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Uji Aktivitas Antibakteri Kombinasi Ekstrak Daun Kersen (*Mutingia Calabura* L.) Dan Daun Kelor (*Moringa Oleifera* L.) Terhadap Bakteri *Staphylococcus Aureus*

Antibacterial Activity Test of Combination of Cherry Leaf (*Mutingia calabura* L) and Moringa Leaf (*Moringa oleifera* L.) Extracts Against Staphylococcus aureus Bacteria

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ABSTRAK

Daun kersen *(Muntingia calabura* L.) dan daun kelor *(Moringa oleifera* L.) mengandung senyawa yang memiliki sifat antibakteri diantaranya flavonoid, saponin, tanin, dan alkaloid. Tujuan dari penelitian ini adalah untuk mengetahui potensi kombinasi ekstrak daun kersen *(Muntingia calabura* L.) dan ekstrak daun kelor *(Moringa oleifera* L.) dalam menghambat pertumbuhan *Staphylococcus aureus.* Metode yang digunakan dalam penelitian ini adalah uji dilusi dan difusi cakram. Kombinasi yang paling efektif kemudian diuji dengan uji pita kertas untuk mengetahui pola kombinasi bersifat sinergis, aditif, atau antagonis. Analisis data dilakukan secara statistik (SPSS). Penelitian menunjukkan bahwa ekstrak daun kersen memiliki nilai KHM sebesar 10% dan daun kelor sebesar 5%. Pada uji pengenceran, diameter penghambatan paling efektif adalah kombinasi (1:2) dengan diameter 19,06 ± 0,37 mm. Efek dari kombinasi (1:2) menunjukkan pola kombinasi sinergis.

ABSTRACT

Cherry leaves (*Muntingia calabura* L.) and Moringa leaves (*Moringa oleifera* L.) contain compounds that have antibacterial properties, including flavonoids, saponins, tannins, and alkaloids. The purpose of this study was to determine the potential of a combination of cherry and moringa leaf extracts in inhibiting the growth of *Staphylococcus aureus*. The method used in this study was dilution and disc diffusion method. The most effective combinations were then subjected to paper tape tests to determine the pattern of synergistic, additive, or antagonistic combinations. Data results were analyzed with SPSS test. The study showed that cherry leaf extract had an MIC value of 10% and moringa leaf had an MIC of 5%. In the dilution test, the most effective inhibition diameter was the combination (1:2) with a diameter of 19.06 ± 0.37. The effect of the combination (1:2) showed a synergistic combination pattern.

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1. INTRODUCTION

Infectious diseases are a serious health issue in developing countries such as Indonesia, as they contribute to high mortality and morbidity rates[1]. Antibiotics are used as substances that can inhibit the growth, reproduction and kill bacteria and fungi [2]. Antibiotics are the primary agents used to treat bacterial infections; however, their improper use has led to bacterial resistance, including resistance to *Staphylococcus aureu*s. The increasing prevalence of antibiotic resistance has driven the search for new treatment alternatives, one of which is the utilization of natural compounds from medicinal plants. Indonesia, as a tropical country, possesses rich biodiversity with great potential as a source of herbal medicines. Alternative herbal medicine provides the following benefits: reduced costs, reduced side effects, and increased efficacy and efficiency [3]. Natural ingredients have long been used for treatment, as they are considered safer than synthetic drugs and have fewer side effects. One plant with antibacterial potential is cherry (*Muntingia calabur*a L.), which contains flavonoids, alkaloids, saponins, and tannins [4]. These compounds have been shown to exhibit antibacterial activity against S. aureus. Research indicates that cherry leaf extract at a 10% concentration can inhibit the growth of *S. aureus,* with an average inhibition zone of 13.7 mm.

