Evaluation of inhibitory potential of mangrove leaves extract *Avicennia marina* for bacteria causing *ice-ice* diseases in seaweed *Kappaphycus alvarezii*

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**Abstract.** Today, the problem of seaweed is the emergence of ice-ice disease, which causes economic losses. The solutions offered at this time are not significant, so there is a need for technology that can control the ice-ice disease. The effort is to use mangrove leaves to extract *Avicennia marina*. This study aimed to evaluate the inhibitory potential of mangrove leaves extract *A. marina* against bacteria that cause ice-ice disease in seaweed. Four pathogenic bacteria that cause ice-ice in seaweed, namely *Stenotrophomonas maltophilia* strain IAM 123, *Shewanella haliotis* strain DW01, *Vibrio alginolyticus* strain ATCC 17749 and *Pseudomonas aeruginosa* strain SNP0614 were tested using *A. marina* leaves extract with methanol solvent, the method used is the maceration method and antibacterial activity test using the *Kirby-Bauer* method with seawater complex (SWC) agar. The results showed phytochemical compounds found in *A. marina* leaves extract, namely alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. *A. marina* leaves extract using methanol solvent shows potential as an antimicrobial which is indicated by inhibition zones in each of the bacteria that cause ice-ice.

1. Introduction

*Avicennia marina* is one of the mangrove plants that live in coastal areas, is widely distributed, and able to live in a wide range of salinity. Halidah and Kama [1], *A. marina* can grow on coarse sand substrate, fine or deep mud.

The benefits of mangrove leaves are widely used for various treatments, especially against pathogenic bacteria. Rahman et al. [2] reported that the fermented liquid from mangrove leaves was able to inhibit the bacteria that cause ice-ice in seaweed. Wibowo et al. that the active compounds identified from *A. marina* are antibiotics or antimicrobial. Anam et al. [3], mangrove leaves extract has anticancer and antiviral activity, seeds have antimalarial activity, while the stem and fruit are used as a treatment for rheumatism, skin diseases, and inflammation.
A. marina leaves have various contents, namely endophytic bacteria, which can inhibit the bacteria that cause ice-ice in seaweed [4] and phytochemical content [3,5,6]. Ho et al. [7] that phytochemical compounds can play a key role in the antioxidant activity of medicinal plants. This potential can be used by the pharmaceutical, medical, and health food industries. Abeysinghe and Wanigatunge [8] and Abeysinghe [9] reported mangrove leaves and bark containing alkaloids, steroids, triterpenoids, and flavonoids.

The compounds found in A. marina leaves have antimicrobial effects, so they can be used to overcome problems in seaweed. The problem in seaweed is the emergence of ice-ice disease caused by several factors, one of which is a bacterial disease factor. Losses caused by ice-ice disease, namely seaweed production, decreased around 24-60%, and economically, the farmers suffered losses [10]. The spread of ice-ice infection can be vertical (from seedlings) or horizontally through the mediation of water. The infection of this disease is very fast, ranging from two to three days and causes seaweed death.

This study aims to evaluate the inhibition of mangrove leaves extracts A. marina against bacteria that cause ice-ice disease in seaweed. This research is expected to be the right solution to overcoming the problem of seaweed disease.

2. Material and Method
The study was conducted from September to December 2017. A. marina leaves sampling in Bojo Village, Mallusetasi District, Barru Regency, South Sulawesi Province (â 4.095795 °, and E 119.612332 °). The leaves extraction process is carried out at the Fish Health Laboratory, Department of Aquaculture, and the Inter-University Center for Food and Nutrition, Bogor Agricultural University. Phytochemical analysis was conducted at the Laboratory of the Spice and Medicinal Plants Research Institute, Agricultural Research and Development Agency, Ministry of Agriculture. The study was conducted in two stages; namely, A. marina leaves extract and tests the antibacterial activity of the extract.

Mangrove leaves extraction A. marina using methanol; at this stage, the leaves are washed, then sorted and dried at room temperature. The extraction process is done by maceration. This method is done by weighing a dry sample of 300 grams, then blending until smooth. The crushed sample was then soaked using a 1:3 ratio of methanol and allowed to stand for 24 hours at room temperature. Then filtered using Whatman filter paper No. 4, then evaporated using a vacuum rotary evaporator until extracts are obtained in powder form.

