



EFFECTIVENESS TEST OF BIDARA LEAF EXTRACT (*Ziziphus mauritiana*) AS ANTIBACTERIAL AGAINST *Staphylococcus aureus* AND *Escherichia coli*

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Abstrak

Ekstrak daun bidara memiliki potensi aktivitas imunomodulator dari segi humoral maupun seluler pada sistem kekebalan tubuh, sehingga diharapkan dapat memberikan respons terhadap infeksi yang disebabkan oleh bakteri. Ulasan ini diarahkan untuk mengeksplorasi aktivitas farmakologis, fitokimia, serta potensi antibakteri dari daun bidara yang diambil dari desa Klagen. Ekstraksi dengan metode maserasi menggunakan pelarut etanol 70%. Uji zona hambat dengan metode difusi cakram pada konsentrasi 40%, 50%, 60%, 70%, 80%, 90%, dan 100% serta kontrol positif tetrasiklin dan kontrol negatif aquades steril. Hasil penelitian diameter uji zona hambat paling lebar yaitu pada konsentrasi 100%. Diameter zona hambat pada *Staphylococcus aureus* sebesar $10,97 \pm 2,47$ mm, sedangkan pada *Escherichia coli* sebesar $5,26 \pm 0,45$ mm. Uji KHM dengan menggunakan konsentrasi 100%, 50%, 25%, 12,5%, dan 6,25% yang kemudian dilanjutkan uji KBM. Hasil dari uji KHM dengan metode mikro dilusi diketahui dengan mengamati perubahan kekeruhan media setelah diinkubasi, kemudian dilanjutkan dengan uji KBM dengan metode pour plate. Nilai KHM diperoleh pada konsentrasi 50%, pada bakteri *Staphylococcus aureus* dengan total koloni sebanyak $1,2 \times 10^6$ CFU/mL dan pada *Escherichia coli* dengan total koloni $1,0 \times 10^7$ CFU/mL. Nilai KBM yang diperoleh pada bakteri uji *Staphylococcus aureus* dan *Escherichia coli* yaitu pada konsentrasi 100%.

Kata kunci: Antibakteri, Daun Bidara, Ekstrak, *Escherichia coli* dan *Staphylococcus aureus*

Abstract

Bidara leaf extract is known to have potential immunomodulatory activity, this suggests that it may provide a response to infections caused by bacteria. The results of phytochemical screening of bidara leaves taken from Klagen village, showed that there were secondary metabolite compounds in the form of flavonoids, tannins, and saponins. The inhibition zone test with the disc diffusion method using extract concentrations of 40%, 50%, 60%, 70%, 80%, 90%, and 100% as well positive control and negative control. The results showed that the diameter of the inhibition zone test was the widest of the two bacteria at *Staphylococcus aureus* was 10.97 ± 2.47 mm, while in *Escherichia coli* it was 5.26 ± 0.45 mm. The next test is the MIC test using concentrations of 100%, 50%, 25%, 12.5%, and 6.25% which is then followed by the MBC test. The results of the MIC test with the micro dilution method are known by observing changes in media turbidity after incubation. Then continued with the MBC test as confirmation of the presence of microbes after the incubation period through the pour plate method. The KHM value was obtained at a concentration of 50%, on *Staphylococcus aureus* bacteria with a total colony of 1.2×10^6 CFU/mL and on *Escherichia coli* with a total colony of 1.0×10^7 CFU/mL. The KBM value obtained on *Staphylococcus aureus* and *Escherichia coli* test bacteria is at a concentration of 100%.

Keywords: Antibacterial, Bidara Leaf, Extract, *Escherichia coli* and *Staphylococcus aureus*

1. INTRODUCTION

The resistance of some pathogenic bacteria to antibiotics is currently increasing, new antibacterial compounds that can work effectively as antibacterial agents are sought. The resistance of pathogenic bacteria to existing antibiotics is a big problem for the

world of health. The search for antibacterial drugs is very meaningful to avoid the development of pathogenic bacteria that can cause disease in humans. Therefore, the search for sources of antibacterial compounds has also been carried out on several types of plants that may contain

secondary metabolite compounds that can work as antibacterial compounds.

