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ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF KERSEN LEAF (*Muntingia calabura L.*) TOWARDS *Escherichia coli* BACTERIA

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ABSTRACT

Escherichia coli is a bacteria that causes infection. In Indonesia, it has been reported that *Escherichia coli* is resistant to several types of antibiotics; therefore, other alternatives are needed to inhibit the growth of this Escherichia coli. One alternative method is to use medicinal plants, such as cherry leaves, which contain flavonoids, saponins, and tannins as antibacterials. Aim to determine the antibacterial activity of an ethanol extract of cherry leaves (*Muntingia calabura L.*) against Escherichia coli bacteria. The research used a post-test-only control group design. This study contained 7 groups: 1 positive control group, 1 negative control group, and 5 treatment groups. The treatment group consisted of an ethanol extract of cherry leaves with concentrations of 1%, 2%, 4%, 8%, and 10%. The control group was a positive control (K(+) with co-trimoxazole and a negative control (K(-) namely dimethyl sulfoxide (DMSO) at 10%. The data used was One-way ANOVA. The results of this study were the average zone of inhibition. The largest is at a concentration of 10%, namely 6 mm, while the smallest average inhibition zone is at a concentration of 4%, namely 4.37 mm. Concentrations of 2% and 1% do not indicate the presence of an inhibition zone. From these results it is concluded that there are significant differences between The concentration of cherry leaf ethanol extract against Escherichia coli bacteria and the minimum inhibitory level of cherry leaf ethanol extract against Escherichia coli bacteria is at a concentration of 4% with an average inhibition zone of 4.37mm.

Keywords: Kersen Leaf Extract, Escherichia coli, antibacterial activity

INTRODUCTION

The problem of infectious diseases continues to grow in the health sector and is the main cause of death throughout the world, including in Indonesia (Mboi et al., 2022). Infections can be caused by bacteria, fungi, viruses, and parasites (Verhoef et al., 2019). The prevalence of infectious diseases in Indonesia is quite high because it is one of the countries with a tropical climate. Dusty conditions, warm and humid temperatures support microbes to continue to reproduce and ultimately cause infection (Wolkoff, 2018).

The bacteria that currently cause health problems, especially in Indonesia, is Escherichia coli. Escherichia coli can cause infection. Generally, it causes three types of infections, namely digestive tract infections such as diarrhea, urinary tract infections, and neonatal meningitis. *Escherichia coli* often causes infections in the urinary tract, bile duct and causes diarrhea (Jawetz, 2007).

One way to slow the growth of *Escherichia coli* bacteria is by administering antibiotics. Antibiotics are drugs that can kill bacteria, the antibiotics that are often used are ampicillin, sulfonamide, tetracyclin and gentamicin. The use of antibiotics which have been widely used for *Escherichia coli* bacteria has increased in the last few decades, causing

organ damage, immunosensitivity and also causing resistance (Tadesse et al., 2012). Previous research results showed that *E. coli* was 100% resistant to ampicillin, 96.2% resistant to erythromycin, 94.3% resistant to nalidixic acid, 92.3% resistant to tetracycline and 92.3% resistant to oxytetracycline (Kasem et al., 2021). A total of 781 patients treated in hospital showed that 81% of *Escherichia coli* has the highest resistant to ampicillin and low resistance to gentamicin (18%) (Tadesse et al., 2012). To reduce this problem, use active bacteria-killing substances contained in medicinal plants, one of which is basil leaves (*Ocimum basilicum* L).

Based on research, cherry leaf juice with concentrations of 90% and 100% does not cause germ growth at an extract concentration of 5%, as can be seen from the formation of a clear zone (Pia, 2023). This study aims to examine the antibacterial activity and determine the minimum inhibitory content of cherry leaf ethanol extract. This study aims to examine the antibacterial activity and determine the minimum inhibitory content of cherry leaf ethanol extract. MIC is used to determine the minimum concentration of antimicrobial substances needed to inhibit the growth of a microorganism. In previous research, it was found that ethanol extract of cherry leaves at a concentration of 5% still had antibacterial ability; therefore, this study used concentrations of 1%, 2%, 4%, 8%, and 10%.