The phytochemical extract qualitative analysis included alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, glycosides, and tested the antibacterial activity of the extract against bacteria that cause ice-ice. The test bacteria used were bacteria that cause ice-ice isolated by Achmad et al. [11], among others were Stenotrophomonas maltophilia strain IAM 123, Shewanella haliotis strain DW01, Vibrio alginolyticus strain ATCC 17749 and Pseudomonas aeruginosa strain SNP0614 with each concentration of 10^6 CFU/mL, scattering amount of 100 µL. This antibacterial activity test uses seawater complex (SWC)-agar media so that by the Kirby-Bauer method [12], 6 mm disc paper is dripped with extraction solution with a concentration of 15 µL; the test concentration used refers to Syafitri et al. (2017) is 2.5 mg / mL, 5 mg / mL, 10 mg / mL, 15 mg / mL and control without using extraction. It is incubated for 24 hours at 28°C. The diameter of inhibition was measured and analyzed.

3. Results and Discussion
Mangrove leaves A. marina has several antibacterial substances that can suppress pathogenic bacteria. Phytochemical tests were carried out to determine secondary metabolite compounds found in A. marina leaves extract.

| Table 1. Phytochemical compounds of mangrove leave A. marina |
|-------------------------------------------------------------|
| Compound | Results |
| Qualitative | Quantitative |

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Based on phytochemical tests, several compounds can inhibit pathogenic bacteria. *A. marina* leaves extract with polar methanol solvent produces active compounds, namely alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides (Table 1). The results obtained are in line with Thatoi et al. [5], bioactive compounds detected in mangrove leaves Avicennia to include alkaloids, flavonoids, phenols, saponins, tannins, glycosides and terpenoids, Anam et al. [3], reported *A. marina* containing flavonoids, tannins, quinones, saponins, and steroids, whereas Danata and Yamindago [6], phytochemicals found in *A. marina* extracts, namely alkaloids, flavonoids, and saponins from both locations. However, the content of Terpenoid and Tannin compounds is only found at stations with certain conditions.

The active ingredient which is owned by plants generally has many roles as antimicrobial compounds. Alkaloids have the potential as an antibacterial because they can damage cell walls by inhibiting cell wall synthesis, which will cause lysis so that cells will die [13]. The well-known function of saponin is as a bactericide, fungicide, amubasida, and insect eradication, anesthetic agents or sedatives and as an anxiety reliever (antianxiety), while madekasosida compounds can stimulate collagen production [14], while the mechanism of action of saponins as an antibacterial includes inhibition of bacterial colonization, decreased surface tension in the extracellular medium, or by lyzing bacterial cell membranes [15]. Other bioactive are flavonoids, a group included in the most phenol compounds in nature. Flavonoids in plants have functioned as color pigments, pathological functions, pharmacological activation, and flavonoids in food [14], and flavonoids are antibacterial polyphenol compounds, tannins are thought to bind to bacterial cell walls so they will activate the ability to attach to bacteria and inhibit bacterial growth. [16], tannins are also capable of contracting bacterial cell walls so that they disrupt cell membrane permeability by forming tannin complexes with bacterial enzymes and substrates, causing cells to not be able to carry out living activities [13,17]. Phenolic compounds have antibacterial activity [18], triterpenoid compounds are also known to have high antioxidant activity [19], whereas glycoside compounds are antibiotic aminoglycoside groups, these compounds diffuse in bacterial cell walls in aerobic conditions [20].

The results of the inhibition zone testing of *A. marina* extract against bacteria that cause ice-ice are shown in Table 2. The inhibition zone test was carried out to see the ability of compounds present in *A. marina* leaves extract to inhibit or kill pathogenic bacteria.

**Table 2. Inhibition activity of *A. marina* leaves extract against bacteria causing ice-ice disease**

| Isolate | Extract concentration (mg/mL) |
|---------|-----------------------------|
|         | 2.5 | 5  | 10 | 15 | Control |
| Alkaloid| +   |    |    |    | --      |
| Saponin | +   |    | 0.32% |   | --      |
| Tannin  | +   |    | 0.23% |   | --      |
| Phenolic| +   |    | --  |   | --      |
| Flavonoid| + |    | 0.0034% | | --      |
| Triterpenoids| + |    | --  |   | --      |
| Steroids| -   |    | --  |   | --      |
| Glycoside| +  |    | --  |   | --      |

Notes: (+): Existed, (-): Not existed, (--): Not analyzed
Concentration. The effectiveness of activity is influenced by vesves; ves compound ve, while compouds.