The results of phytochemical screening of bidara leaves extracted by maceration method using 96% ethanol solvent have several secondary metabolite compounds including phenolic compounds, tannins, and saponins (Nasr *et al.*, 2017). In addition, the results of phytochemical screening that bidara leaves have several groups of active compounds including alkaloids, flavonoids, terpenoids, glycosides, phenolic compounds and saponins (Alekhya *et al.*, 2021).

The extraction method used in this test is the maceration procedure. Maceration is an extraction method that is often used because it is simple and uses room temperature (Xu Z *et al.*, 2020). Maceration is the most commonly used way of extracting samples using a suitable solvent at room temperature. When secondary metabolites are isolated, intracellular and extracellular pressure can destroy the plant cell wall and dissolve the active substances in the cytoplasm in the solvent (Abd-Rabo *et al.*, 2019).

Secondary metabolite compounds are compounds that can be used to inhibit the growth of a bacterium (Othman *et al.*, 2019). In recent years, many secondary metabolite compounds from plants have been used as antibacterial agents. These compounds can act as antibacterial or antibacterial by damaging the bacterial cell wall and changing the permeability of the cell membrane. Some secondary metabolites that can damage bacterial cell walls are phenols, alkaloids, and flavonoids. These compounds can be utilized for natural antibacterial against pathogenic bacteria such as *S. aureus* and *E. coli* (Zhang *et al.*, 2021).

Antibacterial is a compound that can be used to inhibit the growth of a bacteria. In recent years, many secondary metabolite compounds from plants have been used as antibacterial agents. These compounds can act as antibacterial or antibacterial by damaging the bacterial cell wall and changing the permeability of the cell membrane. Some secondary metabolites that can damage bacterial cell walls are phenols,

alkaloids, and flavonoids. These compounds can be utilized for natural antibacterial against pathogenic bacteria such as *S. aureus* and *E. coli* (Septiani, *et al.*, 2017).

Previous research has also discussed the use of thyme leaf extract as an antibacterial agent against *S. aureus* and *E. coli* bacteria. Muharrami *et al.* (2019) also reported that the research they conducted had the aim of knowing the results of phytochemical screening and antibacterial activity of bidara leaf ethanol extract against *S. aureus* and *E. coli* bacteria. The concentrations used from the ethanol extract of bidara leaves are 1%, 10%, 20%, 30%, and 40%. This study uses the disc paper method (agar plate). The results of the antibacterial activity test obtained that 40% concentration can prevent the growth of *S. aureus* bacteria by 0.03-1.68 mm which falls into the weak category, while the *E. coli* bacteria showed no antibacterial activity.

This study is a follow-up to previous research to test the ethanol extract of bidara leaves as an antibacterial agent against *S. aureus* and *E. coli*. The treatment concentrations of bidara leaf extract used in this study were 40%, 50%, 60%, 70%, 80%, 90%, and 100%. In addition to antibacterial testing of bidara leaves, testing of the active compound content of the bidara leaves was also carried out. The phytochemical test was conducted to determine the secondary metabolite compounds contained in bidara leaves taken from Klagen village, Rejoso sub-district, Nganjuk Regency, East Java.



Figure 1 Bidara leaf

Kingdom : Plantae
Subkingdom : Tracheobionta
Superdivision : Spermatophyta



Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Rosidae
Order : Rhamnales
Family : Rhamnaceae
Genus : Ziziphus
Species : *Ziziphus mauritiana* Lamk.

2. RESEARCH METHOD

The research was conducted in Microbiology Laboratory and Plant Physiology Laboratory, Biology Study Program, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University Malang. Bidara (*Ziziphus mauritiana*) leaf samples were taken from Klagen village, Rejoso sub-district, Nganjuk district, East Java.

Research Tools

The research tools needed in this study, namely for the extraction process and phytochemical screening tests, are maceration containers, rotary vacuum evaporators, analytical scales, vials, aluminum foil, beaker glass, filter paper, drop pipettes, test tubes, measuring cups, tube racks, and bunsen. In addition, the tools needed for antibacterial testing include ose, autoclave, micropipette, erlenmeyer, petri dish, laminar flow, hot plate, tissue, label paper, incubator, and tube rack.