Another alternative way that can be done to prevent resistance due to the long-term use of antibiotics is to use active substances that inhibit bacterial growth found in medicinal plants, one of which is the Kersen plant (*Muntingia calabura* L.). Secondary metabolites in cherry leaves are; tannins, flavonoids, terpenoids, saponins, and polyphenols. Flavonoid compounds contained in cherry leaves can function as antibacterials.

Based on the above background, the author wants to test the activity of compounds in Kersen leaves (*Muntingia calabura* L.) in inhibiting the growth of *Escherichia coli* bacteria.

RESEARCH METHODS

Tools and Materials

Tools

Autoclave (HIRAYAMA), micropipette (DRAGON MED), blender, erlenmeyer, mortar and pestle, petri dish, 5cc syringe, cotton swab, incubator, laminar air flow (ESCO LHS 4AG), rotary evaporator (AeLAB), scale caliper (VERNIER CALIPER), water bath (MEMMERT).

Materials

Kersen leaves (*Muntingia calabura L*), test bacteria (*Escherichia coli* ATCC 8739), cotrimoxazole 480 mg tablets, 96% ethanol, distilled water, filter paper, dimethyl sulfoxide (DMSO), Nutrient Agar (MERCK).

Research Procedure

1. Plant determination

   The cherry plants used as research material were determined at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Semarang State University. No. 117/UN 37.1.4.5/LT/2023.
2. Tools sterilization

   The equipment was sterilized using an autoclave at 121 ^{o}C with a pressure of 15 psi for 15-20 minutes.
3. Extraction

   The sample was macerated with 96% ethanol solvent, stirred until homogeneous, and left for 3x24 hours. Next, filter it using filter paper. Maceration is carried out on the remaining filter results. then macerated again for 3x24 hours. The filtrate resulting from maceration and remaceration is combined and then put into a rotary evaporator at a temperature of 60 ^{o}C to remove the ethanol-free extract. To get a thick extract, use a water bath at a temperature of 60 ^{o}C.
4. Phytochemical test

a. Identify flavonoids
Magnesium powder and 2 ml of 2N HCl in 2 ml of extract solution. Flavonoid compounds will show an orange-to-red color.
b. Identify alkaloids
This was done by adding 3 ml of the extract solution to 1 ml of 2N HCl and 6 ml of distilled water, then heating for 2 minutes, cooling, and filtering. The filtrate was examined with Mayer's reagent to form a white precipitate.
c. Identify saponins
The extract is added distilled water. Then shake vertically for 10 seconds. The test result is positive if stable foam appears for several minutes (Kasem et al., 2021).
d. Identify tannins
This is done by reacting 1 ml of the test extract solution with 10% $FeCl_3$. The presence of tannin is indicated by the formation of a dark blue or greenish-black color.
e. Identify terpenoids and steroids
This is done by dissolving the test material in chloroform, and then adding 0.5 ml of anhydrous acetic acid. Next, 2 ml of concentrated sulfuric acid was added through the tube wall. The presence of triterpenoids is indicated by the formation of a brownish or violet ring at the border of the solution, while the presence of steroids is indicated by the formation of a greenish-blue ring.

5. Control solution preparation
The negative control was made from 10 ml of liquid with 10% DMSO. The positive control was cotrimoxazole 480 mg. One cotrimoxazole tablet is crushed, then weighed, and equal to 400 mg. Then the cotrimoxazole powder was dissolved in 100 ml of distilled water to obtain a 40 µg/40 µl cotrimoxazole solution.
6. Test solution preparation
The result of pure extract from Kersen leaves (*Muntingia calabura L*) was subjected to multilevel dilution using DMSO to obtain the required concentration. The 10% test solution was made by weighing 1 g of cherry leaf ethanol extract and then dissolving it in DMSO solution to a volume of 10 ml.
1) The 8% test solution is made by pipetting 8 ml of the 10% test solution, and then adding DMSO solution to 10 ml.
2) The 4% test solution is made by pipetting 5 ml of the 8% test solution, and then adding DMSO solution to 10 ml.
3) The 2% test solution is made by pipetting 5 ml of the 4% test solution, and then adding DMSO solution to 10 ml.
4) The 1% test solution is made by pipetting 5 ml of the 2% test solution, and then adding DMSO solution to 10 ml.
7. *Nutrient Agar* Preparation
5 grams of nutrient agar (NA) dissolved in 250 ml of distilled water. Next, it is homogenized on a hot plate until it boils. The homogenized media is sterilized in an autoclave at 121 ^oC for 15 minutes, then cooled to a temperature of approximately 45-50 ^oC. Test media is used for testing media (Dima et al., 2016).
8. Bacterial suspension Preparation
1 ml of the diluted test bacteria was taken using a syringe and then suspended in a tube containing 9 ml of 0.9% NaCl solution until a turbidity similar to the standard Mc turbidity was obtained. Farland 0.5 (Dima et al., 2016).
9. Antibacterial Activity Test
In the wells, the positive control Cotrimoxazole (25 µg), the negative control 10% DMSO, and the treatment group with ethanol extract of cherry leaves with several concentrations (1%, 2%, 4%, 8%, and 10%) were dropped in 35 µl each. After that, it was incubated in an incubator for 24 hours at 35°C.