In addition to cherry, moringa (*Moringa oleifera* L.) also possesses antibacterial activity and has been used in traditional medicine in various countries. Moringa contains 539 active compounds that are beneficial for treating more than 300 types of diseases. Research has shown that moringa leaf extract at a 5% concentration can inhibit *S. aureus* growth, with an average inhibition zone of 12 mm [5]. The use of plant extract combinations in treatment is believed to be more effective than single-component usage, as it can create a synergistic effect [6]. Although numerous studies have explored herbal medicines as antibacterial agents, specific research on the combination of cherry and moringa leaf extracts agains*t S. aureus* remains limited. Therefore, this study aims to evaluate the effectiveness of combining cherry and moringa leaf extracts in ratios of 1:1, 1:2, and 2:1 in inhibiting the growth of S. aureus. The methods used include dilution to determine the Minimum Bactericidal Concentration (MBC) and diffusion to measure the inhibition zone diameter and identify the most effective combination ratio. Synergistic effects can be expected from such combinations, hence the importance of seeking safer alternatives by combining antibacterial agents that are thought to have synergistic effects [7]. This research is expected to contribute to the development of herbal-based alternative treatments for bacterial infections and support efforts to combat antibiotic resistance.

2. METHOD

2.1 *Tools*

The tools used in this study include: analytical scales, sieve no. 40, glass funnel, separating funnel, filter paper, porcelain cup, Laminar Air Flow (LAF), Beaker glass, horn spoon, micropipette, maceration bottle, test tube, measuring cup, oven, vortex mixer, vacuum rotary evaporator, moisture balance, tweezers, spiritus lamp, object glass, incubator, petri dish, ose needle, autoclave, microscope, and vernier caliper.

2.2 *Material*

Materials used in this study include cherry leaves and moringa leaves, 96% ethanol, Mayer reagent, 3% callium tellurite, amyl alcohol, distilled water, acetic acid (CH3COOH), 10% DMSO, BHI media, NA (Nutrient Agar) media, VJA media (Vogel Johson Agar), MHA media (Mueller Hinton Agar), Wagner's reagent, lugol solution, Fe3Cl, Dragendorff reagent, concentrated hydrochloric acid (HCI), concentrated sulfuric acid (H2S04 p), and crystal violet paint.

2.3 *Preparation of Extracts from Cherry Leaves and Moringa Leaves*

Weighing as much as 11,000 g of cherry leaves and weighing as much as 10,500 g of moringa leaves that have been cleaned, washed with running water, and dried in the sun and covered with black cloth to dry. The dried leaves were crushed using a pollination tool to obtain moringa leaf powder, then sieved with a 40 mesh sieve to obtain powdered cherry leaves and moringa leaves with a homogeneous degree of fineness. Then weighing each powder and macerated with 96% ethanol solvent then the filtrate obtained is heated using a rotary evaporator at 60^{o}C evaporated so that a thick extract is obtained.

2.4 *Identification of Chemical Content*

Identification of flavonoids was carried out by putting the extract into a test tube along with 4 ml of ethanol to dissolve. Then heated filtered, and shaken. Filtrate as much as 2 ml was added with 0.1 mg of magnesium powder and 10 drops of concentrated HCl. The appearance of red colour indicates the presence of positive flavonoids[8]. The identification process of saponins is done by entering the extract sample then, add 10 ml of hot distilled water and allow it to cool. After that, shake the mixture vigorously for 10 seconds. Positive saponin if the foam does not disappear when 1 drop of HCl is inserted. Identification of tannins is done by entering the extract sample then add 15 ml of distilled water and boil for 5 minutes on a water bath. After that, add a few drops of FeCl3 1%. If a violet green colour is formed, it shows a positive result for tannins[9]. Identification of alkaloids using *mayer, wagner and dragendroff* reagents. Terpenoid and steroid tests, 1 mL of extract is put into a test tube and then dissolved in chloroform, and added 0.5 mL of HCl and 2 drops of concentrated sulfuric acid. Positive results include terpenoids if a blue- black colour is formed and steroids if a yellow precipitate is formed.