Research Materials

The research materials needed are Bidara (*Ziziphus mauritiana*) leaves, *E. coli* bacteria and *S. aureus* bacteria. The extraction process material is 70% ethanol. Materials needed in antibacterial testing are Mueller-Hiton Broth (MHB) media, Mueller-Hiton Agar (MHA), NaCl 0.9%, distilled water, tetracycline, 70% alcohol, aluminum foil, cotton, gauze, and plastic wrap. Materials used for phytochemical screening were concentrated HCl, magnesium powder, Burchard reagent, Mayer reagent, Dragendorff reagent and FeCl₃ 1%.

Sample Extraction

The biadara leaves were picked in fresh condition, picked when photosynthesis was at its maximum at 9-10 am. The biadara

leaves that have been picked are then sorted and washed (Taufiq, 2018). Bidara leaves were dried using an oven at 50°C for 3-4 days (until the simplisia when squeezed can be destroyed). Then the dried bidara leaves were ground into powder and sieved and weighed as much as 100 grams.

The extraction method used was the maceration method. Powder from Bidara leaves was weighed 100 grams and placed into a maceration container. Then a solvent of 600 mL of 70% ethanol was added, after which the sample was stored in a place where there was no sunlight. Immersion of the sample was carried out within a period of 3 days and occasionally stirred. After the soaking process, the sample was filtered, and the dregs were re-macerated using a new 70% ethanol solvent of 600 mL. The next stage is the separation of the filtrate from the solvent using a *rotary vacuum evaporator* at a temperature of 50 ° C so that a concentrated extract will be obtained. The evaporation process was carried out for 2-3 hours in order to remove the 70% ethanol solvent. Bidara leaf extract (*Ziziphus mauritiana*) was diluted into concentrations of 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

Phytochemical Screening Test

1) Flavonoids

After the extract is dissolved in heated distilled water, 0.5 ml of extract is added with a pipette, enough Mg powder is added, then 10 drops of concentrated hydrochloric acid are added. If it turns pink, dark red, or orange, it means it contains flavonoids (Nigussie *et al.*, 2021).

2) Alkaloids

The extract was dissolved using heated distilled water then pipetted 0.5 ml into 3 tubes then added Burchard, Mayer, and Dragendorff reagents from each tube. Contains alkaloids with white or yellow precipitate, brown Bouchard precipitate and orange Dragendorff precipitate (Nigussie *et al.*, 2021).

3) Tannin

After the extract is dissolved in heated distilled water, 0.5 ml is added

with a pipette, then 3 drops of 1% FeCl₃ are added. If the color changes to green, blue, purple, dark blue, or blackish brown, it is said to be positive for tannins (Nigussie *et al.*, 2021).

4) Saponins

After dissolving the extract in heated distilled water, add 0.5 ml with a pipette, add 2 ml of hot water, shake vigorously to form bubbles, add 1 drop of concentrated hydrochloric acid. If the foam is strong, it contains saponins (Nigussie *et al.*, 2021).

Tool sterilization

Tools and media were sterilized in an autoclave at 121°C for 15 minutes. Bunsen flames were used to burn tweezers and ose needles.

Mueller Hinton Broth (MHB)

Mueller Hinton Broth (MHB) media as much as 6.3 grams was dissolved into 300 mL of distilled water in an erlenmeyer. The solution was brought to a boil with a temperature of 200° C on a hot plate stirred using a magnetic stirrer until homogeneous clear yellow in color. Close the erlenmeyer tightly using cotton and sterilize for 15 minutes with an autoclave at 121° C.

Mueller Hinton Agar (MHA)

Mueller Hinton Agar (MHA) media is a media specifically formulated for susceptibility testing. MHA media is made using 19 grams of media with solvent used 500 mL of distilled water, then heated while stirring using a magnetic stirrer until homogeneous. MHA media was sterilized in an autoclave with a pressure of 1.5 atm for 15 minutes at a temperature of 121° C.

Bacterial Cultivation

S. aureus and *E. coli* bacteria from stock were prepared. Sterile MHA medium was placed in a sterile petri dish then allowed to solidify. One ose of bacteria was taken from the stock and then inoculated by scraping the media (streak plate). Bacteria were incubated in an incubator at 37° C for 24 hours.

