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# Antibacterial Activity of Ethyl Acetate Fraction of Kersen Leaf (Muntingia Calabura L.) Towards Escherichia Coli Bacteria Growth

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**KEYWORDS**
Kersen Leaf Fraction; Escherichia Coli; Antibacterial Activity.

**ABSTRACT**
Escherichia coli is one of the bacteria that can cause infection. The initial management of infection is through antibiotics; however, inappropriate use of antibiotics can lead to antibiotic resistance. Therefore, alternative methods are needed to inhibit the growth of Escherichia coli bacteria. One possible alternative is the use of medicinal plants, such as kersen leaves, which contain flavonoids, tannins, and saponins as antibacterial compounds. This study analyzed the antibacterial activity of the ethyl acetate fraction of kersen (Muntingia calabura L.) leaves against Escherichia coli bacteria. This research employed a well-diffusion experimental design with a post-test only control group design. The study used seven groups: two control groups and five treatment groups. The treatment groups consisted of the ethyl acetate fraction of kersen leaves with concentrations of 1%, 10%, 30%, 50%, and 70%. The control groups included a positive control (K(+)) with ciprofloxacin and a negative control (K(–)) using 10% Dimethyl Sulfoxide (DMSO). Data were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney test. The largest average inhibition zone, showing a very strong inhibitory response, was found at a 70% concentration (26.9 mm). Strong inhibitory responses were observed at concentrations of 10% (10.5 mm), 30% (13.2 mm), and 50% (19.6 mm), while the smallest average inhibition zone, indicating a weak inhibitory response, was recorded at a 1% concentration (3.8 mm). The ethyl acetate fraction of kersen (Muntingia calabura L.) leaves demonstrated inhibition against the growth of Escherichia coli bacteria, with the highest inhibition level observed at a 70% concentration, producing an average inhibition zone of 26.9 mm.

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## INTRODUCTION

Several studies have examined the antibacterial activity of cherry (Muntingia calabura L.) leaves against various bacteria. Emilia (2023) examined the antibacterial activity of cherry leaf extract against the growth of Escherichia coli and found an average Minimum Inhibitory Concentration (MIC) of 4.37 mm at a concentration of 4% and moderate antibacterial activity with an average inhibition of 6 mm at a concentration of 10%. Another study by Sri Marfuati et al. showed that cherry leaf extract at concentrations of 1%, 2%, 4%, 8%, and 10% produced average inhibition zones of 0 mm, 0 mm, 4.37 mm, 4.87 mm, and 6 mm, respectively, against

Escherichia coli. Meanwhile, research on the ethyl acetate fraction of cherry leaves is still very limited, even though fractionation with semi-polar solvents such as ethyl acetate has the potential to produce more specific and effective active compounds than crude extracts. These studies generally still use crude extracts, and not many have examined the activity of the ethyl acetate fraction with a wider concentration range and levels up to 70% (Federer, 2018; Kruk, 2018; Kruse, 2018; Tenopir & Allard, 2014; Van Panhuis, 2014; Xu et al., 2022).

Infection is an invasive process by microorganisms that proliferate in the body and cause disease in the host. Viruses, bacteria, fungi, and parasites are infectious microorganisms that can live in tropical environments (Bates, 2018; Bohren, 2015; Figueroa, 2019; Marmot, 2020; Shanafelt, 2017). Escherichia coli is a gram-negative, facultative anaerobic bacterium that does not form spores and lives as a natural flora colonizing the human digestive tract, and it can cause diseases such as diarrhea, pneumonia, sepsis, meningitis, and urinary tract infections.

The United Nations Children's Fund (UNICEF) estimates that there are two billion cases of diarrhea in the world each year, and 1.9 million children under the age of 5 die from diarrhea, most of which occur in developing countries. According to the Indonesian Ministry of Health, in 2019 the diarrhea morbidity rate for all ages was 270/1000 population, while for toddlers it was 843/1000 population. Treatment of diarrhea can be carried out by administering antibiotics.

Antibiotics are drugs used to treat infections caused by bacteria. In drug administration for infections caused by gram-negative bacilli, gentamicin, ciprofloxacin, penicillin, and sulfadiazine are given in cases of urinary tract infections caused by Escherichia coli, Klebsiella, and Proteus mirabilis (Anggraini et al., 2021; Campbell, 2016; Greenhalgh, 2017; Mochammad Maulidie Alfiannor Saputera, Tio Widia Astuti Marpaung, 2019; Suhaenah, 2021; Tannenbaum, 2016). However, improper use of antibiotics can lead to antibiotic resistance. For this reason, alternative traditional medicines with natural ingredients containing antibacterial active compounds derived from medicinal plants are needed. The use of plants as traditional medicine in the community is considered quite effective, because the side effects of traditional medicines are relatively small and they are more effective for metabolic and degenerative diseases.

The kersen plant can be used as a traditional medicine. Kersen plants are often found on roadsides and in house yards. The kersen plant remains green, flowers, and bears fruit throughout the year. The parts of the kersen plant that are widely used are the leaves and fruits. The kersen plant (Muntingia calabura L.) is a plant native to South America that has spread throughout Asia, including Indonesia. The results show that kersen leaves contain various bioactive compounds, including flavonoids, saponins, triterpenes, and tannins, some of which are natural ingredients with antibacterial, antioxidant, and anti-inflammatory properties. Kersen leaf extract (Muntingia calabura L.) has anti-inflammatory, antipyretic, and antibacterial activity. This kersen leaf contains many flavonoids, namely flavones, flavanones, and flavans, so it can act as an antibacterial agent with the greatest antibacterial activity against Staphylococcus aureus. In addition, the antibacterial activity of kersen leaf extract can also inhibit the growth of Escherichia coli and Shigella sonnei bacteria.