2.5 *Identification of S. aureus bacteria*

Identification of *S. aureus* bacteria was identified by taking one ose and then transmitted through Vogel Johson Agar (VJA) media. For 18 to 24 hours, the medium was incubated at 37oC. Golden yellow coloured colonies indicate positive results of S. aureus growth[10]. Identification of bacteria by gram staining. Gram staining of S. aureus bacteria uses several steps, namely Gram A (crystal violet), Gram B (lugol iodine), Gram C (alcohol), and Gram D (safranin). Positive results of S. aureus can be seen under a microscope with a magnification of 100x, marked by clustered round-shaped bacteria and purple in colour[11].

2.6 *Antibacterial activity testing dilution method*

The dilution method was used to determine the Minimum Kill Concentration

(KBM) and determine the Minimum Inhibitory Concentration (KHM) of the samples. The test was conducted using 12 sterile tubes. The concentration series started from 40 to 0.07%. Each tube was incubated for 24 hours at 37°C and observed for turbidity.

2.7 *Antibacterial Activity Testing Disc Diffusion Method*

This method uses paper discs that have been soaked with a combination of cherry leaf and moringa leaf extracts in a ratio of (1:1); (1:2); (2:1). The negative control used 10% DMSO, while the positive control used ciprofloxacin discs. Incubation lasted for 24 hours at 37°C. Next, observe the clear zone formed and measure the diameter of the inhibition zone. Measurement of the diameter of the inhibition zone was carried out using a caliper or ruler with an accuracy of 1 mm.

2.8 *Data Analysis*

The data obtained were then analysed using statistical tools (SPSS) Statistical Product and Scandisis with version 27. To ensure that the data were normally distributed or not, the Shapiro- Wilk test was used to test the normality distribution. the analysis was continued with Levene Satistic to check the homogeneity of the data ($p > 0.05$). The test continued with the ANOVA method using one way for significant values ($P < 0.05$). Further analysis uses Tuckey's Post Hoc test to determine which treatments are significantly different.

2.9 *Combination pattern test using the paper tape method*

The combination properties were tested using the paper tape method based on the concentration ratio of the most effective combination of cherry leaf and moringa leaf extracts. The paper tape uses Whatman 1 paper cut to a size of 1 x 3 cm. Place the dry paper tape on the MHA that has been scratched by *S. aureus* with one side together forming a 90° angle (vertically forming the letter 'L') and connect one end to the other tape and attach it to the media. Petri dishes were incubated for 18-24 hours at 35 ± 2°C.

3. RESULT

The extracts of cherry leaves and moringa leaves were obtained by maceration. The extract yield of cherry leaves and moringa leaves in this study was obtained at 24% and 18%. The results obtained from each study may differ from one another due to several actors, including the location of material collection, the month of collection, and the season during which the material was collected.

Table 1. Chemical identification test results of cherry leaf extracts and moringa leaf extract

Chemical content	Library	Result	
		Cherry leaf leaf extracts	Moringa leaf extract
Flavonoids	formation of red, yellow, or orange colours[12]	formation red-orange	formation red-orange
Saponins	formation of stable froth[12]	formation of stable froth	formation of stable froth
Tannins	formation of a greenish brown or blue-black colour[12]	blue-black formation	blue-black formation
Alkaloids	*Mayer* : formation of a white precipitate	*Mayer* : no white precipitate	*Mayer* : no white precipitate
	Wagner : formation of brown precipitate	*Wagner* : formation of brown precipitate	*Wagner* : formation of brown precipitate
	Dragendroff : formation of orange precipitate[12]	*Dragendroff :* formation of orange precipitate	*Dragendroff :* formation of orange precipitate
Terpenoids	formation of an intense green colour[12]	formation of an intense green colour	formation of an intense green colour

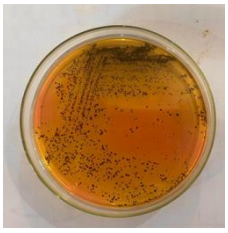

Figure 1. Macroscopic test result of *S. aureus* bacteria

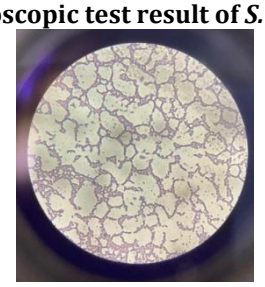

Figure 2. Microscopic test results of *S. aureus* bacteria

Table 2. Results of biochemical identification of bacteria using catalase and coagulase tests

