

Bioautographic Analysis And Antibacterial Activity Test Of Mengkudu Leaf (*Morinda Citrifolia* L.) Ethanol Extract On The Bacteria *Propionibacterium Acnes*

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Abstract.

Noni contains alkaloids, flavonoids, tripertinoid, protein, lime, iron, carotene, glycosides antarkiron. The content of anthraquinin, scaloptin, flavonoid and adaptogen compounds are active compounds that have antifungal and antimicrobial effects. The antibiotics commonly used are antibiotics such as tetracycline, clindamycin and others. These chemical-based antibacterials have many negative side effects, therefore other alternatives are needed as antibacterials, one of which is by using noni. This study aims to analyze bioautography and determine the antibacterial activity of noni leaf ethanol extract against *Propionibacterium acnes* bacteria. This research was conducted experimentally using noni leaf ethanol extract with concentrations of 30%, 40%, 50% as independent variables and bioautographic analysis and antibacterial activity as dependent variables. The bioautography analysis was performed using TLC and the antibacterial activity test was performed using paper discs. The results of antibacterial testing of noni leaf ethanol extract with a concentration of 30% had an inhibition zone of 8.36 mm with a medium category, a concentration of 40% had an inhibition zone of 10.3 mm with a strong category and a concentration of 50% had an inhibition zone of 11.1 mm in a strong category. positive control tetracycline 30µg had an inhibition zone of 20.8 mm which was categorized as very strong. The results of the bioautography test using contact bioautography using the mobile phase of chloroform: n-hexane (7:3). The inhibition zone was obtained at an Rf value of 0.17, and the characteristics of the chromatogram spots were carried out by spraying 10% FeCl₃ suspected that the spots were flavonoid compounds.

Keywords: *morinda citrifolia* L, antibacterial, *propionibacterium acnes*, bioautography test.

I. INTRODUCTION

Noni leaves contain protein, lime, iron, carotene, and ascorbin. Meanwhile, noni flowers contain inter-chiron glycosides. The content of anthraquinin, scaloptin, and adaptogyn compounds are active compounds that have antifungal and anti-microbial effects (Hartanti, 2020). *Propionibacterium acnes* is a Gram positive bacterium which is a normal floral part of the skin and causes opportunistic infections that produce lipase as a contributor to acne formation. *Propionibacterium acnes* plays a role in the pathogenesis of acne by producing lipases that break down free fatty acids from skin lipids (Afifi, 2018). The population of *P acnes* bacteria can be reduced by giving antibiotics such as erythromycin, clindamycin and tetracycline. Tetracycline class antibiotics show a broad antibacterial spectrum, especially in affecting microorganisms that are dividing. Tetracyclines are widely used for the treatment of infections caused by several types of gram-positive and gram-negative bacteria.

Although the use of antibiotics is quite effective in treating acne, the use of antibiotics as the main choice for healing acne must be reviewed to limit the development of bacterial resistance to antibiotics. Meanwhile, excessive use of antibiotics can cause bacteria that were originally sensitive to become resistant. Therefore, it is necessary to search for natural antibacterial compounds that do not have a negative impact on humans, namely by utilizing active bacteria-killing substances contained in plants (Afifi, 2018). The active compounds found in plants as antibacterial are alkaloids, flavonoids, and tannins. Flavonoids function as bacteriostatic and their mechanism of action is to form complex compounds with proteins and dissolve so that they can damage bacterial cell walls. Tannins have activity as an antibacterial. The mechanism of action is by shrinking the cell wall itself, the cell cannot carry out life activities so that growth is inhibited or even dies. Alkaloid compounds have an inhibitory mechanism by interfering with the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not fully formed and causes cell death (Budianti, 2018), (Susanti, 2020).

II. RESEARCH METHOD

2.1 Phytochemical Screening

2.1.1 Alkaloids Examination

The simplicia powder and ethanol extract of noni leaf were weighed as much as 0.5 g, then added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate used for the alkaloid test is as follows:

1. 3 drops of filtrate is added with 2 drops of Mayer reagent, a positive reaction is indicated by the formation of a white or yellow lumpy precipitate.
2. 3 drops of filtrate was added with 2 drops of Bouchardat reagent, a positive reaction was indicated by the formation of a brown to blackish precipitate.
3. 3 drops of filtrate is added with 2 drops of Dragendorff's reagent, a positive reaction is indicated by the formation of a red or orange color precipitate.

Alkaloids are considered positive if there is a precipitate or turbidity in at least 2 reactions from the 3 experiments above (Depkes RI, 1989), (Emelda, 2021).

2.1.2 Flavonoid Examination

The simplicia powder and ethanol extract were weighed as much as 10 g and then added 100 ml of hot water, boiled for 5 minutes and filtered in hot conditions. To 5 ml of the filtrate was added magnesium powder, 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the appearance of a red, yellow or orange color on the amyl alcohol layer (Susanti, 2019).

