The effect of kersen (*Muntingia calabura* L) leaf extract on bacteria *Aeromonas salmonicida smithia* in vitro

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Abstract. Kersen (*M. calabura* L) leaf can be used as a medicine because kersen leaf contains compounds flavonoids, saponins, polyphenols, and tannins. This study aims to determine the inhibitory power and the smallest concentration of a solution of kersen (*M. calabura* L) leaf extract to *A. salmonicida* in vitro. This research uses experimental methods and studied the activity of kersen leaf extract as an antibacterial to *A. salmonicida* with a comparison between treatment and control. The treatments were concentrations of kersen leaf extract of 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml, positive control (chloramphenicol) and negative control, dimethyl sulfoxide (DMSO 5%). The parameters were observed in this study the antibacterial activity of kersen leaf extract to *A. salmonicida*, and dilution of the concentration of the smallest kersen leaf extract can inhibit *A. salmonicida* in vitro. The results showed that kersen leaf extract had antibacterial power to *A. salmonicida* growth. Cassava leaf extract has the ability to inhibit the in vitro growth of *A. salmonicida* at the smallest concentration of 0.5 mg/ml with inhibiting zone of 10 mm Because at that concentration is more effective when compared with chloramphenicol.

1. Introduction

Activity aquaculture in practice, many are faced with several obstacles. One of the obstacles cause of failure is a disease fish farming. Various attempts have been made to overcome the problem of disease. Handling of started to create an optimal environment, quarantine, vaccination, disinfection plague, to the use of antibiotics [1, 2]. The use of antibiotics has a bad effect on the survival of the aquaculture organism that there is resistance in these organisms, as well as causing environmental pollution.

Need their substitutes to disease with natural alternatives is by using herbs or so-called itofarmaka [3, 4, 5, 6]. The use fitofarmaka or herbs as preventive and alternative medicine was already widely used. Fitofarmaka material is a potent plant for the treatment of disease. The advantage of using such fitofarmaka firstly, fitofarmaka be a natural material substitute for antibiotics to control diseases caused by bacteria. Second, fitofarmaka an environmentally friendly material, easily destroyed, and does not lead to residues in fish and humans. Third, fitofarmaka material readily available and quite a lot. Fifth, fitofarmaka economical price, and very cheap [2].
The success of aquaculture related to environmental preservation and aquaculture organism resistance to disease. Disease in fish cultivation is caused by fungi, parasites, viruses, and bacteria [7, 8]. One type of bacteria that needs serious response is *A. salmonicida*. *A. salmonicida* bersifatsangat pathogenic, causing disease furunculosis in fish accompanied dengan terjadinya ulcers and acute septicemia resulting in death [9]. Furunculosis is a disease that has characteristics that necrosis of muscle injuries, swelling under the skin layer with open sores filled with pus and damaged tissue in the wound like a basin [10]. *A. salmonicida* outbreaks have occurred in October of 1980, especially in West Java and the surrounding area. Losses incurred to approximately 4 billion rupiah [11].

Attacks *A. salmonicida* seen at the fish body resistance decreased due to stress caused by declining water quality and handling of the lack of proper feeding. *A. salmonicida* is the most important cause of disease in salmonid fish, also be pathogenic to non-salmonid fish such as goldfish, koi, catfish, catfish, and others [9]. *A. salmonicida* is an obligate pathogenic bacteria in fish that can be isolated from fish that are sick or healthy fish which acts as a carrier or carriers of disease. The bacteria can live several weeks outside the host, depending on the salinity, pH, temperature and water quality [12]. *A. salmonicida* is a gram-negative, non-motile, produce a positive reaction in the oxidase test, acidify glucose, and some isolates produce brown pigment [13]. Kersen (*M. calabura* L) is a plant that is often found on the side of the road, at the edge of the drainage and places less. Based on several studies; Kersen leaves can be used as a drug for Kersen leaf contains flavonoids, saponins, polyphenols, and tannins. Kersen leaf can is used as an antioxidant, antibacterial and anti-inflammatory.

The content of these compounds that make Kersen leaves have the potential antioxidant and antibacterial activity. This compound is obtained by extraction of ethanol [14]. According to [15], the levels of flavonoids contained in the leaves of Kersen that is equal to 0.66% of which has been tested by spectrophotometry.