Testing	Results	Explanation
Catalase	Gas bubbles are formed	+ (Gas bubbles are formed)
Coagulase	White lumps form in the plasma	+ (White lumps form in the plasma)

Table 3. antibacterial test results by dilution

Consentration (%)	cherry leaf extract			Consentration (%)	Moringa leaf extract		
	I	II	III		I	II	III
40	-	-	-	40	-	-	-
20	-	-	-	20	-	-	-
10	-	-	-	10	-	-	-
5	+	+	+	5	-	-	-
2,5	+	+	+	2,5	+	+	+
1,25	+	+	+	1,25	+	+	+
0,625	+	+	+	0,625	+	+	+
0,312	+	+	+	0,312	+	+	+
0.156	+	+	+	0.156	+	+	+
0,07	+	+	+	0,07	+	+	+
Control +	+	+	+	Control +	+	+	+
Control -	-	-	-	Control -	-	-	-

Information:
Control (+): Bacterial suspension
Control (-) : Extract
+ : There is bacterial growth on the VJA media
- : There is no bacterial growth on VJA media

Table 4. Disc Diffusion test result

Sample	Diameter of inhibition zone (mm)			
	Replication I	Replication II	Replication III	Avarage ±SD
Control -	0	0	0	0,00±0,00
Control +	31,3	31,7	30,6	31,2±0,57
Single cherry leaf	13,2	12,6	11,9	12,57±0,65
Single moringa leaf	14,6	13,4	13,25	13,75±0,73

Sample	Diameter of inhibition zone (mm)			
	Replication I	Replication II	Replication III	Avarage ±SD
DKS + DKR (1:1)	16,1	15,7	15,3	15,7±0,4
DKS + DKR (1:2)	19,5	18,9	18,8	19,06±0,37
DKS + DKR (2:1)	16,7	16,2	17,3	16,73±0,55

Information :
Control (-): DMSO 10%
Control (+): Ciprofloxacin
DKS:DKR (1:1) : cherry leaf extractt (10%) : moringa leaf extratt (5%)
DKS:DKR(1:2) : cherry leaf extractt (10%) : moringa leaf extratt (10%)
DKS:DKR(2:1) : cherry leaf extractt (20%) : moringa leaf extratt (10%)

Table 5. Homogeneus subset

Sample	Subset *for alpha* 0.05				
	1	2	3	4	5
Control -	,0000				
Single cherry leaf		12,5667			
Single moringa leaf		13,7500			
DKS + DKR (1:1)			15,7000		
DKS + DKR (1:2)			16,7333		
DKS + DKR (2:1)				19,0667	
Control +					31,2000
Sig.					1,000

Information :
Control (-): DMSO 10%
Control (+): Ciprofloxacin
DKS:DKR (1:1) : cherry leaf extractt (10%) : moringa leaf extractt (5%)
DKS:DKR(1:2) : cherry leaf extractt (10%) : moringa leaf extractt (10%)
DKS:DKR(2:1) : cherry leaf extractt (20%) : moringa leaf extractt (10%)

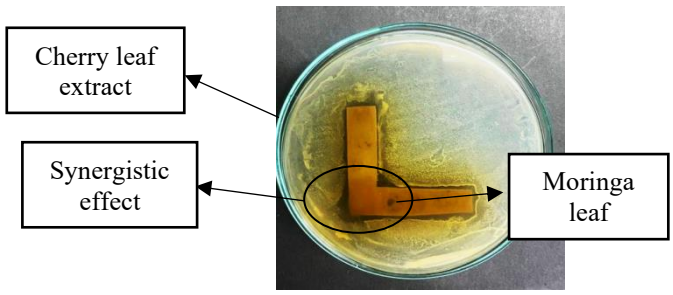

Figure 3. Paper tape test result

4. DISCUSSION

4.1 *Chemical content Identification*

Cherry leaves contain chemical compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids that function actively as antibacterials. Moringa leaves contain chemical compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids that function actively as antibacterials. The results of the chemical content identification test of the kaffir lime leaf and cherry leaf extracts can be seen in table 1.