Data Analysis

The statistical analysis of this research uses normality tests, namely the *Shapiro-Wilk test* and the one-way Anova parametric statistical test.

RESULTS AND DISCUSSION

The phytochemical test aims to determine the content of secondary metabolite compounds contained in a sample by testing the color reaction when adding a color reaction. Phytochemical tests were carried out on the ethanol extract of the Kersen leaf. The results of observations of the phytochemical test of Kersen leaf extract include alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids, which are shown in **Table I**.

Table I. Phytochemical Screening Results

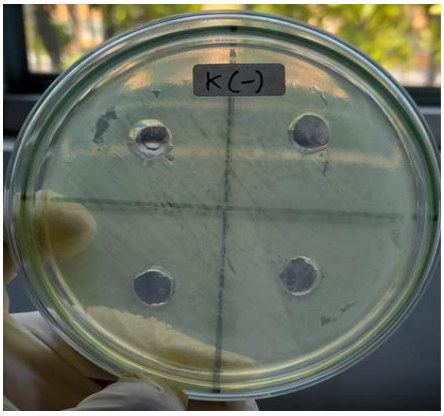
Compound	Result
Flavonoids	++
Tannins	+++
Saponins	++
Alkaloid	-
Steroids	++
Triterpenoids	+

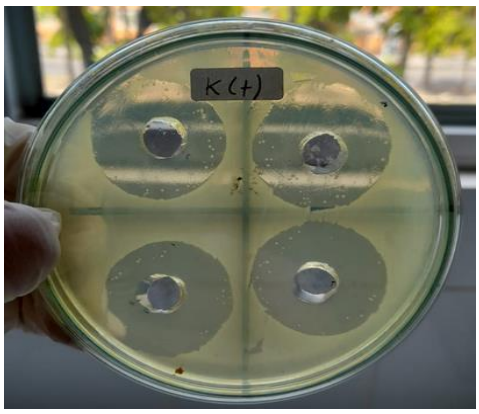
Note: + = there is a compound; - = there is no compound.

Determination of kersen (*Muntingia calabura* L.) plants was carried out to determine the correct identity of the plants to be studied, and then various concentrations of ethanol extract of kersen leaves (*Muntingia calabura* L.) were tested against *Escherichia coli* bacteria using the well diffusion technique. Bacterial growth inhibition testing was carried out with 7 treatment groups.

Table II. Measurement results and average diameter of the inhibition zone of ethanol extract of Kersen leaves (*Muntingia calabura* L.) on the growth of *Escherichia coli* bacteria.

Treatment	Repetition to				Average	P Value
	I	II	III	IV		
Control (+)	24,5 mm	22,5 mm	23 mm	22,5 mm	23,1 mm	0,00
Control (-)	0	0	0	0	0	
1%	0	0	0	0	0	
2%	0	0	0	0	0	
4%	3,5 mm	4 mm	4,5 mm	5,5 mm	4,37 mm	
8%	5,5 mm	4,5 mm	5 mm	4,5 mm	4,87 mm	
10%	5,5 mm	6,5 mm	7,5 mm	4,5 mm	6 mm	