2. Material and methods

2.1. Materials research

The materials will be used in this study is 96% ethanol, tripticase soya agar (TSA), paper discs (paper disc), distilled water, dimethyl sulfoxide (DMSO) 5%, and leaves of Kersen derived from Campus Area C of Universitas Airlangga, Surabaya.

2.2. Materials testing

The bacteria used are *A. Salmonicida* which is obtained Fish Quarantine of Quality Control and Safety of Fishery Class I Surabaya I.

2.3. Research methods

The research method that will be used the method of experiments conducted in vitro by measuring the inhibition zone large Kersen leaf extract that is around the paper disc, then comparing large inhibition zone that uses Kersen leaf extract with the amount of inhibition zone using chloramphenicol.

2.4. Manufacture simplicia

Samples Kersen leaf obtained from around the back of the campus C UNAIR. Kersen leaf is used which grew up and had not yellowed and is 3-5 on the leaf petiole. Kersen leaves that have been selected according to the criteria of then cut as small as possible and then dried using an oven with a temperature of 450°C for 24 hours. Kersen leaf that has been in the oven and then pulverized using a juicer. Kersen leaf that has been mashed weighed as much as 150 grams for the preparation of the next process is extraction.
2.5. Extract kersen leaf
The method used in kersen leaf extract that is by maceration method. Maceration mechanism that is soaking Kersen leaf powder were weighed and then soaked with 96% ethanol solvent. Kersen leaf that has been dried and ground to a powder weighed 150 grams and inserted into the tube Erlenmeyer 1 L which was then added 96% ethanol solvent as much as 600 ml (dry kersen leaf: ethanol 96% at 1: 4). For 1 × 24 hours at room temperature of 25°C. Kersen leaves that have been added solvent 96% ethanol, and then homogenized using a shaker with a speed of 250 rpm (revolution per minute). The resulting mixture of the two materials is a liquid, then filtered and transferred into the bottle. Furthermore, the mixture of leaves of kersen and 96% ethanol solvent is evaporated using a vacuum rotary evaporator at 40°C and produces a kersen leaf extract. Extraction results in paste form that is ready for use in the next process are dilution.

2.6. Sterilization
Sterilization is any process that kills all microorganisms. The equipment used in the study were sterilized using an autoclave at 121°C and high pressure 15 psi (pounds per square inch) for 15 minutes in order to be free of contamination. Sterilization aims to kill all life forms of microorganisms and to eliminate contamination by microorganisms both living and dead [16].

2.7. Kultur Aeromonas salmonicida
Pure culture of A. salmonicida used KIPM obtained from the laboratory of Class I Surabaya I, in the subculture by taking a pure culture of A. salmonicida ose being planted into the media TSA and then incubated at 28°C for 24 hours. This is done aseptically in order to avoid contamination of bacteria to be cultured. The bacteria were incubated for 24 hours, then the identification and biochemical tests.

2.8. Identification bacteria and biochemical test
Identification bacteria is an effort to be able to determine the type of bacteria found in pure cultures of bacteria. Bacterial identification method that is often done conventionally [17]. Identification of the bacteria begins with the individual microscopic morphological observation, then observation of the morphology of bacterial colonies.

Morphology bacteria have two properties namely individual morphology which include size, Bentu, a series of cells, the presence or absence of flagella, the number of flagella, the presence or absence of spores, the spores position and whether or not there is a capsule. Colony morphology which includes the shape, size, texture, and color of the colonies [18]. Forms of bacterial colonies such as roots, dots, round, irregular, stringy, and others. The colors on the bacterial colony is also an assortment of some white, milky white, light gray, pale, yellow, white, transparent, translucent white, light yellow, ocher, yellow, shiny, pale white, yellowish-white and orange [19]. A. salmonicida bacterial colonies are white, small, round and convex [20]. These bacteria biochemical properties have positive and ferment glucose oxidase [11].

