Investigation of potential aquatic plants as herbal biomedicine to treat fish diseases

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Abstract. Fish disease caused by bacteria is a big problem in the aquaculture industry. Solutions for handling this disease have been done a lot, one of which is the use of plants as herbal biomedicine. The purpose of this study was to find potential aquatic plants in the prevention of bacterial fish diseases. Aquatic plants have more advantages including being able to absorb contaminants in the waters and provide additional oxygen to the waters. The research was carried out in-vitro tested on 22 species of aquatic plants from South Sulawesi Indonesia with concentrations of 0.1 gr and 0.2 gr of ethanol extract. The fish disease bacteria tested ie: Aeromonas hydrophila, Flexibacter columnaris, Streptococcus agalactiae, Edwardsiella ictaluri. The results showed: 22 aquatic plant species tested, 8 of them have the potential to control fish diseases, they are Pleocnemia sp, Phymatosorus scolopendria (Burm.f.) Pic.Serm, Selaginella plana (Desv. Ex Poir) Hieron, Humata repens (L.f.) J. Small ex Diers Syn, Donax canniformis (G. Forst.) K. Schum, Hymenachne amplexicaulis (Rudge) News, Ludwigia adscendens (L.) H. Hara, Eclipta prostrata (L.) L. Two species i.e Selaginella plana (Desv. Ex Poir) Hieron and Eclipta prostrata (L.) L., are capable of inhibited the growth 3 type of bacterial fish disease tested.

1. Introduction
The biggest issue in aquaculture cultivation is the diseases caused by microorganism. Eventually, these microorganisms are becoming more resistant to conventional treatment due to the excessive use of anti-bacterial and anti-fungi drugs. People have been using different ways to treat fish diseases, i.e., by using antibiotics or herbal medicines [1–3]. In a related study, Citarasu [4] asserts that herbal products possess beneficial characteristics, including their ability to increase growth and provide supplements to boost the immune system, they are also known to increase appetite, as well as possessing antimicrobial and anti-stress properties, which are beneficial in plant cultivation without any environmental or dangerous side effects. Compounds contained within the plants such as phenolic, polyphenol, alkaloid, quinone, terpenoid, lectin and polypeptide have been known as the effective alternatives to inhibit the growth of microorganism’s pathogens without causing resistance effect in bacteria as in antibiotic use [5].
A number of herbal plants such as *Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens*, and *Zingiber officinalis*, *Cynodon dactylon*, *Beal aegle marmelos*, *Winter cherry withania somnifera* and *Ginger (Zingiber officinale)* have been tested for their efficacy in treating fish diseases [6,7]. Other potential plants that may be used in treating fish diseases are aquatic plants. Several previous studies have explored the potential of aquatic plants as herbal medicine to treat fish diseases as discussed in the studies by [8–11]. The use of aquatic plants in fish cultivation is an environmentally friendly alternative that balances the need to have a sound ecosystem and preventing fish diseases. In this regard, the use of aquatic plants may provide an environmentally friendly aquatic solution; moreover, it does not compete with human needs for herbal medicine with the added bonus of promoting a sustainable fish health management. Aquatic vegetation is quite beneficial since they are able to absorb ammonia, as well as heavy metal contaminants and other pollutants in the water. The sites where fish lay their eggs will provide oxygen to the surrounding aquatic environments and remove allelochemical compounds such as alkaloids, phenols, flavonoids, anthraquinone and others that ultimately will contribute to the health of aquatic fauna [12].

In the current study, we are searching for potential herbal medicine candidates from aquatic plants that can be used to treat fish diseases. The sheer number of aquatic plants and their potential in aquaculture that have not been identified has persuaded us to carry out this study. An aquatic plant can coexist quite well alongside fish and other aquatic fauna; therefore, using aquatic plant as herbal medicine for treating fish diseases will bring an additional benefit in the form of a symbiotic mutualism between the aquatic flora and fauna in a healthy aquatic ecosystem that is free from contamination.