Based on research by Emilia (2023), kersen leaf extract (Muntingia calabura L.) has antibacterial activity against the growth of Escherichia coli, with an average Minimum Inhibitory Concentration (MIC) value of 4.37 mm at a concentration of 4% and moderate antibacterial activity with an average inhibition value of 6 mm at a concentration of 10%. (9) Based on the description above, further purification is needed in research on kersen leaves (Muntingia calabura L.) to determine the effectiveness of inhibition against Escherichia coli bacteria. Therefore, the author aimed to test the activity of the compounds in the ethyl acetate fraction of kersen leaves (Muntingia calabura L.) in inhibiting the growth of Escherichia coli bacteria.

This research offers novelties that distinguish it from previous studies. First, substantively, this study specifically tested the antibacterial activity of the ethyl acetate fraction of cherry (Muntingia calabura L.) leaves, not the crude extract, so it is expected to obtain more purified active compounds with more optimal activity. Second, this study used a wider and graded concentration range (1%, 10%, 30%, 50%, and 70%) to observe the relationship between increasing concentration and increasing inhibition zone diameter, which has not been widely explored in previous studies. Third, this study conducted a phytochemical analysis of the ethyl acetate fraction to identify the content of secondary metabolites that play a role in antibacterial activity, thus providing a more comprehensive understanding of the mechanism of action of the fraction. Fourth, this study used the well diffusion method, which allows the active compounds to diffuse optimally into the medium, in contrast to the disc method commonly used in previous studies (Alouw et al., 2022; Hadi & Permatasari, 2019; Handoko, 2020; Nuryah et al., 2019; Shamsudin et al., 2022). Thus, this study not only provides empirical data regarding the potential of the ethyl acetate fraction of cherry leaves as an antibacterial agent, but also contributes to the development of phytopharmaceuticals based on Indonesian natural ingredients.

Based on the above background, the researcher formulated the research problem as follows: “How effective is the antibacterial activity of the ethyl acetate fraction of kersen leaves (Muntingia calabura L.) on the growth of Escherichia coli bacteria?” The purpose of this study is to determine the antibacterial activity of the ethyl acetate fraction of kersen leaves (Muntingia calabura L.) against the growth of Escherichia coli bacteria. The results of this study can add to knowledge and insight regarding the antibacterial activity of the ethyl acetate fraction of kersen leaves (Muntingia calabura L.) against the growth of Escherichia coli bacteria.

This research has theoretical implications for the development of pharmaceutical science and microbiology, particularly in enriching the understanding of the potential of the ethyl acetate fraction of cherry leaves as an alternative source of antibacterial compounds against Escherichia coli. Practically, the findings of this study can serve as a basis for the development of phytopharmaceutical preparations or standardized traditional medicines to treat bacterial infections, especially in efforts to reduce dependence on synthetic antibiotics and to address the problem of antibiotic resistance. The results of this study also contribute to the pharmaceutical industry and future researchers in determining the effective concentration of

the ethyl acetate fraction of cherry leaves for further testing, both in vitro and in vivo, and open opportunities for exploring antibacterial activity against other pathogenic bacteria.

## METHOD

### Scope of Research

The scope of this research is included in the scope of Pharmacology, Microbiology, and Biochemistry.

### Place and Time of Research

This research was conducted in March 2024. This research will be carried out at the Research Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon in March-June 2024 (Kuuire & Dassah, 2020).

### Types and Research Designs

This study is a laboratory experimental research with *a post-test only control group research* design. The groups in this study consisted of:

1. Group 1: the negative control group, namely *Escherichia coli bacterial suspension,* was given 10% DMSO as much as 10 ml.
2. Group 2: the positive control group, namely *Escherichia coli* bacterial suspension, was given Ciprofloxacin 500 mg.
3. Group 3: treatment group 1 was given a fraction of ethyl acetate of kersen leaves (*Muntingia calabbura* L.) with a concentration of 1%.
4. Group 4: treatment group 2 is given a fraction of ethyl acetate of kersen leaf (*Muntingia calabura* L.) with a concentration of 10%.
5. Group 5: treatment group 3 is given a fraction of ethyl acetate of kersen leaf (*Muntingia calabura* L.) with a concentration of 30%.
6. Group 6: treatment group 4 is given a fraction of ethyl acetate of kersen leaf (*Muntingia calabura* L.) with a concentration of 50%.
7. Group 7: treatment group 5 was given a fraction of ethyl acetate of kersen leaves (*Muntingia calabura* L.) with a concentration of 70%.

### Population and Sample

The population of this study is *Escherichia coli bacteria.* The sample of this study is pure culture of *Escherichia coli* bacteria in the seeding of *Nutrient agar media*.

### Inclusion and Exclusion Criteria

Bacterial colonies that grow on agar media after exposure to the treatment are identified as *Escherichia coli*. Contaminated *Escherichia coli* cultures.

### Cara Sampling

The sampling method of this study is carried out by *means of simple random sampling* where sampling is done randomly or randomly.