According to [18] Identification of bacteria selanjunya ie, the biochemical tests carried out by pure isolates taken using a needle ose who have been sterilized before use a bunsen flame; then pure isolates were grown in several biochemical test media. The results of the biochemical test can be observed based on changes that occur in A. salmonicida media. Hasil biochemical tests, according to [21] can be seen in Table 4.1 below.

| No. | Parameter biochemistry test | Biochemistry test |
|-----|-----------------------------|-------------------|
| 1.  | Gram                        | -                 |
| 2.  | Motile                      | -                 |
| 3.  | Catalase                    | +                 |
| 4.  | Oxidase                     | +                 |
5. Glucose +
6. O / F F
7. H2S -
8. Sucrose -
9. Lactose -
10. Maltose +
11. Simon Citrate +
12. MR +
13. VP -
14. Indole -
15. Nitrate Reduction +
16. Gelatin +

Information: (+) = Positive, (-) = Negative, (F) = fermentative

2.9. Setup bacteria suspension
Manufacture the suspension is done by taking 4-5 colonies of bacteria that were incubated for 24 hours using a needle ose and put into a test tube containing 10 ml physiological saline solution and then it is homogenized using a vortex. Furthermore, the seed was standardized by using Mc. Farland is by way of equated with the turbidity standard solution Mc. Farland no. 0.5. Turbidity of a bacterial suspension will be adjusted to the density of bacteria at concentrations of 106 CFU/ml. Use a concentration of 106 CFU/ml is virulent bacteria to fish.

2.10. Implementation research
Preparation of solutions leaf extract kersen with different concentrations method used is the disk diffusion method. Make a concentration dilution increments. Things to do in making the concentration must first set up a test tube that has been sterilized as many as six pieces are then each tube labeled 1-7 corresponding concentration used. The treatment given is to kersen leaf extract concentration of 2 mg/2 ml, 1 mg/ml, 0.5 mg/mL, 0.25 mg/ml, and 0.125 mg/ml, the negative control using DMSO 5% and positive control using chloramphenicol 30 mg. Various concentrations of the kersen leaf extract, which is then compared with the antibiotic chloramphenicol.

Manufacture solution to the tube to 1 with a concentration of 2 mg/2 ml solution that is done mixing kersen leaf extract as much as 2 mg added to the tube and 5% DMSO 2 ml. Tubes concentrations were mixed with the second material is stirred by using a vortex until homogeneous. Tubes to 2 that a concentration of 1 mg/ml carried out mixing the same by taking 1 ml from tube one and add 1 ml DMSO 5%, then in the vortex until homogeneous. The solution is then performed the same thing by taking 1 ml of solution from the previous test tube and add 5% DMSO 1 ml. After dilution to tube to 5 with final concentration is 0.125 mg/ml. The solution concentration of 0.125 mg/ml, 1 ml was taken and discarded because it was unused. Tubes to 6 containing 5% DMSO in 1 ml of solution is used as a negative control and a positive control using chloramphenicol 30 mg.

Treatments will used in this study are as follows:
Tube 1 (2 mg / 2 ml): 2 mg Kersen leaf extract solution 2 ml DMSO + 5%
Tube2 (1 mg / ml): 1 ml of Kersen leaf extract of tube 1 + 1 ml DMSO 5%
Tube3 (0.5 mg / ml): 1 ml of Kersen leaf extract of tube 2 + 1 ml DMSO 5%
Tube4 (0.25 mg / ml): 1 ml of Kersen leaf extract of tube 3 + 1 ml DMSO 5%
Tube5 (0.125 mg / ml): 1 ml of Kersen leaf extract of tube 4 + 1 ml DMSO 5%
Tubes 6 : 1 ml DMSO 5% as negative control (-)
Tube7 : chloramphenicol as a positive control (+)

After the process of dilution terraced Kersen leaf extract, and then do the immersion process paper disc into each Kersen leaf extract concentration. Paper discs soaked for ± 10 minutes to infuse. Paper
discs that have been soaked, taken aseptically with tweezers, then wind-dried at a temperature of space. Paper discs have dried, then transferred into a petri dish that already contains TSA media that have been planted \( A. \text{salmonicida} \). Immersion paper disc was also performed on a 5% DMSO chloramphenicol as a negative control and a positive control which is then transferred into a petri dish containing TSA media that have been planted \( A. \text{salmonicida} \). Media TSA has given paper disc soaked Kersen leaf extracts with varying concentrations and negative and positive controls, then incubated at 30 °C for 24 hours.

2.11. Determination zoneresistor
Determination inhibition zone is done by observing the clear zone on the colony. \( A. \text{salmonicida} \) that has been given the paper discs containing Kersen leaf extract solution. The greater the inhibition zone, the greater the ability to Kersen leaf extract inhibits the growth of \( A. \text{salmonicida} \). The inhibition zone formed then measured using a ruler (mm).