2. Material and Method

2.1. Aquatic Plant Material

The aquatic plants from the region of South Sulawesi were collected in October 2018. They were identified according to the standard botanical techniques for species identification at the Research Centre of the Indonesian Institute of Science (LIPI) in Cibinong, West Java. The fresh samples were washed using tap water to remove any impurities on their surface and later dried at room temperature. The dry samples were pulverized into powder using a blender. Analysis of the antibacterial fish pathogen was carried out in the laboratory Installation of the Agency for Research and Control of Fish diseases (BRPBATTP) Ministry Marine and Fisheries Republic Indonesia.

| No | Species name                                      | Family name   |
|----|--------------------------------------------------|---------------|
| 1  | Schismatoglottis calyptrata (Roxb.) Zoll & Moritzi | Araceae       |
| 2  | Pleocnemia, sp                                   | Tectariaceae  |
| 3  | Elatostema, sp                                   | Urticaceae    |
| 4  | Phymatosorus scolopendria (Burm.f.) Pic.Serm     | Polypodiaceae |
| 5  | Davallia repens (L.) Kuhn                        | Davalliaceae  |
| 6  | Stenosemia aurita (Sw.) C.Presl                  | Tectariaceae  |
| 7  | Hemigraphis reptans (G.Forst.) T. Anderson ex Hemsl| Acanthaceae   |
| 8  | Vesicularia montagnei (Schimp.) Broth            | Hypnaceae     |
| 9  | Selaginella plana (Desv. Ex Poir) Hieron Daun   | Selaginellaceae|
| 10 | Elatostema integrifolium (D.Don) Wedd            | Urticaceae    |
| 11 | Humata repens (L.f.) J. Small ex Diels Syn.      | Davalliaceae  |
| 12 | Ischaemum timorense Kunth                        | Commelinaceae |
| 13 | Donax canniiformis (G.Forst.) K. Schum           | Marantaceae   |
| 14 | Amphineuron, sp                                  | Thelypteridae |
| 15 | Hymenachne amplexicaulis (Ridge) News             | Poaceae       |
| 16 | Ludwigia adscendens (L.) H. Hara                 | Onagraceae    |
17. Limnocharis flava (L.) Buchenau
   - Alismataceae
18. Eclipta prostrata (L.) L.
   - Compositae
19. Adiantum diaphanum Blume
   - Pteridaceae
20. Ceratophyllum demersum L.
   - Ceratophyllaceae
21. Azolla, sp
   - Salviniaceae
22. Staurogyne elongata Kuntze
   - Acanthaceae

2.2. Preparation of Plant Extracts
Aquatic plants samples were extracted according to the procedures described by Indonesian Materia Medika Departemen Kesehatan Republik Indonesia (1995) and Harborne, (1998). The dried powder sample (1 g) was diluted in 10 mL ethanol 70% and extracted using a sonicator for an hour. Then, the filtrate and the residue were separated using 10 µm funnel and 58x58 cm width.

2.3. Antibacterial test with paper disc diffusion method
The bacteria strains were obtained from the Agency for Research and Control of Fish Diseases in West Java. The bacteria were cultivated on a growth medium so they will multiply before test. First, *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *Flexibacter columnaris* were cultivated on Trypton Soy Broth (TSB) media. Afterward they were incubated for 24 hours at 28°C with the exception of Edwardsiella ictaluri, which was incubated for 48 hours. Meanwhile, *Streptococcus agalactiae* was cultivated on Brain Heart Infusion (BHI) then incubated for 48 hours at 28°C (the growth period of both bacteria is 48 hours). They were later harvested and diluted with 5 mL sterile saline buffer solution with a cell density of 107 CFU/mL (Mac Farland standard).

Antibacterial susceptibility tests were carried out and diameter of the antibacterial inhibitory zone was determined in accordance with the Bauer method [13]. The number of colonies was calculated using [14]. Antimicrobial agent diffuses into the agar and inhibits the growth of the tested microorganism, the diameter of inhibition growth zones was measured in millimeters (mm).

Table 2. Result of the bacteria sensitivity test with regard to the tested aquatic plants

| No. | Specie name | Part of plant | Aeromonas Hydrophilla | Flexibacter columnaris | Streptococcus agalactiae | Edwardsiella ictaluri |
|-----|-------------|---------------|-----------------------|------------------------|--------------------------|-----------------------|
| 1.  | Schismatoglottis calyptrate (Roxb.) Zoll & Moritzi | Herbs         | -                     | -                      | -                        | -                     |
| 2.  | Pleocnemia, sp | Herbs         | -                     | +                      | -                        | -                     |
| 3   | Elatostema, sp | Herbs         | -                     | -                      | -                        | -                     |
| 4   | Phymatosorus scolopendria (Burm.f.) Pic.Serm | Herbs         | -                     | -                      | +                        | +                     |
| 5   | Davallia repens (L.f.) Kuhn | Herbs         | -                     | -                      | -                        | -                     |
| 6   | Stenosemia aurita (Sw.) C.Presl | Herbs         | -                     | -                      | -                        | -                     |
| 7   | Hemigraphis reptans (G.Forst.) T. Anderson ex Hemsl | Herbs         | -                     | -                      | -                        | -                     |
| 8   | Vesicularia montagnei (Schimp.) Broth | Herbs         | -                     | -                      | -                        | -                     |
| 9   | Selaginella plana (Desv. Ex Poir) Hieron | Leaf, Herbs, Root and steam | -                     | +                      | +                        | +                     |
3. Results and Discussion

