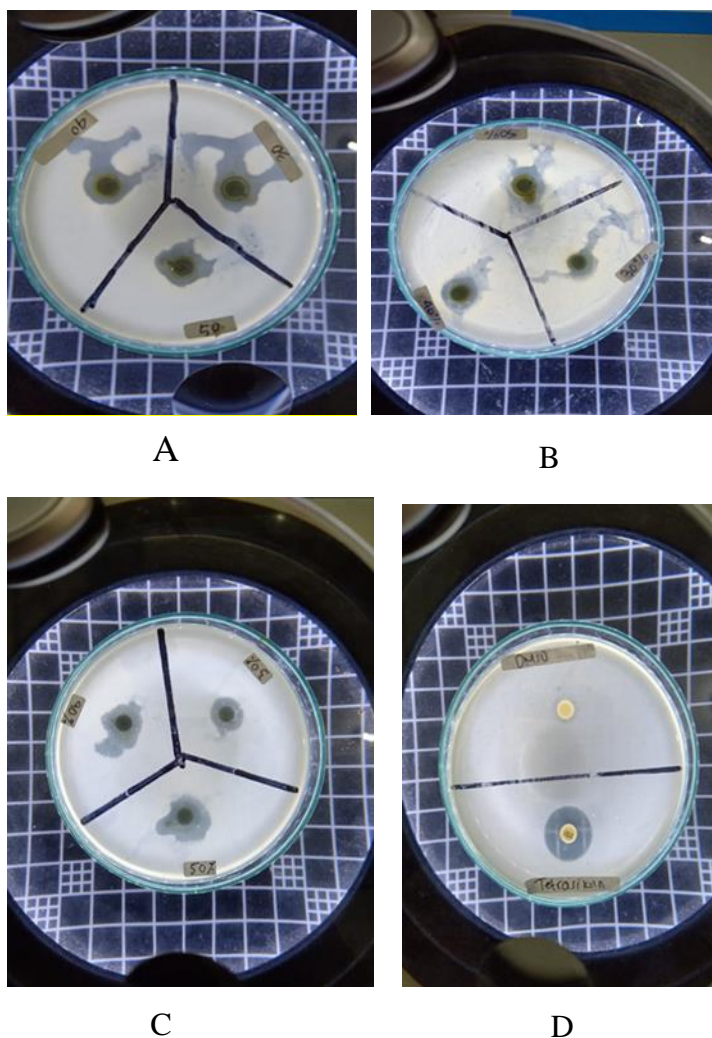


Fig 1. Antibacterial Activity Test Results of Ethanol Extract of Kari Leaves (*Archidendron pauciflorum* Benth.) Against *Propionibacterium acnes* Bacteria.

Description: A. First Repetition (1)
B. Second Repetition (2)
C. Third Repetition (3)
D. Control Positive and Negative

Discussion bioautography analysis

Bioautography is a detection method to find antibacterial compounds and serves to determine what chemical components provide antibacterial activity from curry leaf extract (Gritter, 1991). In this study, the contact bioautography method was used, this method was chosen because it was considered simple in testing and the results were clearly visible. This test was carried out using a thin layer chromatographic plate which had been marked with the lower boundary and the upper mark as the elution boundary mark. The elution distance made is 8.5 with the hope that this distance can separate the compounds that will be eluted on the TLC plate. The results of TLC-Bioautography showed that there was an inhibition zone

against the bacteria *Propionibacterium acnes* at an Rf value of 0.17. Curry leaves have antibacterial activity are flavonoid compounds. Flavonoids function to form complex compounds with proteins and can damage bacterial cell membranes (Widya, 2019).

Identification of chemical components was carried out to determine the class of compounds contained in the extract using a TLC plate that had been sprayed with reagents. FeCl₃ reagent shows flavonoid compounds if the stain appears black due to the formation of a complex between the phenol group and Fe contained in the FeCl spray reagent. The reaction is analogous to the reaction between phenol groups and flavonoid compounds. Based on the visible color of the reagent, curry leaf extract is thought to contain flavonoid compounds (Lukman, 2016).

IV. CONCLUSION

Based on the results of the study, it was concluded that:

1. The ethanol extract of curry leaves (*Murraya koenigii* (L) Spreng) has a chemical compound that inhibits antibacterial activity, namely flavonoid compounds marked in black by the bioautography method.
2. Curry leaves (*Murraya koenigii* (L) Spreng) can be used as antibacterial because they have a strong clear inhibition zone at 50% concentration of 13.6 mm, 40% of 11.7 mm and 30% concentration of 10.7 mm.

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