2.12. Parameter research
The parameters were observed in the study; this is the Kersen leaf extracts for antibacterial activity against \( A. \text{salmonicida} \) in vitro. Results of Kersen leaf extract can inhibit \( A. \text{salmonicida} \) and diluting the concentration of the smallest Kersen leaf extract can inhibit \( A. \text{salmonicida} \). Interpretation of bacterial growth inhibition area refers to a common standard drug of plant origin that is the diameter of the inhibition zone measuring 10-20 mm [12].

2.13. Data analysis
Data results from the study will be presented in the form of data tables (Descriptive) and images to get an overview of research data, make it easier to read and understand [22].

3. Results and discussion
Identification bacteria were performed to ensure the correctness of the identity of \( A. \text{Salonica} \) obtained from Fish Quarantine Laboratory Quality Control and Safety of Fishery Class I Surabaya I. The method used for bacterial identification is by observation of bacterial colony morphology and microscopic observations using Gram staining and biochemical tests of \( A. \text{salonicida} \). Based on the Gram stain test, observations, and test colony morphology and properties of \( A. \text{salonicida} \) biokimiadari pure culture, bacterial identification results obtained can be seen in Table 2 below.

| No. | Character         | Result         |
|-----|------------------|----------------|
| 1.  | Color            | White          |
| 2.  | Edge             | flat           |
| 3.  | Elevation        | Convex         |
| test Gram |                |                |
| 4.  | Form             | rod            |
| 5.  | Color / Gram     | Pink           |
| Biochemistry test |        |                |
| 6.  | Motile           | -              |
| 7.  | Growing at 37 °C | -              |
| 9.  | O / F            | F              |
| 10. | arginine hydrolysis | +            |
| 11. | lysine decarboxylase | -          |
| 12. | ornithine dekardoksilase | -      |
| 13. | Simon citrate    | -              |
14. H2S - 15. Urea - 16. Indole - 17. Vp / MR + 18. gelatin hydrolase + Test Sugars 19. Arabinose + 20. Glucose + 21. Inositol - 22. Mannitol + 23. sucrose - 24. CBB-A blue

Result bacterial identification Table 2 above shows that the bacteria in accordance with the characteristics of *A. salmonicida*. *A. salmonicida* are facultative anaerobic bacteria that can live in the presence of oxygen or no oxygen. *A. salmonicida* subsp. salmonicida, chromogens, masoucida, and smithies can grow at a temperature of 20-37°C [11].

Research This method is by paper disc diffusion. Kersen leaf extract has activity in inhibiting the growth of *A. salmonicida*. Inhibition of *A. salmonicida* can be seen by measuring the inhibition zone around the paper discs contained. Presence clear zone is an area around the paper disc diffusion in influencing the growth of bacteria. Antibacterial strength can be determined by measuring the diameter of inhibition zone formed by extracts were tested [23]. How to measure the inhibition zone is done by measuring the straight central inhibitory zone or measured from the right edge to the left edge of the clear zone is formed. Results of inhibition zone of Kersen leaf extract solution and chloramphenicol as a comparison can be seen in Table 3 below.

| Concentration extract leaf Kersen | Diameter inhibition zone (mm) Average | A. salmonicida |
|----------------------------------|-------------------------------------|----------------|
| 2 mg / 2 ml                       | 14.6                                |                |
| 1 mg / ml                         | 12                                  |                |
| 0.5 mg / ml                       | 11.3                                |                |
| 0.25 mg / ml                      | 9                                   |                |
| 0.125 mg / ml                     | 7                                   |                |
| Control (-) 5% DMSO               | -                                   |                |
| Control (+) Chloramphenicol       | 15                                  |                |

Table 3 shows that from any concentration used in the test, bacteria are a wide range of inhibition zone size. The results can be seen from the size of the inhibition zone formed around the paper disc. Kersen leaf extract can be said to be resistant in inhibiting the *A. salmonicida*. Inhibition zone formed with various concentrations can be compared with the positive control, namely chloramphenicol. Every aquaculture activities would certainly there are obstacles that can lead to reduced productivity in a business. The main cause of failure of the production of farmed fish is usually caused by the presence of pests and diseases that attack in a container of fish farming. Because the sick fish will not experience optimal weight gain, and this is very detrimental to the farmers.