Phytochemical analysis results showed that mangrove leaves contained potential as an antimicrobial because it phytochemical tests, namely alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. However, all test bacteria showed a sensitivity to the extract, so the activity of extracts is influenced by the content of active compounds that have been identified in A. marina leaves extract using methanol as solvent by Saravanan and Radhakrishnan [21], showed the best antimicrobial activity against Staphylococcus aureus and Pseudomonas aeruginosa. Danata and Yamindago [6], A. marina mangrove leaves extract were able to inhibit the growth of S. aureus and V. alginolyticus, with clear zones of 4.43-5.79 mm and 4.25-5.48 mm, respectively. This inhibition zone value is different from the one obtained. This is presumed by differences in strains of pathogens, especially V. alginolyticus, while S. aureus is a Gram-positive bacterium that has a higher sensitivity than Gram-negative bacteria and a difference in the content of antibacterial compounds obtained, both qualitatively and quantitatively. The extract was tested to have the best antagonistic activity against S. haliotis strain DW01; this indicates that S. haliotis strain DW01 was more sensitive than S. maltophilia strain IAM 123, V. alginolyticus strain ATCC 17749 and P. aeruginosa strain SNP0614. The biggest inhibition occurred in the pathogenic S. haliotis strain DW01, which was 15.55 ± 1.10 at the highest concentration of 15 mg/mL, while the lowest was on the V. alginolyticus strain ATCC 17749 and P. aeruginosa strain SNP0614. Variance analysis results showed a significant difference (P>0.05) between S. maltophilia IAM 123 isolates, S. haliotis strain DW01, V. alginolyticus strain ATCC 17749 and P. aeruginosa strain SNP0614. The inhibitory ability of extracts is influenced by the content of the active compounds that have been identified in phytochemical tests, namely alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. However, all test bacteria showed a sensitivity to the extract, so the active compound contained in the extract was potential as an antimicrobial because it can kill or inhibit the growth of pathogenic bacteria. The amount of inhibition zone is influenced by the level of bacterial sensitivity, the concentration of antibacterial compounds. Fardiaz et al. [22], antimicrobial compounds at certain concentrations can inhibit growth and kill microbes, and no one antimicrobial compound is ideal for all purposes because there are differences in the sensitivity of microbial cells to antimicrobial compounds. Furthermore, Pelezar and Chan [18], the size of inhibitory zone activity is influenced by antibacterial activity, a small inhibitory zone shows low antibacterial activity, whereas a large inhibitory zone shows high antibacterial activity.

4. Conclusion
Phytochemical analysis results showed that mangrove leaves extract A. marina contained alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. A. marina extract using

| Species                        | Concentration (mm) | Concentration (mm) | Concentration (mm) | Concentration (mm) | Concentration (mm) |
|-------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Stenotrophomonas maltophilia  | 8.70±0.21          | 8.95±0.21          | 9.40±0.34          | 11.60±1.98         | 0.00±0.00          |
| strain IAM 123                |                    |                    |                    |                    |                    |
| Shevanella haliotis strain    | 9.00±1.02          | 12.20±0.21         | 14.85±1.58         | 15.55±1.10         | 0.00±0.00          |
| DW01                          |                    |                    |                    |                    |                    |
| Vibrio alginolyticus strain   | 8.10±0.22          | 8.75±0.59          | 8.90±0.22          | 9.10±0.14          | 0.00±0.00          |
| ATCC 17749                    |                    |                    |                    |                    |                    |
| Pseudomonas aeruginosa strain | 8.05±0.11          | 8.15±0.22          | 8.70±0.33          | 8.80±0.33          | 0.00±0.00          |
| SNP0614                       |                    |                    |                    |                    |                    |

Note: Different superscript in same column and bar showed significantly different result (P<0.05)
methanol as a solvent showed potential as an antimicrobial as indicated by inhibition zones in each of the bacteria that cause ice-ice.

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