**Large Sample**

The sample size of each group for the experimental test was determined using Federer's formula. When calculated as follows:

Description:

n: number of repetitions

Q: Total Treatment

Because this study used 7 treatment groups, then:

$$(n-1)\ (t-1) > 15$$
$$(n-1)\ (7-1) > 15$$
$$(n-1)\ (6) > 15$$
$$6N-6 > 15$$
$$6n > 21$$
$$n = 3.5$$

From the results of the calculation, it can be concluded that the number of repetitions to be carried out is as many as 4 repetitions.

**Table 1. Research Repeat Design**

| Treatment | Repetition | | | |
|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** |
| K (-) | K (-) I | K (-) II | K (-) III | K (-) IV |
| K (+) | K (+) I | K (+) II | K (+) III | K(+) IV |
| P1 | P1I | P1II | P1III | P1IV |
| P2 | P2I | P2II | P2III | P2IV |
| P3 | P3I | P3II | P3III | P3IV |
| P4 | P4I | P4II | P4III | P4IV |
| P5 | P5I | P5II | P5III | P5IV |

Source: Primary data from research results (2024).

**Description:**

K (-) : DMSO 10%

K (+) : Ciprofloxasine

P1 : Ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) with a concentration of 1%

P2 : Ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) with a concentration of 10%

P3 : Ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) with a concentration of 30%

P4 : Ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) with a concentration of 50%

P5 : Ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) with a concentration of 70%

## Research Variables

### 1. Independent Variables

The independent variable in this study was the ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.).

### 2. Bound Variables

The bound variable in this study was the antibacterial activity against *Escherichia coli* bacteria in agar media.

## Operational Definition

**Table 2. Operational Definition**

| Yes | Variabel | Operational Definition | Measuring Instruments | Measurement Results | Scale |
|---|---|---|---|---|---|
| 1 | Ethyl acetate fraction of kersen leaf (*Muntingia calabura L.*) | The result of fractionation using ethyl acetate solvent made with a concentration of (1%, 10%, 30%, 50%, 70%). | Scales and measuring cups | Concentration 1%, 10%, 30%, 50%, and 70%. | Nominal |
| 2 | Bacterium *Escherichia coli* | The growth of *Escherichia coli* bacteria in NA media by the sewage diffusion method was treated with ethyl acetate fraction and then the bacterial inhibition was seen. | Calipers | Diameter of the barrier zone (mm) | Ratio |

Source: Modified from research by Emilia (2023) and Marfuati et al. (2023).

## Data Collection Techniques

### 1. Tools and Materials

The tools used in this study include: analytical scales, ovens, micropipettes, *Blender*, erlenmeyer, beakers, measuring cups, test tubes, test tube racks, lumps and pestles, spatulas, porcelain cups, Buchner funnels, stirring rods, ose needles, petri dishes, syringes, cotton swabs,

incubators *Mac. Jar*, *laminair air flow,* The Bunsen, the Buns, *rotary evaporator,* scaled calipers, *Mc. Farland* 0,5, cookborer, dan *waterbath.* (Alouw et al., 2022)

The materials used in this study include: kersen leaves (*Muntingia calabura* L.) taken from the Kalijaga area, Harjamukti District, Cirebon City, aquadest, 96% ethanol, ethyl acetate, agar media, bacterial suspension (*Escherichia coli*), tablet Ciprofloxacin 500 mg, filter paper, label paper, aluminum foil, *Dimethyl Sulfoxide* (DMSO 10%,) Merck, *Nutrient Agar* (NA).(Alouw et al., 2022)

**Research Procedure**

**a. Plant Determination and Sample Preparation**

Plant determination was carried out at the Plant Taxonomy Research Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Semarang, to be tested and see the results of plant taxonomy. The results of the taxonomic test showed that the plant used was a kersen plant (*Muntingia calabura* L.)

Preparation of kersen leaf samples (*Muntingia calabura* L.*)* with Plant lifespan is at least 4 months. Then, separated from unnecessary parts such as stems and petioles and sorted. Then wash it thoroughly and dry it in the oven. The dried sample is then blended until a fine powder is obtained, then the weight is weighed.(Alouw et al., 2022)

**b. Sterilization Tools**

The tools used in the study were sterilized first. Sterilization is generally carried out by heating at high temperatures. Glass tools and media are sterilized in an autoclave at a temperature of 121$^{oC}$ at a pressure of 15 psi or about 1 ATM for 15-20 minutes. The ose needles were sterilized over the Bunsen fire.(Alouw et al., 2022)

**c. Manufacture of Kersen Leaf Ethanol Extract (Muntingia calabura L.)**

Cherry leaf extract (*Muntingia calabura* L.) is made using the maceration method with ethanol solvents. The 5 kg kersen leaves are cleaned of dirt, washed with running water until clean and dried in the sun for 48 hours. Once dry, the kersen leaves are mashed using a blender. (Alouw et al., 2022)

Kersen leaf simplicia powder (*Muntingia calabura* L.) as much as 200 grams are put into a container and then add solvent, then macerate with a ratio of sample weight to solvent which is 1:5. The solvent used is 96% ethanol as much as 1000 ml, then stirred using a stirring for 10 minutes until the solution is homogeneous and massed in a vessel then closed flat and soaked for 3x24 hours protected from sunlight while occasionally stirring. After being macerated for 3x24 hours, it is then filtered using filter paper, so that the filter results in the form of filtrate and residue will be obtained. Next, the residue is macerated back with 96% ethanol extract for 3 days. The filtrate resulting from maceration and remaceration is combined and evaporated with the aim of freeing from ethanol solvents and removing moisture content by using *Vacuum rotary evaporator* at 60$^{oC.}$ To obtain a thick extract, it is carried out *waterbath* with a temperature of 60OC. (Alouw et al., 2022; Mochammad Maulidie Alfiannor Saputera, Tio Widia Astuti Marpaung, 2019)