Cultivation fish to avoid pests and diseases of fish in a container cultivation then prior to farming activities should be carried out treatment on the container to be used as cleaning container cultivation, use of water both in quality and quantity, the equipment will be used for farming activities have been disinfected, do not ill keep fish healthy fish simultaneously, immediately discard fish that are sick. If the fish has been attacked by pests and diseases of fish, then steps must be done is to the treatment of...
diseased fish. DiseaseFish is a result of the interaction of three components: the environment, the fish itself, and the agent that causes the disease farmed fish becomes ill and can cause death. This fish disease can be caused by various things in between fish diseases caused by viruses, bacteria, fungi, parasites and food.

Based on the results obtained, it is known that the Kersen leaf extract has antibacterial activity marked by a clear zone on the determination of the diameter of the inhibition zone. Test concentrations used in this study that is with a concentration of 2 mg / 2 ml, 1 mg / ml, 0.5 mg / mL, 0.25 mg / ml, and 0.125 mg / ml. Selection of the concentrations used in this study is based on previous research and also based on the literature that says that the extract is said to be potentially as antimicrobial if the administration level ≤ 1000 pg/ml can inhibit the growth of antimicrobial [24].

ControlNegative used was 5% DMSO. The negative control using DMSO 5% in some bacteria showed little clear zone on the test inhibitory zone diameter. It is caused by a paper disc soaked with a 5% DMSO at planting on inhibition zone diameter test has not yet dried, causing the apparent zone diameter of inhibition zone test. According to [25], DMSO has antibacterial activity at concentrations above 5%. So in this study formed an apparent zone diameter of Kersen leaf extract are added to the solvent DMSO 5% is considered not affect the appearance of the bacterial inhibition zone test and is considered the diameter of the clear zone at 5% DMSO does not exist.

Controlpositive were used in this study was chloramphenicol at a concentration of 30 ug. Chloramphenicol can work in inhibiting bacterial protein synthesis in cells [25]. Selection of the positive control with the use of chloramphenicol in this study because in aquaculture are still many farmers who are still using these antibiotics. It is known that the use of chloramphenicol is very dangerous to human life. These antibiotics can cause cancer, liver damage, kidney damage, and anemia and interfere with metabolic processes. Chloramphenicol antibiotic residues there in products and consumed by humans continuously then over time, the residue is left in the body and may result in aplastic anemia [27].

Results research showed that the Kersen leaf extract has antibacterial activity against A. salmonicida is indicated by a clear zone at a concentration of 2 mg / 2 ml with a diameter of 14.6 mm, 1 mg / ml with a diameter of 12 mm, 0.5 mg / ml with a diameter of 11.3 mm, 0.25 mg / ml with a diameter of 9 mm, and 0.125 mg / ml with a diameter of 7 mm. Results from the five concentration was tested on A. salmonicida showed that the smallest concentration of Kersen leaf extract solution is shown at a concentration of 0.5 mg/ml with a large zone of inhibition of 11.3 mm, compared with inhibition zone formed of chloramphenicol at 15 mm. Interpretation of bacterial growth inhibition area refers to a common standard drugs of plant origin that is the diameter of inhibition zone measuring 10-20 mm [28]. Kersen leaf extract at concentrations of 0, 5 mg / ml expected to be more effective when used as a drug, because the small concentration of A. salmonicida was able to inhibit in vitro. According to [29], drug therapy is beneficial, namely the provision of adequate concentration and not excessive as a critical condition in the treatment. It was concluded from this study Kersen leaf extract with a concentration of 0.5 mg / ml have been able to inhibit bacterial growth.

ExtractKersen leaf active as an antibacterial due to the chemical components contained in the extract. Kersen leaf extract containing flavonoids, tannins, and saponins are thought to have an activity potentially. Antibacterial. This is consistent with the literature that states that the chemical compounds are potentially antibacterial flavonoids, saponins, tannins, glycosides, phenols, and steroids [30].

4. Conclusion

Based on the results of analysis and discussion in this study, can be summed up as follows: 1) kersen leaf extract has antibacterial activity against the growth of A. salmonicida and 2) kersen leaf extract has the ability to inhibit the growth of A. salmonicida in vitro in the smallest concentration of 0.5 mg/ml with an inhibition zone of 11.3 mm because at these concentrations will be effective when compared with chloramphenicol.
5. References

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