2.1.3 Saponin Examination

The simplicia powder and ethanol extract were weighed as much as 0.5 g were put into a test tube, added 10 ml of hot water, cooled and shaken for 10 seconds. If foam is formed as high as 1-10 cm which is stable for no less than 10 minutes, and does not disappear with the addition of 1 drop of 2 N hydrochloric acid, it indicates the presence of saponins (Depkes RI, 1989).

2.1.4 Tannin Examination

The simplicia powder and ethanol extract were weighed as much as 0.5 g and added with 10 ml of distilled water and then filtered. The filtrate was diluted with water until it was colorless. 2 ml of the solution was taken and 1-2 drops of 1% FeCl₃ reagent were added. If a blue or blackish green color occurs, it indicates the presence of tannins (Depkes RI, 1989).

2.1.5 Glycoside Examination

The simplicia powder and ethanol extract were weighed as much as 5 g, dissolved in 96% ethanol solvent, evaporated on a water bath, dissolved the remainder in 5 ml of anhydrous acetic acid, and added 10 drops of sulfuric acid P. Blue or green color formed indicates the presence of glycosides (Susanti, 2019).

2.1.6 Steroid/Triterpenoid Examination

Simplicia powder and ethanol extract were weighed as much as 5 g, each was macerated in 20 ml of ether for 2 hours and then filtered. 5 ml of filtrate was evaporated in an evaporating dish to dryness. To the residue was added 20 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid (Lieberman-Bouchard reagent). The formation of purple or red color that changes to blue-green indicates the presence of steroids/triterpenoids (Harbone, 1987).

2.2 Sterilization of Tools and Materials

Growth media were sterilized by autoclaving at 121°C for 15 minutes and glassware sterilized in an oven at 160-170°C for 1-2 hours, except for materials made of rubber which were sterilized by immersing them in 70% alcohol and using a needle on a Bunsen flame. until it turns red (Zulvia, 2017)

2.3 Preparation of Mueller Hinton Agar (MHA) Media

A total of 38 g of MHA media was dissolved into sterile distilled water little by little, then the volume was made up to 1 L and heated until completely dissolved. The media was sterilized in an autoclave at a temperature of 121 °C for 15 minutes.

2.4 Bioautography Test

a. Preparation of the Mobile Phase (Eluent). Before elution, the eluents used were chloroform and n-hexane in a ratio of (5:5), (6:4), (7:3), (8:2), (9:1), (10:0) The eluent in one vessel is saturated first, each mixture of the mobile phase is inserted into the chamber and then closed tightly and saturation is carried out. This saturation is done to equalize the vapor pressure in the entire vessel.

b. Fraction Sample Highlighting. The sample is spotted using a capillary tube on the TLC plate in each comparison eluent, the TLC plate is inserted into the chamber and then dried by aerating.

c. Bioautographic bacterial activity test. The bioautography test was carried out to detect active compounds that have antibacterial activity. A total of 1 ml of the suspension was put into a sterile petri dish. Pour 20 ml of MHA medium. Furthermore, the cup is shaken on a flat table surface, so that the media and bacterial suspension are mixed evenly and let stand until solid. The chromatograms resulting from the separation of compounds by TLC were placed on the solidified medium. It was allowed to stand for 30 minutes, the chromatogram plate was removed and removed from the medium. Then incubated for 1 x 24 hours at a temperature of 37°C. sprayed with 10% FeCl₃ reagent if it produces a black/grey color, it indicates a positive flavonoid compound (Andarini, 2019).

2.5 McFarland Standard Solution 0.5

Composition : 1% sulfuric acid solution 9.95 ml

Barium chloride solution 1.75% 0.05 ml

Ways of making :

9.95 ml of 1% sulfuric acid solution was pipetted and 1.75% barium chloride solution was pipetted as much as 0.05 ml, put into a test tube and shaken homogeneously. Until a standard Mc Farland solution of 0.5 was obtained (Andarini, 2019).

2.6 Preparation of Bacterial Suspension

To make a suspension of *Propionibacterium acnes* bacteria, by culturing *Propionibacterium acnes* taken with sterile wire, then suspended into a test tube containing 10 ml of 0.9% NaCl. Furthermore, it is compared with the standard turbidity of the McFarland solution of 0.5 (Andarini, 2019), (Sari,2015).

2.7 Activity Test of Noni Leaf Ethanol Extract Against *Propionibacterium acnes*

The inhibition test used the agar diffusion method, namely disc. A total of 1 ml of the suspension was put into a sterile petri dish. Pour 20 ml of MHA medium. Furthermore, the cup is shaken on a flat table surface, so that the media and bacterial suspension are evenly mixed. Let stand until solid, then take the paper disc using tweezers which was previously heated over a Bunsen fire. Disc paper was inserted into the noni leaf extract with a predetermined concentration of 30%, 40%, 50%, DMSO as a negative control and Tetracycline as a positive control. After that it is placed on the surface of the media so that it is carefully used with tweezers and marked each location of each concentration. Then the media was incubated in an incubator at 37°C for 24 hours. After that, the inhibition zone formed around the disc was measured using a digital caliper. Marked by a clear zone around the disc (Retno, 2020), (Nasution, 2022).

III. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

Based on the results of the phytochemical screening examination of noni leaf powder and extract, it showed that noni leaves contain chemical compounds of alkaloids, saponins, tannins, flavonoids, steroids/triterpenoids, and glycosides as evidenced by chemical tests.

3.2 Bioautography Test Results

This test was carried out using a TLC plate which had been marked with the lower and upper boundary of the plate as a sign of the elution boundary. The elution distance made was 8.5 cm in the hope that this distance was sufficient to separate the compounds to be eluted on the TLC plate.

Data Table of Thin Layer Chromatogram Results of Noni Leaf Ethanol Extract

No	Mobile Phase	Rf value
1	Kloroform : n-heksana = 6:4	0,17 0,23

2	Kloroform : n-heksana = 7:3	0,17 0,23 0,32 0,38
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The results of the bioautography test showed that the best mobile phase was the ratio of Chloroform: n-hexane 7:3, which was 4 stains. A good eluent is characterized by the number of spots that appear, spots that are tailless, and the distance between one spot and another is clear. Furthermore, bioautography testing is carried out which is a follow-up test. In this study, the contact bioautography method was used, this method was chosen because it was considered simple in operation, and the results were more clearly visible. In contact bioautography, the process of transferring the active compound into the medium in order to produce a zone of inhibition and the ability to distinguish between active compounds with the same Rf value. The eluted plate was contacted on the surface of solid media containing a suspension of *Propionibacterium acnes* bacteria for 30 minutes. The plate was then removed from the media and the media was incubated for 18-24 hours, in order to obtain a zone of inhibition.

3.3 Bacterial Growth Inhibition Examination Results

In this study, the antibacterial activity was tested in inhibiting the growth of *Propionibacterium acnes* bacteria using the agar diffusion method, namely disc paper. The basis for choosing this method is because it is fast, easy and simple in its operation and does not require special equipment.

Bacterial Growth Inhibitory Zone Results Table

Test sample	Concentration (%)	Obstacles zone (mm)			Average inhibition zone (mm)
		Replication			
		1	2	3	
Negative Control	-	0	0	0	-
Noni Leaf Ethanol Extract	30%	9,4	8,2	7,5	8,36
	40%	10,6	10,2	10,1	10,3
	50%	11,2	11,0	11,1	11,1
Positive Control	-	21,3	20,8	20,3	20,8

The results of the examination of bacterial growth inhibition showed that the ethanol extract of noni leaves with concentrations of 30%, 40%, and 50% each had an inhibitory zone against the bacteria *Propionibacterium acnes*. The average clear zone diameter formed around the paper disc at each concentration was: 30% (8.36 mm), 40% (10.3 mm), 50% (11.1 mm). Each concentration, namely 50% and 40%, has a strong resistance response. While at a concentration of 30% has a moderate resistance response. The largest inhibition zones were produced in extracts with concentrations of 50% and 40% and then the inhibitory power decreased again at concentrations of 30%.

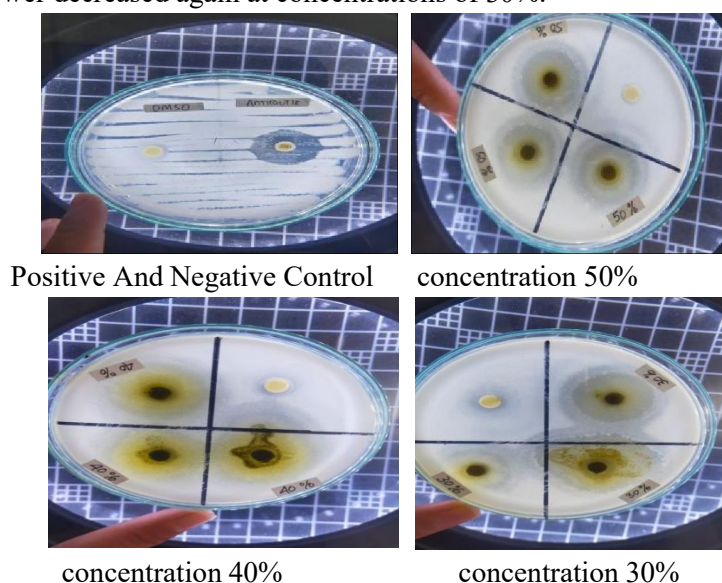


Fig 1. Results of Antibacterial Activity of Noni Leaf Ethanol Extract (*Morinda citrifolia* L.) Against *Propionibacterium acnes*.

IV. CONCLUSION

1 Ethanol extract of noni leaf (*Morinda citrifolia* L.) has a class of antibacterial compounds, flavonoids which are characterized by the appearance of a blackish ash color with 10% FeCl₃ spraying.

2 The ethanolic extract of noni (*Morinda citrifolia* L.) leaves has antibacterial activity against *Propionibacterium acnes*, with the highest bacterial inhibitory activity at 50% concentration with an inhibition zone of 11.1 mm categorized as strong.

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