Preparation of Bacterial Suspension

After preparing bacterial cultures of *S. aureus* and *E. coli*, they were inoculated in MHB media and then incubated in an incubator at 37°C for 24 hours. After incubation, one bacterial colony was taken and inoculated into 10 ml of 0.9% NaCl solution. The tube is then shaken until homogeneous and the turbidity standard is Mc Farland 0.5 (10⁸ CFU/mL) (Nigussie, 2021) and the liquid microdilution test is 5x10⁵ CFU/mL.

The composition of McFarland standard solution consists of 0.05 ml of 1.175% barium chloride solution and 9.95 ml of 1% sulfuric acid. The working step of making McFarland's standard solution is to place barium chloride and sulfuric acid in a test tube and then homogenize. If the turbidity of the bacterial suspension is the same as the turbidity of McFarland's solution, then the concentration of the bacterial suspension is 10⁸ CFU/mL.

Inhibition Zone Test

S. aureus and *E. coli* bacteria were inoculated on MHB media. *Mueller Hinton Broth* (MHB) media as much as 6.3 grams was dissolved into 300 mL of distilled water in an erlenmeyer. The solution was brought to a boil with a temperature of 200° C on a hot plate stirred using a magnetic stirrer until homogeneous clear yellow in color. Incubated in an incubator at 37°C for 24 hours. After incubation, one bacterial colony was taken and inoculated into 10 ml of 0.9% NaCl solution. The tube was then shaken until homogeneous with Mc Farland 0.5 turbidity standard (10⁸ CFU/mL) (Nigussie, 2021) and liquid microdilution test which is 5x10⁵ CFU/mL.

MHA (*Mueller Hinton Agar*) media was made using 19 grams of media with the solvent used 500 mL of distilled water, then heated while stirring using a magnetic stirrer until homogeneous. Sterile MHA media was poured sufficiently into a sterile petri dish. Inoculated *E. coli* and *S. aureus* bacterial suspensions as much as 100 µL on the surface of the agar media, leveled with a cotton swab and allowed to dry. A triplo zone

was created on the bottom surface of the Petri dish. Prepared leaf extracts with concentrations of 40%, 50%, 60%, 70%, 80%, 90% and 100%, negative control (sterile distilled water) and positive control (tetracycline). Dip the paper disc (6 mm diameter) into the leaf extract for 30 minutes, then place it on the agar medium. Then incubate at 37°C for 24 hours (Balouiri *et al.*, 2016). The clear area around the disc paper indicates that there is antibacterial activity from the leaf extract. The diameter of the inhibition zone was measured in millimeters (mm) with a caliper.

Table 1 Inhibition zone diameter categories

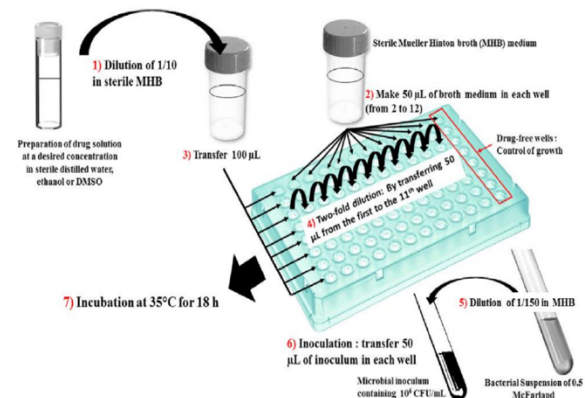
Diameter	Category
≥ 20 mm	Very strong
10-20 mm	Strong
5-10 mm	Medium
≤5 mm	Weak

MIC Test

The Minimum Inhibitory Concentration test used graded concentrations with a concentration of half the previous concentration. In this study, the concentrations of bidara leaf extract used were 100%, 50%, 25%, 12.5%, 6.25%, K+ (tetracycline) and K- (sterile distilled water). The test solution with a concentration of 100% was diluted into sterile MHB media in a ratio of 1:10. Then 200 µL of solution (100% concentration) was pipetted into the first well-96. Multilevel dilution of the extract solution (100% concentration) was carried out by pipetting 100 µL of extract solution and then inserted into the next well which had been filled with 100 µL of sterile MHB media, the same method was carried out until the fifth well. Making a suspension of test bacteria by making a bacterial suspension whose turbidity is the same as McFarland's solution, then diluting the suspension in sterile MHB media in a ratio of 1: 150 (Figure 2).