**d. Manufacture of Ethyl Acetate Fraction of Kersen Leaves (Muntingia calabura L.)**

20 grams of ethanol extract is dissolved with 200 ml of ethanol-water mixture solvent, then partitioned with 200 ml of ethyl acetate solvent, beaten in a separate funnel so that two layers are visible (the upper layer is the ethyl acetate fraction, while the lower layer is the insoluble ethyl acetate fraction). The two layers are separated. The bottom layer is added back to the ethyl acetate solvent. Then shake again until you get a clear top layer/ethyl acetate layer.(Anggraini et al., 2021; Suhaenah, 2021)

The ethyl acetate fraction is evaporated by *vacum rotary evaporator* So that a thick fraction of ethyl acetate is obtained. The insoluble fraction of ethyl acetate is collected and vaporized with *vacum rotary evaporator* to become a water fraction.(Mochammad Maulidie Alfiannor Saputera, Tio Widia Astuti Marpaung, 2019; Nuryah et al., 2019)

**Phytochemical Analysis**

Phytochemical analysis of the ethyl acetate fraction of kersen leaf (*Muntingia Calabura* L.) was carried out on several secondary metabolite compounds, namely: alkaloids, flavonoids, saponins, triterpenoids, steroids and tannins.

2. Alkaloid Test: 0.5 g sample plus 1 ml HCL 2 N plus 9 ml hot aquades for 2 minutes then cooled and filtered for filtrate, then the results are divided into 4 ; In tube 1 apply the Bauchardat reagent wait until the sediment changes color positive result if the sediment is brown or black. Tube 2 is given the Dragen drauf reagent, a positive result when the sediment turns white. Tube 3 plus the Hager reagent results positive when the sediment turns yellow. Tube 4 was given the Mayer reagent, Methanol, and the Bauchardat reagent was said to be positive when the sediment turned brown or black.
3. Saponin test: 0.5 g of sample plus 10 ml of hot water then wait for it to cool, then shake until foam appears, let it sit for 2 minutes and add one drop of HCL 2 N then shake again until a steady foam is formed for ±10 minutes.
4. Tannins Test: 1 g of sample plus 10 ml of hot water wait until it cools, then filter and the filtrate is dripped with $FeCl_3$ 1%, the result is positive if it changes color to dark blue or greenish-black.
5. Phenolic and Flavonoid Test: 0.5 g of sample plus 2 ml of Methanol wait for it to cool, then filter and then the filtrate results are divided into two parts of the tube, the first is given NaOH 10% positive results if it turns red, then in the second tube is given $H_{2SO4}$ concentrates, positive results if it turns red.
6. Triterpenoid and Steroid Test: 0.5 grams of sample plus 2 ml of ethanol wait for it to cool, then filter the filtrate results and add 3 drops of anhydrous acetic acid and 1 drop of $H_{2SO4}$ concentrate positive Triterpenoid results if they change color to red or purple, and positive steroid results if they change color to green.

**Data Analysis**

1. **Univariate Analysis**

The description of the results of the study was carried out testing mean, median, maximum data, minimum data.

**2. Bivariate Analysis**

Bivariate analysis to determine the relationship between the independent variable (the ethyl acetate fraction of kersen leaf (*Muntingia calbura* L.) with a concentration of 1%, 10%, 30%, 50%, 70%) and the bound variable (*growth of Escherichia coli* bacteria). This study used a sample of <50, so the normality test carried out was the *Shapiro-wilk test and used* the One-way Anova *parametric statistical test*. Therefore, the comparative hypothesis test of categorical (nominal) and numerical (ratio) data types was not paired and 2 groups used unpaired T-tests.

If the distribution of data obtained is abnormal, it is carried out with homogeneity tests obtained homogeneously. The next analysis was carried out with a non-parametric *test of Kruskal walls* and continued using *the Mann Whitney differential test.*

**Research Ethics**

This study used *Escherichia coli bacteria* from the Microbiology Laboratory of Stikes Muhammadiyah Cirebon as a test material. This research has received ethical approval from the Health Research Ethics Commission, Faculty of Medicine, Gunung Jati Independent University, Cirebon. With the ethical number 22/EC/FKUGJ/IV/2024.

**RESULT AND DISCUSSION**

**Results of phytochemical test analysis of ethyl acetate fraction of kersen leaf *(Muntingia calabura L.)***

The ethyl asctat fraction of kersen leaf (*Muntingia calabura* L.) was subjected to phytochemical tests to determine bioactive compounds including *alkaloids, tannins, steroids, triterpenoids, saponins, phenolics,* and *flavonoids.*

**Table 3. Phytochemical Test Results**

| Yes | Phytochemical Tests | Remarks | Results |
|---|---|---|---|
| 1 | Alkaloid | | |
| | a. Bauchardrauf | Brown-black deposits | - |
| | b. Dragendrauf | White deposits | - |
| | c. Hager | Yellow sediment | - |
| | d. Meyer, methanol, dan bauchardat | Brown-black deposits | - |
| 2 | Tanin | Dark blue or blackish-green color formed | + |
| 3 | Triterpenoid | Red or purple formation | - |
| 4 | Steroid | Formed green color | + |
| 5 | Saponin | A steady foam is formed over ± | + |
| 6 | Phenolic | Formed red color | + |
| 7 | Flavonoid | Formed red color | - |

Source: Results of phytochemical tests at the Research Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon (2024).