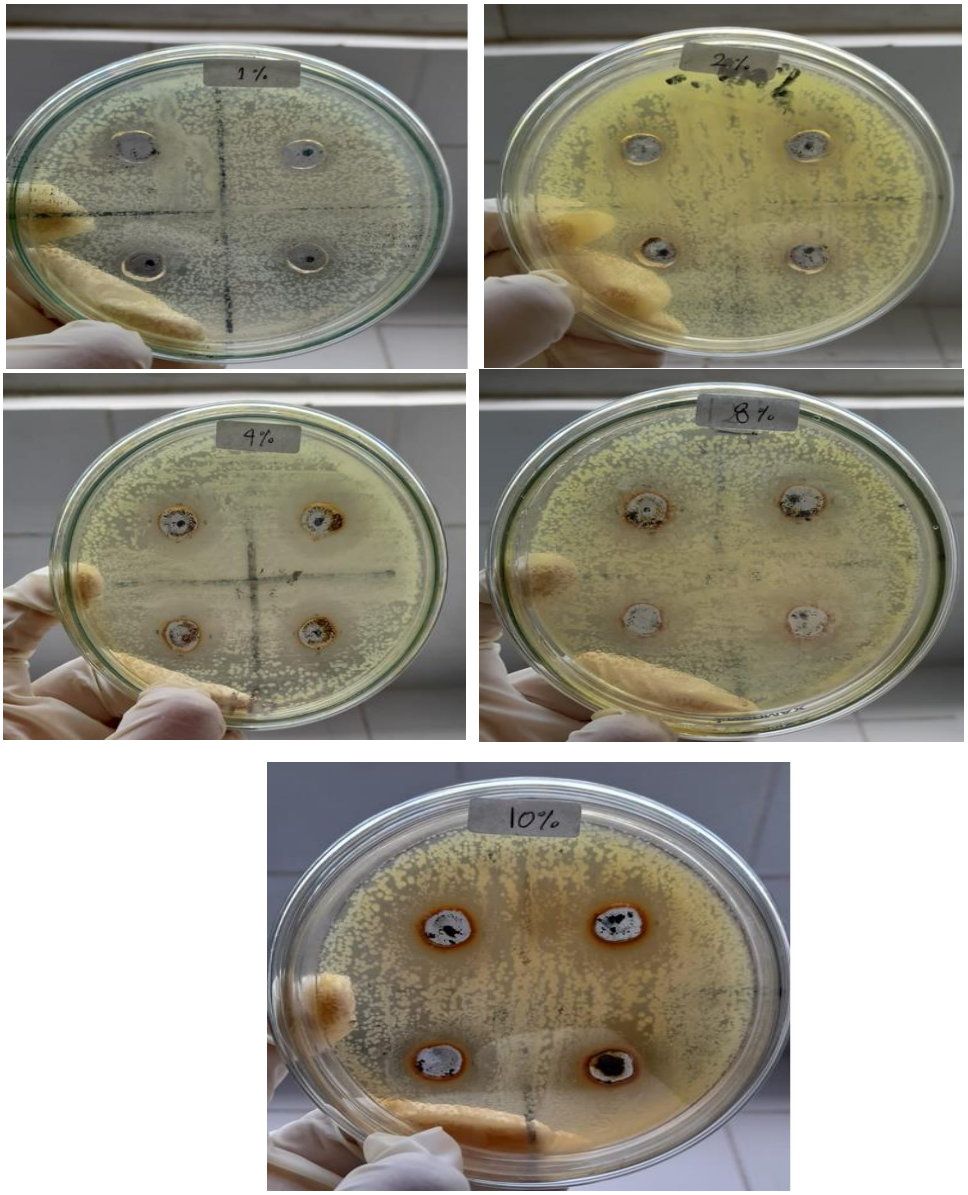


Figure 1. Antibacterial Activity Test Results of Kersen Leaf Ethanol Extract (*Muntingia calabura* L) against *Escherichia coli* bacteria.

Based on the results shown in Figure 1 and Table II. The inhibition zone at 10% concentration was 6 mm, at 4% concentration, it was 4.37% mm, at 2% concentration, and at 1%, there were no inhibitory zones. Kersen leaf extract (*Muntingia calabura* L.) has an antibacterial effect on the growth of *Escherichia coli* bacteria. Based on research, through phytochemical tests, kersen leaves were proven to contain alkaloids, flavonoids, saponins,- and tannins, which are antibacterial (Krisridwany et al., 2022). The results of the one-way ANOVA test for the Kersen leaf extract treatment group had a value of p = 0.000. Because the p value <0.05 (**Table III**), the average value between the ethanol extract treatment groups of kersen leaves is significantly different.