Incubation is carried out for 24 hours in an incubator and then observations are made on the turbidity of the media (Pinta *et al.*, 2017). The growth of bacteria can be known by observing with the naked eye on

cloudy media. The more bacteria that grow on the media, the higher the turbidity of the media will be. The reading of antibacterial activity is done by giving an assessment using the notation (+++) for very turbid media, (++) for media that looks turbid, (+) slightly turbid media, and (-) clear media.

**Figure 2** Minimum inhibitory concentration test work steps**MBC Test**

The Minimum Bactericidal Concentration testing was carried out using the agar dilution method, namely by inoculating bacteria into agar media using the pour plate method. MBC is determined by observing whether or not bacteria grow in agar media after incubation. Positive MBC is seen from the lowest concentration that shows the death of bacteria (no bacteria grow) (Fitriana *et al.*, 2019).

Another purpose of the MBC test is to confirm the results of the MIC test. Each dilution well from the MIC test was pipetted as much as 10 µL and transferred to a sterile petri dish then filled with sterile MHA media (pour plate). Finally, the media was incubated for 24 hours at 37°C. Furthermore, the calculation of bacterial colonies that grew from each Petri dish was carried out. Calculation of bacterial colonies was carried out by directly observing and calculating using a digital colony counter.

3. RESULTS AND DISCUSSION

Phytochemical screening was conducted to obtain information on secondary metabolites contained in bidara leaf extract. Phytochemical screening was carried out qualitatively and the compounds tested were flavonoids, tannins, saponins and alkaloids. The results of qualitative phytochemical screening on bidara leaf extract can be seen in table:

Table 2 Phytochemical screening result

Secondary Metabolites	Bidara Leaf Extract	Description
Flavonoids	+	Orange
Tannins	+	Green-black
Saponins	+	Permanent foam
Alkaloids	-	No white sediment

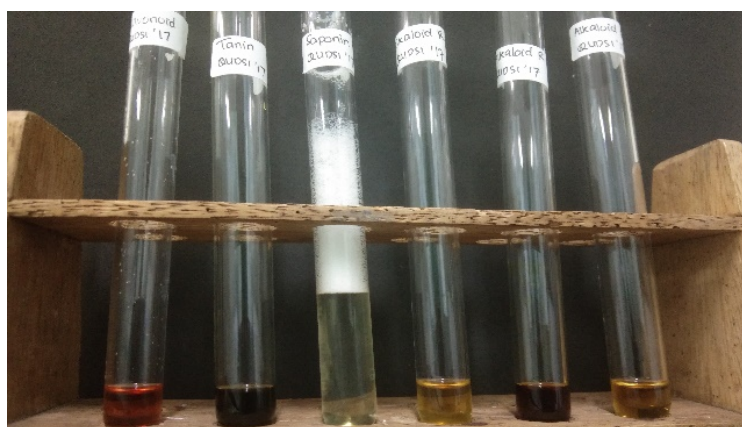


Figure 3 The results of phytochemical screening test

Data analysis of zone of inhibition of *S. aureus* bacteria:

Table 3 Data analysis of zone of inhibition of *S. aureus*

Treatment	Mean (mm) $\pm$ SD	Category
K-	0 $\pm$ 0	Weak
40% Extract	4,00 $\pm$ 0,58	Weak
50% Extract	6,01 $\pm$ 0,42	Medium
60% Extract	6,44 $\pm$ 0,91	Medium
70% Extract	7,42 $\pm$ 0,53	Medium
80% Extract	8,01 $\pm$ 0,48	Medium
90% Extract	9,54 $\pm$ 1,91	Medium
100% Extract	10,97 $\pm$ 2,47	Strong
K+	16,11 $\pm$ 4,13	Strong

Based on the average diameter of the inhibition zone, it can also be concluded the category of the inhibition zone on *S. aureus*. The 40% concentration with an average diameter of the inhibition zone of 4.00 $\pm$ 0.58 mm is categorized into the weak category, while the 50%, 60%, 70%, 80%, and 90% concentrations are included in the moderate category. The 100% concentration falls into the strong category, because it has an average inhibition zone diameter of 10.97 $\pm$ 2.47 mm.