Based on table 3. it shows that the ethyl acetate fraction of kersen leaf (*Muntingia calabura* L.) contains secondary metabolite compounds, namely saponins, tannins, phenolics, and steroids.

**Growth Inhibition *of Eschericha coli***

Various concentrations of ethyl acetate fraction of kersen leaves (*Muntingia calabura* L.) were tested for resistance to *Escherichia coli* bacteria ATCC 25922 using a beam diffusion technique. Seven treatment groups were tested to inhibit bacterial growth.

**Table 4. Treatment results with 4 repetitions**

| Treatment | Repetition to | | | | Average (mm) | Category |
|---|---|---|---|---|---|---|
| | I (mm) | II (mm) | III (mm) | IV (mm) | | |
| Kontrol (+) | 42,8 | 44,1 | 42,6 | 42,8 | 43,1 | Very Powerful |
| Kontrol (-) | 0 | 0 | 0 | 0 | 0 | Weak |
| 1% | 3,4 | 3,5 | 3,9 | 4,4 | 3,8 | Weak |
| 10% | 10,4 | 11,0 | 9,9 | 10,8 | 10,5 | Strong |
| 30% | 13,8 | 12,5 | 14,0 | 12,5 | 13,2 | Strong |
| 50% | 21,5 | 19,0 | 18,0 | 20,0 | 19,6 | Strong |
| 70% | 27,1 | 26,5 | 26,6 | 27,7 | 26,9 | Very Powerful |

Source: Results of antibacterial activity tests at the Research Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon (2024).

Based on table 4, it shows that the results in the negative control, at 4 times of repetition, do not have an inhibition zone. Positive control was obtained, the average inhibition zone of 43.1mm was declared to be very strong resistance. At a concentration of 1%, an average barrier zone of 3.8 mm or weak resistance was obtained, a concentration of 10% on an average of 4 repetitions was obtained, an inhibition zone of 10.5 mm was declared strong, a concentration of 30% was obtained with an average of 13.2 mm of strong resistance, and a concentration of 50% obtained an average of 19.6 mm of strong resistance, and a concentration of 70% obtained an average of 26.9 mm of very strong resistance.

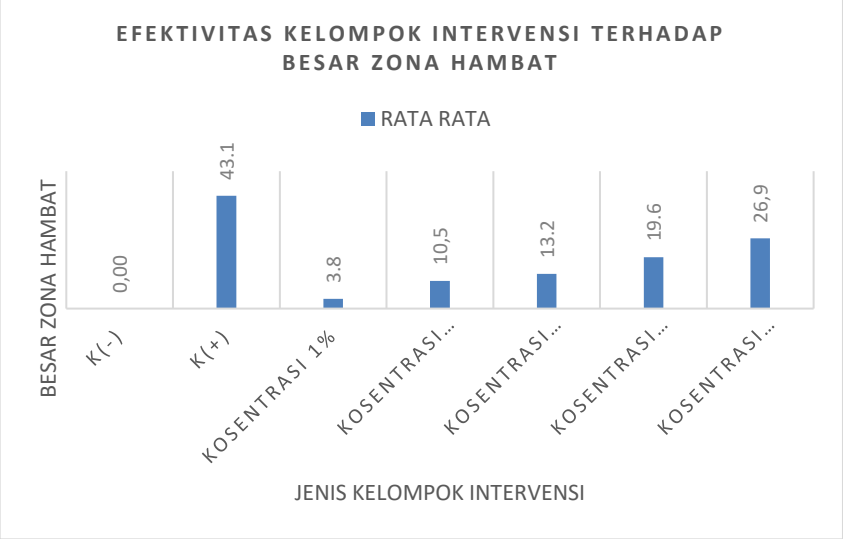


**Image 1. Inhibition Percentage**
Source: Primary data from research results on Escherichia coli at the Research Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon (2024).

Based on figure 6, it can be seen that the strongest inhibition response is found at a concentration of 70% because at this concentration there is an inhibition zone of 26.9 mm, while the weakest inhibition response is found at a concentration of 1% with an inhibition zone obtained of 3.8 mm.

**Data Analysis Results**

**1. Normality**

The normality test is carried out to assess the distribution of data on a data group or variable, normally distributed or not normally distributed. In this analysis, the *Shapiro-Wilk normality test* was used because the sample used was <50.

In this study, the results of the normalias test were obtained at concentrations of 1%, 10%, 30%, 50%, and 70% with (*p-value)* > *0.05* while in the control group (+) (*p-value)* < 0.05, the data was not distributed normally.

**2. Homogeneity Test**

The homogeneity test was carried out to see whether the groups had the same variation (homogeneous) or not before the hypothesis test was carried out. Based on the results of this study, sig. (*p-value)* of 0.025, it can be concluded that the data variant is not homogeneous because n sig. (*p-value*) <0.05.

**3. Uji Hypothesis**

Based on the normality test, the results of the data were not distributed normally, so it was followed by analysis using the non-parametric *test of Kruskal Walls.*

**Table 5. Uji Hypothesis**

| Subject | Mean | N | Say. |
|---|---|---|---|
| Control + | 26,50 | 4 | 0,00 |
| Kontrol - | 2,50 | 4 | |

| | | |
|---|---|---|
| Concentration 1% | 6,50 | 4 |
| Concentration 10% | 10,50 | 4 |
| Concentration 30% | 14,50 | 4 |
| Concentration 50% | 18,50 | 4 |
| Concentration 70% | 22,50 | 4 |

Source: Results of the Kruskal-Wallis test, Swadaya Gunung Jati University, Cirebon (2024).