4.2 *Identification of S. aureus bacteria*

This study aims to identify *Staphylococcus aureus* using Vogel Johnson Agar (VJA) media containing 3% potassium tellurite. Incubation was carried out at 37°C for 18–24 hours[13]. The results showed black-colored colonies with a surrounding yellow medium and a convex bacterial surface. The black coloration is caused by the reduction of potassium tellurite to tellurium metal by *S. aureus*, while the yellow color occurs due to mannitol fermentation, which produces lactic acid, with phenol red acting as an indicator of pH changes to acidity [14].

Gram staining is performed to microscopically identify bacteria and confirm that they are Gram-positive, as Gram-positive bacteria absorb purple dye [15]. The purple coloration in Gram-positive bacteria is due to their ability to retain the primary stain (crystal violet) after washing with 95% alcohol, which is related to the composition of their cell wall. Gram-positive bacteria contain more lipids and peptidoglycan than Gram-negative bacteria [16]. The Gram staining results in this study showed purple-colored, grape-like clustered cocci, indicating that the bacteria were *Staphylococcus aureus.*

The catalase test aims to differentiate *Staphylococcus* species from *Streptococcus* species by detecting the presence of the catalase enzyme produced by *S. aureus*[17]. A positive catalase test is indicated by the formation of gas bubbles (O_2), which occurs because S. aureus produces catalase that breaks down H_2O_2 into H_2O and O_2 [18]. The coagulase test is used to determine the ability of bacteria to produce the coagulase enzyme. A positive result is marked by the formation of white clots in plasma, as coagulase enables plasma coagulation with the help of serum factors [19]. The antibacterial activity test using the dilution method was conducted with three replications by observing the turbidity of the solution to determine the Minimum Inhibitory Concentration (MIC). Due to the dark color of the extract used in this study, turbidity observation was difficult, making MIC determination impossible. Instead, the Minimum Bactericidal Concentration (MBC) was assessed by streaking the solution on Vogel Johnson Agar (VJA). MBC is determined only when *S. aureus* shows no growth at all.

4.3 *Antibacterial activity by dilution*

MIC is the lowest or smallest concentration of a sample that can inhibit the growth of *S. aureus* bacteria which is indicated by a sample that is not cloudy, while MKC is the lowest or smallest concentration of a sample that kills bacteria. MKC is indicated by the absence of bacterial growth that has been etched on selective media at that concentration. The dilution test results of cherry leaf extract had MKC at the lowest concentration of 10%. Research conducted by [20] shows that a single extract of cherry leaves has a MKC value of 10%. This shows the similarity of the research results with previous research.

Testing antibacterial activity using the dilution method aims to determine the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MKC) values of cherry and Moringa leaf extracts. Various concentrations are used, ranging from 40%, 20%, 10%, 5%, 2.5%, 1.25%, 0.625%, 0.312%, 0.156%, and 0.07%. The positive control uses a bacterial suspension, while the negative control uses cherry and Moringa leaf extracts. This research was carried out with three replication tests. The research results showed that the Minimum Inhibitory Concentration (MIC) could not be determined. This is caused by the very dark color of the cherry and Moringa leaf extracts, which ultimately affects the results read in the test tube. Nonetheless, this study provides insight into the

challenges that may be encountered in determining certain parameters in antibacterial testing. The MKC test results show that the MKC from Moringa leaves is 5%. MKC results are determined from the clear area of lowest concentration where bacterial colonies cannot grow in the petri dish. Differences in MKC results are influenced by different solvents and different extract stock solutions used. The higher the concentration of the extract, the higher the potential to inhibit bacteria, where the higher concentration means the number of bacteria will decrease [21].