3.1. Testing the anti-bacterial activity of aquatic plants from Sulawesi against fish diseases

Anti-bacterial test in-vitro method using the paper discs diffusion method is based on the principle that microbes will diffuse into the growth medium, whereas the extract of aquatic plant will inhibit the bacterial growth. Observations were made by examining whether each of the aquatic plant extract will form an inhibition zone around the fish diseases pathogenic bacteria. Next, we will measure the diameter of the inhibition zone formed.

### Table 3. Diameter of the inhibition zone formed from the extract aquatic plants on Aeromonas hydrophila.

| No  | Species name                        | Average zone inhibition measurement (mm) 0.1 gr | Average zone inhibition measurement (mm) 0.2 gr |
|-----|-------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1   | Ludwigia adscendens (L.) H. Hara    | 4.85                                          | 6.15                                          |
| 2   | Eclipta prostrata (L.) L.           | 3.15                                          | 4.55                                          |
| 3   | Donax canniformis (G. Forst.) K. Schum | -                                             | 2.4                                           |

### Table 4. Diameter of the inhibition zone formed from the extract aquatic plants on Flexibacter columnaris.

| No  | Species name                        | Average zone inhibition measurement (mm) 0.1 gr | Average zone inhibition measurement (mm) 0.2 gr |
|-----|-------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1   | Pleocnemia sp                       | -                                             | 1                                             |
| 2   | Selaginella plana (Desv. ex Poir) Hieron (leaf) | 1.2                                          | 2.95                                          |
| 3   | Ludwigia adscendens (L.) H. Hara    | 5.05                                          | 5.75                                          |

### Table 5. Diameter of the inhibition zone formed from the extract aquatic plants on Streptococcus agalactiae.

| No  | Species name                        | Average zone inhibition measurement (mm) 0.1 gr | Average zone inhibition measurement (mm) 0.2 gr |
|-----|-------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1   | Phymatosorus scolopendria (Burm.f.) Pic.Serm | 4.35                                          | 5.3                                           |
| 2   | Selaginella plana (Desv. Ex Poir) Hieron (Leaf) | 2.5                                          | 4.35                                          |
|     | Selaginella plana (Desv. Ex Poir) Hieron (Root and steam) | 4.65                                          | 5                                             |
Table 6. Diameter of the inhibition zone formed from the extract aquatic plants on *Edwardsiella ictaluri*.

| No | Spesies name | Average zone inhibition measurement (mm) 0.1 gr | Average zone inhibition measurement (mm) 0.2 gr |
|----|--------------|---------------------------------------------|---------------------------------------------|
| 1. | Phymatosorus scolopendria (Burm.f.) Pic.Serm | - | 1.55 |
| 2. | Selaginella plana (Desv. Ex Poir) Hieron (Leaf) | 0.6 | 3.7 |
| 3. | Selaginella plana (Desv. Ex Poir) Hieron (Root and steam) | 2.25 | 3.25 |
| 4. | Selaginella plana (Desv. Ex Poir) Hieron (Herba) | 1.15 | 2.15 |
| 5. | Eclipta prostrata (L.) L. | 2.85 | 4.85 |

Total 22 aquatic plant species tested, 8 of them have the potential to control fish diseases, they are *Pleocnemia* sp, *Phymatosorus scolopendria* (Burm.f.) Pic.Serm, *Selaginella plana* (Desv. Ex Poir) Hieron, *Humata repens* (L.f.) J. Small ex Diels Syn, *Donax canniformis* (G. Forst.) K. Schum, *Hymenachne amplexicaulis* (Rudge) News, *Ludwigia adscendens* (L.) H. Hara, *Eclipta prostrata* (L.) L.