In this study, it was found that the data group was significant because it had a (*P-value)* <0.05

**4. Differences Between Concentration Groups**

After the analysis of variance (*Kruskall Walls*) was carried out, a follow-up *Mann Withney* test was carried out which aimed to show significant differences between the two groups.

**Table 6. Differences Between Concentration Groups**

| Concentration | Concentration | P value |
|---|---|---|
| Control + | K- | 0,013 |
| | 1% | 0,019 |
| | 10% | 0,020 |
| | 30% | 0,020 |
| | 50% | 0,020 |
| | 70% | 0,019 |
| Kontrol - | K+ | 0,013 |
| | 1% | 0,013 |
| | 10% | 0,014 |
| | 30% | 0,014 |
| | 50% | 0,014 |
| | 70% | 0,013 |
| 1% | K+ | 0,012 |
| | K- | 0,013 |
| | 10% | 0,020 |
| | 30% | 0,020 |
| | 50% | 0,020 |
| | 70% | 0,019 |
| | | |
| 10% | K+ | 0,020 |
| | K- | 0,014 |
| | 1% | 0,020 |
| | 30% | 0,021 |
| | 50% | 0,021 |
| | 70% | 0,020 |
| 30% | K+ | 0,020 |
| | K- | 0,014 |
| | 1% | 0,020 |
| | 10% | 0,021 |
| | 50% | 0,021 |
| | 70% | 0,020 |
| | K+ | 0,020 |

| | | |
|---|---|---|
| | K- | 0,014 |
| | 1% | 0,020 |
| 50% | 10% | 0,021 |
| | 30% | 0,021 |
| | 70% | 0,020 |
| | K+ | 0,019 |
| | K- | 0,013 |
| | 1% | 0,019 |
| 70% | 10% | 0,020 |
| | 30% | 0,020 |
| | 50% | 0,020 |

Source: Results of the Mann-Whitney follow-up test based on primary research data from the Research Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon (2024).

Based on table 6 above, in each study of each type of group there was a significant difference *(p<0.05),* so that h0 was rejected.

In this study, after preparing kersen leaf (Muntingia calabura L.) extract, the next step was the fractionation process. The fractionation method used by the researcher was the liquid–liquid method. The fractionation process causes the compounds in the extract to bind to solvents corresponding to their polarity. The solvent used was ethyl acetate. Ethyl acetate is a semi-polar solvent that can attract components of active compounds that are both polar and non-polar, based on the principle of "like dissolves like". (41)

Based on the results of this study, it was found that the ethyl acetate fraction of kersen leaves (Muntingia calabura L.) has the ability to exert antibacterial activity. This was demonstrated at concentrations of 1%, 10%, 30%, 50%, and 70%, with mean inhibition zones of 3.8 mm, 10.5 mm, 13.2 mm, 19.6 mm, and 26.9 mm, respectively, indicating its potential as an antibacterial agent capable of inhibiting Escherichia coli bacteria. The higher the concentration, the stronger the inhibition.

Based on the research of Sri Marfuati et al., kersen leaf extract can inhibit the growth of Escherichia coli bacteria at concentrations of 1%, 2%, 4%, 8%, and 10%, with average inhibition zones of 0 mm, 0 mm, 4.37 mm, 4.87 mm, and 6 mm, respectively. In this study, the ethyl acetate fraction of kersen leaves had a larger average inhibition zone diameter than the kersen leaf extract. The large difference in the inhibition zone diameter between the antibacterial activity of the ethanol extract and the ethyl acetate fraction is due to secondary metabolites. Ethyl acetate is able to dissolve compounds with a range of polar and non-polar molarities, enabling it to extract secondary metabolites, such as alkaloids, flavonoids, steroids, triterpenoids, saponins, and phenolics, which are known to have antibacterial activity. Therefore, the ethyl acetate fraction is the most active, forming the largest and most potent inhibition zone compared to the extract. Ethanol extract still contains complex compounds, such as water, protein, fat, carbohydrates, fiber, calcium, phosphorus, iron, and vitamin C, which may cover and hinder the antibacterial activity of the ethanol extract, resulting in a smaller inhibition zone compared with the ethyl acetate fraction. (9

Escherichia coli is a short rod-shaped (coccobacillary), gram-negative bacterium. (40) Escherichia coli is a gram-negative bacillus of the family Enterobacteriaceae. It is a facultative

anaerobe and non-spore-forming. Gram-negative bacteria have a thinner peptidoglycan layer than gram-positive bacteria. The cell wall of members of the Enterobacteriaceae, including Escherichia coli, consists of an inner cytoplasmic membrane and an outer membrane containing lipopolysaccharides (LPS) and lipoproteins. LPS consists of lipid A, polysaccharides, and O antigens. The periplasm is the space between the inner cytoplasmic membrane and the outer membrane. This space contains peptidoglycan chains, which are abundant in gram-positive bacteria, whereas in gram-negative bacteria the peptidoglycan content is lower. Peptidoglycan is an essential component of the bacterial cell wall. This component protects the organism from osmotic pressure, determines cell shape, and is integrated with cell growth. This makes it a weak point in gram-negative bacteria that can be targeted by secondary metabolites. E. coli strains with K1 capsular polysaccharide antigens cause about 40% of cases of septicemia and 80% of cases of meningitis. (40)

