Antibacterial activity of bitter gourd (*Momordica charantia* L.) leaf extract against *Aeromonas hydrophila*

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Abstract. Bacterial resistance against antibiotic in the field of fisheries is mainly caused by the wide usage of antibiotics over the last decade, allowing the bacteria to mutate to adapt against the antibiotic. The most common bacteria that infected freshwater fish is *Aeromonas hydrophila*, which is now resistant to most antibiotics. Bacterial resistance against antibiotics is a problem that can be handled by phytopharmacology, one of them is the bitter gourd (*Momordica charantia* L.) leaf which has antibacterial properties. The aim of this study was to find out the antibacterial activity of the bitter gourd (*Momordica charantia* L.). The parameters observed were antibacterial activity determined from the inhibition zone around the paper disc and the minimal concentration required which produced the biggest inhibition zone. The minimal concentration of bitter gourd (*Momordica charantia* L.) leaf extract that produced the biggest inhibition zone against *Aeromonas hydrophila* based on the CLSI M-45 category was 5 mg/ml with an inhibition zone diameter of 12.3 mm. This is categorized into the intermediate category. The intermediate category indicates that bitter gourd (*Momordica charantia* L.) leaf extracts with the concentrations used in this study wasn’t effective enough to be used as an antibacterial substance.

1. Introduction

Bacterial resistance to antibiotics is a health problem that has spread throughout the world [1,2]. The main causes of bacterial resistance to antibiotics is extensive antibiotic use over the past century, resulting in the bacteria mutating to adapt to the antibiotics [2]. Bacterial infection in freshwater fish is one of the biggest threats to the quality of freshwater fish farming [3,4,5]. Bacterial infections that most commonly attack freshwater fish are *Aeromonas hydrophila*, causing Motile *Aeromonas Septicemia* (MAS), Motile *Aeromonad Infection* (MAI), Hemorrhagic Septicemia, Red pest, and Red-sore (Cannas et al. 1998). This problem is compounded by the antibiotic resistance that occurs in *A. hydrophila* [6].

Bacterial resistance due to antibiotic use can be handled by phytopharmacology use [5,7,8]. One of the plants that can be used as a phytopharmacology is *Momordica charantia* L. This plant has antibacterial properties possessed by the leaf extract [9]. These antibacterial properties are derived from the 32% alkaloid compounds, 22% flavonoids, 1.37 mg / 100gr tannin, 1.6% terpenoids and 5.2% saponins contained in the leaves of *Momordica charantia* L. [10, 11]. The antibacterial activity of the compounds contained in the bitter melon leaf extract (*Momordica charantia* L.) can be used as a reference in the development of the treatment of diseases caused by *A. hydrophila*. 
2. Materials and methods

2.1. Momordica charantia leaves
The materials used included Momordica charantia leaves obtained from Kediri regency, East Java and A. hydrophila obtained from the BKIPM Class I Surabaya I collection. Other materials used included 96% ethanol (polar), Dimethyl Sulfoxide (DMSO) 10%, distilled water, whisman paper no. 93, Mueller Hinton Agar (MHA) media, Tripticase Soya Agar (TSA) media, Tripticase Soya Broth (TSB), physiological NaCl, methylated paper disc blanks, and Gentamycin 10 µg.

2.2. Antibacterial activity
This study used an experimental method that was carried out in vitro in order to test the antibacterial activity of bitter melon leaf extracts against A. hydrophila by comparing the inhibitory zones formed with a control. The concentrations of the Momordica charantia leaf extract used in this study were P1 (20 mg / ml); P2 (10 mg / ml); P3 (5 mg / ml); P4 (2.5 mg / ml); P5 (1.25 mg / ml) and P6 (0.625 mg / ml). The negative control in this study was 10% DMSO while Gentamycin 10 g was the positive control. According to CLSI (2015), A. hydrophila is sensitive to Gentamycin (goals, Aminoglycosides) with a resulting inhibition zone of >15 mm. These results were categorized into sensitive categories.

The first stage in this study was the sterilization process and the manufacture of the media. The sterilization process was carried out using the method of steam, a chemical solution, dry heating or the gas method. The media used included TSA, MHA and TSB, and the dose for making the media was 40 g TSA in 1 L distilled water, 38 g MHA in 1 L distilled water and 30 g TSB in 1 L distilled water [14].

2.3. Extraction process
The extraction process of the bitter melon leaves is that the flour is sifted with 96% ethanol with a ratio of 1: 3 (w / v), or it is perfectly submerged for 72 hours and the maceration results are concentrated using a rotary evaporator. They are then put in an oven at 50°C until the extraction results are semi-solid [15]. Furthermore, the bitter melon extract was tested qualitatively for phytochemical compounds. This was to prove the presence of antibacterial contents including alkaloids, flavonoids, terpenoid and saponins. The alkaloid compounds were tested using the Wagner reagent and the flavonoid compounds were tested using the alkaline reagents [13]. The testing of terpenoids was done using the Salkowki test and the testing of the saponin compounds was done using the foam test [13,14], whereas the tannin compounds were tested using the Ferric chloride reagent (FeCta) [12].

The density of the bacteria in this study was 108 CFU / ml, which was compared to the Mc Farland standard number 0.5 (1.5 x 10^8 CFU / ml). While making the concentration of bitter melon leaf extract using serial dilution with 10% DMSO solvent, the obtained dilution concentration was 20 mg / ml; 10 mg / ml; 5 mg / ml; 2.5 mg / ml; 1.25 mg / ml and 0.625 mg / ml.