4.4 *Disc Diffusion Test*

The antibacterial activity test of cherry (*Muntingia calabura*) and moringa (*Moringa oleifera*) leaf extracts against *Staphylococcus aureu*s using the disk diffusion method revealed that the 1:2 ratio exhibited the highest inhibition zone (19.06±0.37 mm), surpassing the 2:1 (16.73±0.55 mm) and 1:1 (15.7±0.4 mm) ratios. Statistical analysis (SPSS 27) confirmed normal data distribution and homogeneity, with ANOVA indicating significant differences among treatments ($p = 0.000 < 0.05$). Post Hoc Tukey's test showed that the 1:2 combination was the most effective antibacterial formulation, significantly outperforming other tested groups in inhibiting *S. aureus.*

4.5 *Data Analyse*

The data obtained were then analysed using statistical tools (SPSS) Statistical Product and Scandisis with version 27. To ensure that the data were normally distributed or not, the Shapiro-Wilk test was used to test the normality distribution. If the data were normally distributed ($p > 0.05$), the analysis continued with Levene Satistic to check the homogeneity of the data ($p > 0.05$). Testing continued with the ANOVA method using one way for significant values ($P < 0.05$). Further analysis used Tuckey's Post Hoc test to determine which treatments were significantly different.

The homogeneus subsets test was carried out to identify significant mean differences between various subgroups (subsets) in the data. Based on the table above, it shows that the negative control and single extract are the groups with lower values and do not have as high a result as the 1:2 combination of cherry leaf extract and Moringa leaf extract. This shows that the most effective combination and closest to positive control is a combination with a ratio of 1:2 (cherry leaf extract: moringa leaf extract). The combination with a ratio of 1:2 also has a significant difference with the combination with a ratio of 1:1 and 2:1.

4.6 *Paper tape test*

The properties of the extract combination can be observed through the clear area or inhibition zone produced by each paper strip treated with the extract. The results of the combination property test are shown in Figure 12, where the inhibition zone at the meeting point of cherry (*Muntingia calabura*) and moringa (*Moringa oleifer*a) leaf extracts expands at the corner. This differs from the inhibition diameter on the paper strip sides that do not meet. The combination test of cherry and moringa leaf extracts at a 1:2 ratio demonstrated a synergistic effect against *S. aureus*. The active compounds in cherry leaves include flavonoids, tannins, saponins, and alkaloids. Flavonoids can transfer energy to bacterial cytoplasmic membranes, inhibiting bacterial motility [22]. The antibacterial mechanism of flavonoids involves protein denaturation, which damages

bacterial cell walls. Saponins in cherry leaves increase bacterial cell membrane permeability, leading to cell lysis. When saponins react with bacterial cells, they cause bacterial rupture or lysis [23]. Tannins interact by forming complex polysaccharides that damage bacterial cell walls, disrupting cell permeability. This interference prevents bacterial cells from carrying out life activities, ultimately inhibiting bacterial growth and causing bacterial death. Tannins not only damage bacterial cell walls but also alter protein properties and inhibit bacterial nucleic acid synthesis [24].

The active compounds in moringa leaves include flavonoids, tannins, saponins, alkaloids, and steroids [25]. Flavonoids disrupt cell membranes, causing leakage of essential metabolites and deactivating bacterial enzyme systems. Saponins act by increasing membrane permeability, leading to hemolysis and bacterial cell rupture. Alkaloids function as antibacterial agents by damaging peptidoglycan, a crucial component of bacterial cell walls. This destruction prevents the complete formation of cell walls, leading to cell death [26]. The antibacterial mechanism of tannins includes inactivating bacterial cell adhesion, enzyme systems, and protein transport across the inner cell layer. Tannins damage polypeptides in bacterial cell walls, impairing their structure and causing bacterial cell lysis [27]. Steroids contribute to bacterial cell wall destruction by interacting with the membrane, increasing permeability, and ultimately leading to bacterial cell death [28].

5. CONCLUSIONS

The combination of cherry (*Mutingia calabura* L.) leaf extract and Moringa (*Moringa oleifera* L.) leaf extract (1:2) which has the most effective antibacterial activity against *S. aureus* bacteria and has a synergistic effect combination pattern.

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