Different strains of Escherichia coli are associated with a number of characteristic diarrheal diseases. Among them are enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), and Shiga toxin–producing Escherichia coli (STEC). From the STEC group, Escherichia coli O157:H7 is a prototype strain. Each class of Escherichia coli has different somatic (O) and flagellar (H) antigens and specific virulence characteristics. (40)

Saponins are classified as complex, polar glycoside compounds. The mechanism of action of saponins as antibacterial agents is through damage to the bacterial cell wall. Saponins interact with lipopolysaccharides in the bacterial cell wall, increasing cell wall permeability and decreasing the surface tension of the bacterial membrane, which leads to cell wall lysis. Lysis of the bacterial cell wall allows antibacterial substances to enter more easily and interfere with bacterial metabolism, eventually causing bacterial cell death. (41)

Tannins, as antibacterial agents, act by passing through the bacterial cell wall to the inner membrane and then disrupting cell metabolism, leading to bacterial cell death. Tannin activity proceeds rapidly in gram-positive bacteria, whereas in gram-negative bacteria the activity is slower due to the presence of a double-layered membrane. (42)

Steroids have the potential to act as antibacterial agents by causing leakage in bacterial structures, such as lysosomes, due to the sensitivity of the lipid membrane to steroid components. Steroids interact with the phospholipid membrane of bacterial cells. The phospholipid membrane is permeable to lipophilic compounds. Permeability is the property of a membrane that allows certain liquids or compounds to pass through it. This interaction compromises membrane integrity and alters the morphological components of the bacterial cell. As a result, the bacterial cells become fragile and undergo lysis. (40,41)

Phenolics are plant compounds characterized by aromatic rings containing at least one hydroxyl group. The mechanism of phenolic compounds as antibacterial agents involves inhibition of key enzymes in bacterial cells and disruption of cellular balance in the protoplasm, as well as protein precipitation, ultimately acting as toxins. (42)

In this study, ciprofloxacin was used as a positive control because it is a fluoroquinolone-class antibiotic with broad-spectrum activity that inhibits the growth of test bacteria. Ciprofloxacin acts against many different types of bacteria, including Escherichia coli. Ciprofloxacin acts against Escherichia coli by inhibiting the DNA gyrase enzyme, which

plays an important role in bacterial DNA replication and transcription. This enzyme separates and rearranges DNA double strands during genetic synthesis. Ciprofloxacin causes damage to the DNA strand by inhibiting DNA gyrase activity. In addition, ciprofloxacin forms a stable complex with the DNA double helix, preventing strand separation during replication or transcription. This damages the structure and function of genetic material in bacterial cells. Furthermore, ciprofloxacin can interfere with protein synthesis by inhibiting the activity of topoisomerase IV, another enzyme required by Escherichia coli to separate replicated chromosomes into two daughter chromosomes during cell division.

By stopping the activity of these enzymes, ciprofloxacin inhibits protein synthesis, which is an important part of bacterial survival and growth.(Shamsudin et al., 2022) From the activity of enzymes can strengthen the efficacy of drugs and can reduce the risk of resistance strongly. (Hadi & Permatasari, 2019) In positive control, an average inhibition zone of 43.1mm was obtained, which means that the resistance response is very strong. The purpose of the positive control test was to prove that Ciprofloxacin has good inhibition against bacteria *Escherichia coli.* In positive control, a higher inhibition zone was obtained compared to the fraction, this is because the antibiotic drug has gone through the trial stage first until it can work optimally before being marketed, while the fraction is still not tested so it is not optimal in inhibiting bacterial growth. In the results of this study, the antibacterial activity in the fraction had an inhibition against bacteria but was not better than the positive control.(Handoko, 2020)

This study used DMSO 10% as a negative control. DMSO is one of the solvents that has the ability to dissolve almost any compound, both polar and non-polar. Non-toxic, DMSO has analgesic and anti-inflammatory effects. In addition, these solvents are more environmentally friendly and safe for health (Jones et al., 2020). In negative control, no inhibition zone was found during four repetitions; this indicates that this DMSO does not have antibacterial properties and will have no impact on treatment.

## CONCLUSION

The ethyl acetate fraction of kersen leaf (Muntingia calabura L.) has an inhibition to the growth of Escherichia coli bacteria. The highest inhibition of the ethyl acetate fraction of kersen leaf (Muntingia calabura L.) against the growth of Escherichia coli was found at a concentration of 70%. Although these results show the potential of the ethyl acetate fraction of kersen leaves in inhibiting bacterial growth, further research is needed on the use of different concentrations to determine the extent of their effect on the inhibition. In addition, further research is also needed to test the inhibition of the ethyl acetate fraction of kersen leaf (Muntingia calabura L.) against bacteria other than Escherichia coli, in order to get a more comprehensive picture of the antimicrobial potential of this plant.