Table 4 Data analysis of zone of inhibition of *E. coli*

Treatment	Mean (mm) ± SD	Category
K-	0 ± 0	Weak
40% Extract	1,24 ± 0,22	Weak
50% Extract	1,57 ± 0,47	Weak
60% Extract	2,68 ± 0,53	Weak
70% Extract	3,21 ± 0,64	Weak
80% Extract	3,54 ± 0,51	Weak
90% Extract	3,71 ± 0,41	Weak
100% Extract	5,26 ± 0,45	Medium
K+	16,17 ± 3,81	Strong

Concentrations of 40%, 50%, 60%, 70%, 80%, and 90% of the mean diameter of the inhibition zone are classified as weak category. If the average diameter of the inhibition zone at 100% concentration is 5.26 ± 0.45 mm, it is included in the medium category.

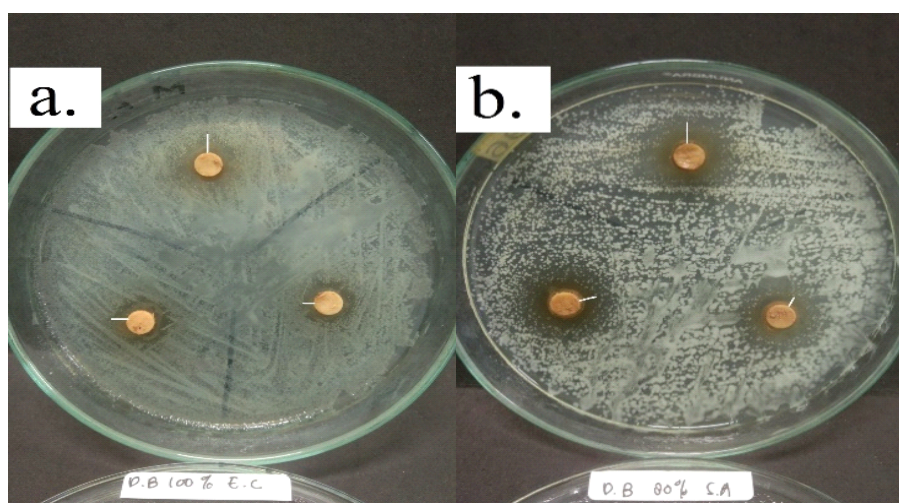


Figure 4 Observation results of inhibition zone test from bidara leaf extract on bacteria a. *E. coli* and b. *S. aureus*

Minimum inhibitory concentration test results:

Table 5 Minimum inhibitory concentration test results

Treatment	Microbial Test	Repeat	Concentration				
			100%	50%	25%	12,5%	6,25%
Bidara leaf extract	<i>E. coli</i>	U1	-	+	++	++	+++
		U2	-	+	++	++	+++
		U3	-	+	++	+++	+++
	<i>S. aureus</i>	U1	-	-	+	+++	+++
		U2	-	-	++	++	+++
		U3	-	-	+	+++	+++
K+	<i>E. coli</i>	U1	-	-	-	-	-
	<i>S. aureus</i>	U1	-	-	-	-	-
K-	<i>E. coli</i>	U1	++	++	++	++	++
	<i>S. aureus</i>	U1	++	++	++	++	++

Description:

(-) : Clear

+ : Slightly Turbid

++ : Turbid

+++ : Very Turbid

K+ (Positive control): Tetracycline

K- (Negative control): Sterile distilled water

Minimum Bactericidal concentration test results:

Table 6 Minimum Bactericidal concentration test results

Treatment	Microbes	Concentration				
		100%	50%	25%	12,5%	6,25%
Bidara leaf extract	<i>E. coli</i>	0	$5,2 \times 10^7$	∞	∞	∞
	<i>S. aureus</i>	0	$6,2 \times 10^6$	∞	∞	∞
K+	<i>E. coli</i>	0	0	0	0	0
	<i>S. aureus</i>	0	0	0	0	0
K-	<i>E. coli</i>	∞	∞	∞	∞	∞
	<i>S. aureus</i>	∞	∞	∞	∞	∞