Minimum Inhibitory Concentration (MIC) is the minimum concentration of an antimicrobial substance that is able to inhibit bacterial growth after incubation for 24 hours, and no bacterial colonies will grow. The MIC can be determined from the resulting inhibition zone. The diameter of the inhibition zone was classified as weak (5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (20–30 mm) (Detha et al., 2018).

The minimum inhibitory content (MIC) kersen leaves is found at a concentration of 4% because, at this concentration, an inhibitory zone of 4.37 mm is obtained, whereas at a concentration of 2% there is no inhibitory zone. The average inhibitory zone of 4.37 mm, if classified, means it has a weak inhibitory response to the growth of Escherichia coli bacteria. The results of testing the ethanol extract of cherry leaves showed that the higher the concentration used, the larger the inhibition zone formed. This is because there are more active compounds contained in the extract. This result is in line with previous research, which found that the greater the extract concentration, the greater the inhibition zone that will be formed, and obtained the minimum inhibitory level of ethanol extract of kersen leaves against Staphylococcus aureus bacteria at a concentration of 45% with an inhibitory zone diameter of 2.133 mm (Kartika & Syarifuddin, 2022).

According to Lingga, the growth of most bacteria will decrease as the concentration of antibacterials added increases (Lingga et al., 2016). Kersen leaf extract contains active compounds of flavonoids, saponins, and tannins that inhibit bacterial growth. Flavonoid compounds can damage the permeability of bacterial cell walls, lysosomes, and microsomes (Fajriani et al., 2022). Saponins also interfere with the surface tension of the cell walls, so when the cell wall tension is disturbed, antibacterial substances can easily enter the cells, disrupting metabolism and causing the bacteria to die (Ngazizah et al., 2017). The mechanism of action of tannins is to inactivate microbial cell adhesion, inactivate enzymes, and disrupt protein transport in the inner layers of cells. Moreover, tannins also target the cell-wall's polypeptides. Therefore, cell wall formation is imperfect. This causes the bacterial cell to lyse due to the osmotic pressure; thus, the bacterial cell will die. Tannins are antibacterial by precipitating the proteins (Hidanah et al., 2022).

The results of the one-way ANOVA test for the Kersen leaf extract treatment group had a value of p = 0.000. Because the p value <0.05 (Table III), the average value between the ethanol extract treatment groups of kersen leaves is significantly different. The analysis is continued with a Post-Hoc test which shows that if the data has a p value <0.05, it means the data is significant or significantly different from other concentrations. If p = > 0.05 then the data is not significant or significantly different from other concentrations.

Table III. Parametric One-way Anova Test

Subject	Mean	N	Sig.
Consentration 1%	0,00	4	0,00
Consentration 2%	0,00	4	
Consentration 4%	4,37	4	
Consentration 8%	4,87	4	
Consentration 10%	6,00	4	

Table IV. Difference Test Between Concentration Groups

Concentration	Concentration	P value
1%	2%	1,000
	4%	0,000
	8%	0,000
	10%	0,000
2%	1%	1,000
	4%	0,000
	8%	0,000
	10%	0,000
4%	1%	0,000

	2%	0,000
	8%	0,862
	10%	0,043
8%	1%	0,000
	2%	0,000
	4%	0,862
	10%	0,234
10%	1%	0,000
	2%	0,000
	4%	0,043
	8%	0,234

Post-Hoc test results show that the diameter of the Escherichia coli bacteria inhibition zone for a concentration of 1% has no significant difference with a concentration of 2%, a concentration of 4% has no significant difference with a concentration of 8%, and a concentration of 10% has no significant difference with a concentration of 8%, but there is a significant difference at a concentration of 1% with a concentration of 4%, 8%, and 10%. For a concentration of 2%, there is a significant difference with a concentration of 4%, 8%, and 10%. A concentration of 4% has a significant difference from 10%.

CONCLUSION

Based on the statistical test above, there was a significant difference in each concentration of cherry leaf ethanol extract against Escherichia coli bacteria, with a p value <0.050. From the Minimum Inhibitory Concentration test of the ethanol extract of cherry leaves against Escherichia coli bacteria, it was obtained at a concentration of 4%, namely with an average inhibitory zone of 4.37mm.

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