## REFERENCES

Alouw, G., Fatimawali, F., & Lebang, J. S. (2022). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kersen (Muntingia calabura L.) Terhadap Bakteri Staphylococcus aureus DAN Pseudomonas aeruginosa Dengan Metode Difusi Sumuran. *Jurnal Farmasi*

*Medica/Pharmacy Medical Journal (PMJ)*, *5*(1), 36. https://doi.org/10.35799/pmj.v5i1.41430

Anggraini, P. H., Septiarini, A. D., & Wardani, T. S. (2021). Uji Daya Hambat Ekstrak Dan Fraksi N-Hekasan, Fraksi Etil Asetat, Fraksi Air Daun Kersen (Muntingia Calabura L) Terhadap Bakteri Staphylococcus aureus ATCC 25923. *Duta Pharma Journal*, *1*(2), 8–19. https://doi.org/10.47701/djp.v1i2.1209

Bates, D. W. (2018). Big data in healthcare: Opportunities and challenges. *Health Affairs*, *37*(3), 368–374. https://doi.org/10.1377/hlthaff.2017.1157

Bohren, M. A. (2015). The mistreatment of women during childbirth in health facilities. *PLoS Medicine*, *12*(6), e1001847. https://doi.org/10.1371/journal.pmed.1001847

Campbell, S. M. (2016). Measuring quality of care in general practice. *BMJ Quality & Safety*, *25*(1), 5–7. https://doi.org/10.1136/bmjqs-2015-004141

Federer, L. (2018). Defining data librarianship: A survey of competencies. *Journal of EScience Librarianship*, *7*(2), e1126. https://doi.org/10.7191/jeslib.2018.1126

Figueroa, C. A. (2019). The use of telehealth during the COVID-19 pandemic. *Journal of Telemedicine and Telecare*, *25*(8), 455–462. https://doi.org/10.1177/1357633X19874949

Greenhalgh, T. (2017). Beyond adoption: A new framework for theorizing and evaluating digital health. *Journal of Medical Internet Research*, *19*(11), e367. https://doi.org/10.2196/jmir.8775

Hadi, K., & Permatasari, I. (2019). Uji Fitokimia Kersen (Muntingia calabura .L) Dan Pemanfaatannya Sebagai Alternatif Penyembuhan Luka. *Prosiding SainsTeKes Semnas MIPAKes UMRi*, *1*, 22–31.

Handoko, A. D. (2020). Uji Efektivitas Antibakteri Ekstrak Daun Kersen (Muntigia Calabura L.) Terhadap Bakteri Escherichia coli. *Repositori.Umsu.Ac.Id*, *10*(2), 1–51.

Jones, H. E., Calixte, C., Manze, M., Perlman, M., Rubin, S., Roberts, L., & Romeo, D. (2020). Primary care patients' preferences for reproductive health services. *Contraception*, *101*(4), 226–230. https://doi.org/10.1016/j.contraception.2019.12.003

Kruk, M. E. (2018). High-quality health systems in the Sustainable Development Goals era. *The Lancet Global Health*, *6*(11), e1196–e1252. https://doi.org/10.1016/S2214-109X(18)30386-3

Kruse, C. S. (2018). Health information technology and healthcare resource utilization. *JMIR Medical Informatics*, *6*(2), e34. https://doi.org/10.2196/medinform.8473

Kuuire, V., & Dassah, E. (2020). Place and Health. In *International Encyclopedia of Human Geography (Second Edition)*. https://doi.org/10.1016/B978-0-08-102295-5.10412-3

Marmot, M. (2020). COVID-19 and health inequalities. *Journal of Epidemiology & Community Health*, *74*(9), 681–682. https://doi.org/10.1136/jech-2020-214401

Mochammad Maulidie Alfiannor Saputera, Tio Widia Astuti Marpaung, N. A. (2019). Konsentrasi Hambat Minimum (Khm) Kadar Ekstrak Etanol Batang Bajakah Tampala (Spatholobus littoralis Hassk) Terhadap Bakteri Escherichia Coli Melalui Metode Sumuran. *Jurnal Ilmiah Manuntung*, *5*(2), 167–173.

Nuryah, A., Yuniarti, N., & Puspitasari, I. (2019). Prevalensi dan Evaluasi Kesesuaian

Penggunaan Antibiotik pada Pasien dengan Infeksi Methicillin Resistant Staphylococcus Aureus di RSUP Dr. Soeradji Tirtonegoro Klaten. *Majalah Farmaseutik*, *15*(2), 123. https://doi.org/10.22146/farmaseutik.v15i2.47911

Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Shah, S. A. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*, *27*(4). https://doi.org/10.3390/molecules27041149

Shanafelt, T. D. (2017). Physician burnout and healthcare system performance. *Mayo Clinic Proceedings*, *92*(1), 129–146. https://doi.org/10.1016/j.mayocp.2016.10.004

Suhaenah, A. (2021). Penetapan Kadar Flavonoid Fraksi Etil Asetat Daun Karet Kebo (Ficus elastica) Dengan Metode Spektrofotometri UV-VIS. *Modul Biokimia Materi Metabolisme Lemak, Daur Asam Sitrat, Fosforilasi Oksidatif Dan Jalur Pentosa Fosfat*, *13*(1), 6.

Tannenbaum, C. (2016). Why sex and gender matter in implementation research. *BMC Medical Research Methodology*, *16*, 145. https://doi.org/10.1186/s12874-016-0247-7

Tenopir, C., & Allard, S. (2014). Research data management services in academic research libraries. *Journal of Academic Librarianship*, *40*(1), 84–91. https://doi.org/10.1016/j.acalib.2013.11.003

Van Panhuis, W. G. (2014). A systematic review of barriers to data sharing in public health. *BMC Public Health*, *14*, 1144. https://doi.org/10.1186/1471-2458-14-1144

Xu, Z., Zhou, X., Kogut, A., & Clough, M. (2022). Effect of online research data management instruction on social science graduate students' RDM skills. *Library & Information Science Research*, *44*(4), 101190. https://doi.org/10.1016/j.lisr.2022.101190