ANTIBACTERIAL ACTIVITY OF *Gynura procumbens* LEAF EXTRACT AGAINST *Pseudomonas aeruginosa* IN VITRO

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Abstract

Intensification of fish farming has caused various impacts, for example diseases in fish. One of the diseases in fish is a bacterial disease. The use of natural compounds as an antimicrobial agent, which is relatively safe and effective, is a strategy to treat the disease. The aim of this study was to evaluate the antibacterial activity of *Gynura procumbens* leaf extract against *Pseudomonas aeruginosa*. Antibacterial activity test was done by using paper disc diffusion. The variations on the test paper disc method used were based on the concentration difference of *Gynura procumbens* leaf extract such as: A (250 ppm), B (500 ppm), C (750 ppm), D (1,000 ppm) dan E (1,250 ppm). The results showed *Gynura procumbens* leaf extract produced antibacterial compounds which inhibited the growth of the bacteria. Effective antibacterial activity was detected in concentration of 1,250 ppm with inhibition zone diameter of 11.01 ± 0.59 mm. It can be concluded that *Gynura procumbens* leaf extract may contribute in controlling the spread of bacterial diseases in fish farming, particularly caused by *Pseudomonas aeruginosa*.

Keywords: Antibacterial activity, *Gynura procumbens*, and *Pseudomonas aeruginosa*

INTRODUCTION

The intensification of fish farming has caused various diseases that have resulted in decreased production [1]. One of the diseases that attack cultivated fish is a bacterial disease. In intensive fish farming, where the fish are often under stressful conditions, bacterial disease will occur and result in serious economic losses. Pathogenic bacteria that often attack freshwater fish is *Pseudomonas aeruginosa*.

*Pseudomonas aeruginosa* is a Gram negative rod-shaped bacterium, moves with flagella and is aerobic [2]. The impact of the intensive use of antibacterial agents worldwide for prophylactic and therapeutic purposes has led to an increase in bacterial resistance. Pathogenic bacteria often develop resistance to drugs, if exposed to long-term antibacterial drugs and this makes the infection more difficult to treat [3,4]. Therefore, an alternative method is needed to control pathogenic bacteria in fish culture systems. The strategy that can be done is to utilize natural materials as biological control agents for fish diseases. One of the substances that can control bacterial disease is *Gynura procumbens* extract.

Phytochemical analysis conducted by Kaewseejan [5] revealed that the contents of *G. procumbens* extract are chlorophyll-a, b and carotenoids in ethanolic extract. Moreover, the ethanolic *G. procumbens* extract showed the presence of alkaloids and volatile oils. Haron and Jusoh [6] found the acidic extract of *G. procumbens* has positive reaction towards *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The aim of this study was to evaluate the antibacterial activity of *G. procumbens* leaf extract against *P. aeruginosa*.

METHOD

**Extraction of G. procumbens**

*Gynura procumbens* leaf samples were collected from local markets located in the Malang, East Java, Indonesia. The extraction method uses the extraction method from Wang [7], with minor modifications. 500 g dry sample of *G. procumbens* leaves was macerated into 1,500 ml of 96% ethanol for 24 hours. Furthermore, the extract sample was cooled and deposited at room temperature for 36 hours, then filtered using filter paper. Extract samples that have been filtered are then evaporate using a rotary evaporator at a
temperature of 50 °C, which removed the ethanol and left an aqueous solution containing ethanol-soluble precipitate. The extracts can be stored at 20 °C for further use.

**Bacterial test strain and growth conditions**

For study, *P. aeruginosa* was used and obtained from the Biology Service Unit, Faculty of Science and Technology, Airlangga University, Surabaya.

According to Ulloa-Urizar [9], the cultivation medium was trypticase soy agar (trypticase soy broth) (Oxoid). Cultures were grown for 24 h at 32 °C. For antibacterial activity assay, the colonies were inoculated in 3 mL of trypticase soy broth and incubated without agitation for 24 h at 32 °C. The cultures were later diluted with fresh medium to approximate the density of 0.5 McFarland standard, which represented an estimated concentration of $10^7$ CFU/mL. The McFarland standard was prepared by inoculating colonies of the bacterial test strain in sterile saline and adjusting the cell density to the concentration specified before.

**Disc Diffusion Test**

Disc diffusion method was done according to Haron and Jusoh [6]. The blank discs (6 mm in diameter) were immersed with different concentration of *G. procumbens* extracts, namely 250 ppm, 500 ppm, 750 ppm, 1,000 ppm and 1,250 ppm and were left dried. After the discs were completely dried, they were placed over trypticase soy agar poured into the plates. It had been spread with the microorganisms by using sterile forceps before. The plates were incubated at 32 °C for 24. The diameter of inhibition zone around each discs were then measured by using a millimeter ruler.

**RESULT AND DISCUSSION**

The antibacterial activity of *G. procumbens* extract can be observed by measuring the diameter of the growth inhibition zone in *P. aeruginosa*. The results are shown in the following figure.

![Figure 1. Inhibition Zone Diameter of G. procumbens extract](image)

The graph above present the area of inhibition zone diameter (mm) at different concentrations of *P. aeruginosa* bacteria. Figure 1 above shows that with increasing the concentration of *G. procumbens* extract, the area of the inhibition zone diameter also increases. Furthermore, it shows that *G. procumbens* extract has antibacterial compounds that can inhibit bacterial growth, where the E concentration (1,250 ppm) has an inhibition zone diameter of $11.01 \pm 0.59$ mm.

Haron and Jusoh [6] found the acidic extract of *G. procumbens* has positive reaction towards *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Moreover, Methanol extract of *G. procumbens* showed antimicrobial activity against *Staphylococcus aureus* and methicillin-resistant *S. Aureus* [9]. Also, Nawi et al [10] found the methanol extract of *G. procumbens* leaves showed antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* compared to hexane extract.

It may be caused by active compounds in the crude extract which can possess their antimicrobial properties in a sufficient amount of concentration [11]. Phytochemical analysis conducted by Kaewseejan, et al [5] revealed that the contents of *G. procumbens* extract are chlorophyll-a, b and carotenoids in ethanolic extract. Moreover, the ethanolic *G. procumbens* extract showed the presence of alkaloids and volatile oils.

**CONCLUSION**

This present study can be concluded that *G. procumbens* extract has potential of antimicrobial effects proven in *G. procumbens*
extract concentration of 1.250 ppm with inhibition zone diameter of 11.01 ± 0.59 mm.

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REFERENCES