DISCUSSION

Based on the results of phytochemical screening on bidara leaves taken from Klagen village, Rejoso sub-district, Nganjuk district, it is known that the bidara leaves contain several secondary metabolite compounds including flavonoids, saponins, and tannins. According to previous research by Usman *et al.* (2021) who have also conducted phytochemical screening on bidara leaves taken from Mattiro Langi village, Sarappo Lompo Island, Pangkep, South Sulawesi, positively contains active compounds in the form of tannins, saponins, flavonoids, and alkaloids. Li Y (2020) also added that the results of phytochemical screening of bidara leaves taken from the city of Pekanbaru, Riau are known to have secondary metabolite compounds of alkaloids, flavonoids, tannins, and saponins.

The results of phytochemical screening in this study taken from Klagen village, Rejoso sub-district, Nganjuk district did not find secondary metabolite compounds in the form of alkaloids, while in studies taken from different locations found alkaloid compounds. This can occur due to

the influence of biotic and abiotic factors and genetic factors. According to Radusiene *et al.* (2012) plants of the same species growing in different environments may have differences in the concentration of certain secondary metabolites. This is because plants always interact with the surrounding environment, they come into contact with different abiotic components such as water, light, temperature, soil and chemicals (minerals/fertilizers). These varying amounts of abiotic components cause stress to the plants which in turn causes variations in the production or accumulation of their secondary metabolite compounds.

Differences in location have a major influence on the content of secondary metabolite compounds of a plant. At the location of bidara leaf sampling there is a lowland area with an altitude of 45 m above sea level. According to Sholekah (2017) the height of the place is one of the factors that affect the growth of a plant. As a result, a series of metabolic processes in the plant will also be disrupted so that the compounds produced from the process will be different at each altitude.



The first secondary metabolite compound found in bidara leaf extract is flavonoids. Previous research says that flavonoids are the compounds that play the most important role in antibacterial testing. As said by Adamczak *et al.* (2020) that secondary metabolite compounds that play an important role in antibacterial mechanisms are flavonoids, this is because flavonoids are able to inhibit nucleic acid synthesis, inhibit cytoplasmic membrane function by affecting permeability and disrupting the performance of several important enzymes.

In addition to flavonoids, there are also secondary metabolite compounds in the form of tannins in bidara leaf extract. In previous studies, it has also been explained that tannin is one of the secondary metabolite compounds that can function as an antimicrobial or antibacterial. As said by Kaczmarek (2020) tannins can inhibit the growth of various microbes, such as bacteria, fungi, and yeasts. The nature of the tannin structure contributes to its antibacterial activity. Ekambaram (2016) added that as macromolecular polyphenols, tannins contain many hydroxy phenolics, which is one of the reasons why tannins show strong antibacterial activity.

The last secondary metabolite compound found in bidara leaf extract in this study is saponin. Saponin itself is a secondary metabolite compound that almost all plants have, this is because saponins function to protect these plants from pests and pathogenic microbes. Ravi *et al.* (2016) explain that saponins are secondary metabolite compounds that are spread throughout the plant kingdom, this is characterized by the presence of saponins in plant tissues which are mostly susceptible to fungal or bacterial attacks. Zhao *et al.* (2020) added that saponins have a good bacteriostatic effect, which can change membrane permeability, destroy cell membrane structures, and inhibit bacterial growth.

Inhibition zone test was conducted to determine the inhibition of bidara leaf extract against test bacteria based on the diameter of the inhibition zone formed. This

inhibition zone test uses the disc diffusion method. The extract concentrations used in the inhibition zone test were 40%, 50%, 60%, 70%, 80%, 90%, and 100%. The results of the inhibition zone test show that bidara leaf extract can work as the largest antibacterial on *S. aureus*, on *E. coli* bacteria there is antibacterial activity but not as great as on *S. aureus*. This happens because *S. aureus* and *E. coli* have different cell wall structures, so the ability of the extract to penetrate the cell wall to carry out antibacterial activity is also different (Prats-Ejarque *et al.*, 2019).