The antibacterial activity test was done using a paper disc blank with a diameter of 6 mm. The Blank disc paper was inserted into test tubes P1 - P6 and soaked for 24 hours [11]. Inoculation was done using the spread method, adding 100 ml of A. hydrophila suspension to the MHA media and this was flattened using a cotton swab [8, 11]. The paper discs that already contained the extract of bitter melon were placed on the inoculated media, and then stored in a refrigerator for 2 hours, which was intended to promote the presence of the extract on the media. The results of the inoculation were incubated at 28°C for 24 hours [11].

2.4. Analysis data
The results of the research data have been presented in the form of tables, graphs and descriptive explanations. The descriptive analysis was performed to describe the diameter of the inhibitory zone formed and to compare it with the control treatment and antibiotic sensitivity standards. The diameter of the inhibitory zone was grouped into sensitive, intermediate or resistant.
3. Result and discussion

The qualitative tests of the phytochemicals from the bitter melon were obtained alkaloids (+), flavonoids (+), tannin (+), terpenoids (-), and saponins (+). These results showed that the bitter melon leaf extract in this study contained alkaloid, flavonoid, tannin and saponin compounds, while the terpenoid test results were negative.

The average inhibitory zone from the results of antibacterial activity test of the bitter melon leaf extract against *A. hydrophila* was interpreted into several categories, namely resistance and intermediate, in accordance with the CLSI-45 standards (CLSI, 2015). The CLSI-45 standard used was the interpretation of the gentamycin 10 µg inhibition zone against *A. hydrophila*. The results of the interpretation average diameter inhibition zone of the Gentamycin 10 µg can be seen in Table 1.

| Treatment | Average (mm) | Resistance (x <12 mm) | Intermediate (12mm < x <15mm) | Sensitive (x >15mm) |
|-----------|--------------|------------------------|-------------------------------|-------------------|
| P1        | 13.6         | Y                      |                               |                   |
| P2        | 12           | Y                      |                               |                   |
| P3        | 12.3         | Y                      |                               |                   |
| P4        | 11.3         | Y                      |                               |                   |
| P5        | 7.6          | Y                      |                               |                   |
| P6        | 6.3          | Y                      |                               |                   |
| K -       | 6            | Y                      |                               |                   |
| K +       | 16.6         |                        |                               | Y                 |

The concentration of the bitter melon leaf extract in treatment P1 (20 mg / ml), P2 (10 mg / ml), and P3 (5 mg / ml) obtained an average inhibition zone diameter of 13.6 mm; 12 mm and 12.3 mm. The three treatments showed the presence of antibacterial activity that was considered to be in the intermediate category. The intermediate category indicates that the treatment can be more effective if the dose used is higher, so then the antibacterial activity in a sensitive category can be obtained [17].

The concentration of the leaf extract showed as being in the category of resistance in the P4 treatment (2.5 mg / ml), P5 (1.25 mg / ml), and P6 (0.625 mg / ml), as well as a negative control (DMSO 10%). The resistant category showed that the bacterial isolates were not clinically inhibited by the antibacterial concentration used [17].

The use of antibacterials in medicine must meet certain principles such as the use of drugs in general, namely the exact diagnosis, the right medicine, the right dosage form, the right dose and it being delivered on time. The antibacterial use of bacteria with an insensitive sensitivity can cause cure bacterial infections and increases the risk of antibacterial resistance [18].

Based on the test data, the antibacterial activity of the bitter melon extract against *A. hydrophila* was determined. The minimum concentration of bitter melon leaf extract which produces the largest inhibitory zone against *A. hydrophila* was the P3 treatment, which was 5 mg / ml which produced an average inhibition zone with a diameter of 12.3 mm. This antibacterial activity was produced through the mechanism of action of all phytochemical compounds contained in the pare leaves, including alkaloids, flavonoids, tannins, and saponins [13,15]. The mechanism of action of alkaloids in inhibiting the growth of *A. hydrophila* is to inhibit the nucleic acid synthesis together as well as inhibiting the dihydrofolate reductase enzyme [19]. The mechanism of action of flavonoids in inhibiting the growth of *A. hydrophila* is focused on inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and inhibiting the energy metabolism of the bacteria [20]. Alkaloids and flavonoids work synergistically in inhibiting the growth of *A. hydrophila*, in which the two compounds have almost the same mechanism and work objectives, resulting in more optimal results when the two compounds are used together compared to when they are given separately [21].
The mechanism of action of tannin in relation to inhibiting the growth of *A. hydrophila* is focused on inhibiting the extracellular enzymes released by microbes, taking over the substrate needed for microbial growth, or through direct effects on the microbial metabolism through the inhibition of oxidation phosphorylation [22]. The mechanism of action of the saponins in inhibiting the growth of *A. hydrophila* causes a leakage of proteins and some enzymes from the bacterial cells [23]. Tannin and saponins work additively in inhibiting the growth of *A. hydrophila*, which involve two different mechanisms and work goals. The effect when the two compounds are used together compared to when they are given separately is the same [24].

4. Conclusion

Based on the results of the study, it can be concluded that the bitter melon leaf extract (*Momordica charantia L.*) has antibacterial activity against *A. hydrophila* with the category of resistance being in intermediate. The minimum concentration which produced the largest inhibitory zone was produced by a concentration of 5 mg / ml, which produced an inhibitory zone with a diameter of 12.3 mm, which is an antibacterial in the intermediate category.

5. References

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