The difference in the antibacterial activity of bidara leaves against *S. aureus* and *E. coli* bacteria is due to differences in the cell walls of the two bacteria. The cell wall of gram-positive bacteria consists of a large amount of peptidoglycan. This peptidoglycan layer consists of a network that has many pores, which causes exogenous penetration into the cell (Mohammad *et al.*, 2019). In contrast to the cell wall in gram-positive bacteria, gram-negative bacteria cell wall consists of a peptidoglycan membrane in the inner layer and an outer membrane consisting of phospholipids, lipoproteins, and lipopolysaccharides. This bilayer cell wall structure in gram-negative bacteria is one of the strong barrier factors against foreign molecules that will try to penetrate the bacterial cell wall (Sarjito *et al.*, 2021).

Based on the results of the qualitative MIC test, it is known that the positive value of MIC from bidara leaf extract against *S. aureus* bacteria is found at a concentration of 50%, while in *E. coli* bacteria MIC results are found at a concentration of 100%. Antibacterial activity works better on gram-positive bacteria because of the relatively simple cell wall monolayer structure, allowing antimicrobial compounds to penetrate more easily into the cell (Zhang *et al.*, 2021). The cell wall of *S. aureus* bacteria does not have a lipopolysaccharide layer, antibiotic compounds more easily penetrate cells and lyse bacterial cells (Nikolic *et al.*, 2023).

MIC test results on *E. coli* bacteria, the minimum concentration of bidara leaf

extract that can work to inhibit bacterial growth is at a concentration of 100%. This is because the cell wall in *E. coli* bacteria has a bilayer structure consisting of a peptidoglycan membrane in the inner layer and an outer membrane containing phospholipids, lipoproteins, and lipopolysaccharides. This bilayer cell wall structure acts as a strong barrier factor against attempts by foreign molecules to penetrate the bacterial cell wall, especially in gram-negative bacteria such as *E. coli* (Mohammad *et al.*, 2019).

The MBC test results have a positive value at a concentration of 100%, this is because the agar media at a concentration of 100% with the test bacteria *S. aureus* and *E. coli* were not found at all bacterial colonies that grew in it. The minimum kill concentration is the concentration that is able to inhibit bacterial growth. This is indicated by the absence of *E. coli* and *S. aureus* growing on the petri dish, which indicates that the bacteria are killed by the test solution at that concentration (Wang *et al.*, 2021).

MIC quantitative results were found at a concentration of 50%, namely in the test bacteria *S. aureus* found bacterial colonies growing on agar media as much as 6.2×10^6 CFU/mL, while in *E. coli* test bacteria as much as 5.2×10^7 CFU/mL. The difference in KHM test results from the two test bacteria is because the two bacteria have different cell wall structures. Gram-negative bacteria are more resistant to antibacterial agents than gram-positive bacteria. This is because gram-negative bacteria have more complex cell walls. The cell wall is formed by a thin peptidoglycan layer adjacent to the cytoplasmic membrane and an outer membrane composed of phospholipids and lipopolysaccharides. This makes it difficult for secondary metabolite compounds to enter the cell (Jubeh *et al.*, 2020).

4. CONCLUSION AND SUGGESTIONS

The results of phytochemical screening of bidara leaves taken from Klagen village, Rejoso sub-district, Nganjuk district show that there are secondary metabolite compounds in the form of flavonoids,

tannins and saponins. The inhibition zone value of the best bidara leaf extract is at a concentration of 100%. Inhibition zone test on *S. aureus* bacteria with 100% concentration has a diameter of 10.97 ± 2.47 mm which is classified as a strong category, while on *E. coli* bacteria with 100% concentration treatment has an inhibition zone diameter of 5.26 ± 0.45 mm which falls into the weak category.

The MIC value of bidara leaf extract against *S. aureus* bacteria was obtained at a 50% concentration of 6.2×10^6 CFU/mL, while the MIC value of *E. coli* bacteria was obtained at a 50% concentration of 5.2×10^7 CFU/mL. The MBC value of bidara leaf extract on *S. aureus* and *E. coli* bacteria is at a concentration of 100%. Phytochemical screening tests should be carried out on other compounds from bidara leaves. It is necessary to test the antibacterial activity of bidara leaves taken from other regions. Antibacterial test should be conducted on other species of bacteria.

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