We tested the *Selaginella plana* (Desv. Ex Poir) Hieron species by dissecting it into three parts, i.e. the herbs, stem + root and leaves. The leaves of *Selaginella plana* (Desv. Ex Poir) Hieron potentially has the ability to inhibit three pathogenic diseases, i.e. *Flexibacter columnaris*, *Streptococcus agalactiae*, and *Edwardsiella ictaluri*. Section of the herb (the entire body of *Selaginella plana* (Desv. Ex Poir) Hieron) are able to inhibit two pathogens, i.e. *Streptococcus agalactiae* and *Edwardsiella ichtalurie* [10].

There are Three potential species capable of inhibiting diseases caused by *Aeromonas hydrophila*, *Donax canniformis* (G. Forst.) K. Schum, all are potentially able to inhibit *Aeromonas hydrophyla* at a dose of 0.2 gr extract. *Ludwigia adscendens* (L.) H. Hara has a bigger potential in controlling *Aeromonas hydrophyla* with a moderate inhibiting power. *Eclipta prostrata* (L.) L is also able to inhibit the growth of *Aeromonas hydrophyla* (moderately), however, its potential may increase in line with the increased dose of the extract provided (Table 3).

The tested aquatic plant that are able to inhibit the growth of *Flexibacter columnaris* are *Pleocnemia* sp, *Selaginella plana* (Desv.ex Poir) (leaves), and *Ludwigia adscendens* (L.) H. Hara. The inhibition power of *Ludwigia adscendens* (L.) H. Hara is considered moderate at a dose between 0.1 gr to 0.2 gr. The remaining three species have a weak inhibiting power up to a dose of 0.2 gr concentrate extract.

In the test to measure the power to inhibit the growth of *Streptococcus agalactiae* bacteria, we uncover five potential aquatic plants (Table 5). *Phymatosorus scolopendria* (Burm.f.) Pic.Serm species, root section of *Selaginella plana* (Desv. Ex Poir) Hieron, and *Eclipta prostrata* (L.) L. has a moderate inhibiting power at a dose up to 0.2 gr concentrate extract. Other species with a weak inhibiting power include sections of the leaves and herb of *Selaginella plana* (Desv. Ex Poir) Hieron, *Humata repens* (L.f.) J. Small ex Diels Syn, *Hymenachne amplexicaulis* (Rudge) Nees (Table 5).

When testing the effectiveness of aquatic plants in the prevention of *Edwardsiella ictaluri*, we uncover three aquatic species that are capable to inhibit the growth of *E. ichtaluri*, i.e.,...
Phymatosorus scolopendria (Burm.f.) Pic.Serm, Eclipta prostrata (L.) L., and Selaginella plana (Desv. Ex Poir) Hieron (herbs, root + stem and leaves), with a weak inhibiting power at a dose up to 0.2 gr of extract (Table 6). The leaves section of Selaginella plana (Desv. Ex Poir) Hieron inhibits the growth of three bacteria causing fish diseases, i.e. Flexibacter columnaris, Streptococcus agalactiae, and Edwardsiella ictaluri. Moreover, Eclipta prostrata (L.) L and Selaginella plana also inhibits the growth of three fish diseases pathogens, i.e. Aeromonas hydrophila, Streptococcus agalactiae, and Edwardsiella ictaluri (Table 2). [10] assert that Selaginella plana (Desv. Ex Poir) Hieron positively contains Flavonoid, Phenol, Saponin, and Glycosides. Ethyl acetate, ethanol extracts and water extract of Eclipta prostrata (L.) L are capable to inhibit the growth and act as an antibacterial agent against Salmonella typhi [15]. In traditional medicine Eclipta prostrata (L) very famous as traditional medicines, in a screening of antibacterial and antioxidant activities of Eclipta prostrata (L) leaves [16]. [17] state that the extract of Eclipta prostrata (L.) may reduce asthma symptoms. Throughout Asia and South Africa, E. Prostata (L) has long been used as a traditional remedy for treating haemorrhagic ailments (such as haemoptysis, hematemesis, haematuria, epistaxis and bleeding of the uterus), skin disorder, respiratory disorder, coronary diseases, hair loss, vitiligo, snake bites and diseases associated with liver and kidney deficiencies. This aquatic plant contains Triterpenoids, Flavonoids, Thiopenes, Coumestans, Steroids, Sesquiterpene lactones, terthienyl aldehyde, fatty alcohols, polycacetlylenes and phenolic acids [18,19]. Aquatic plants have the potential as antibacterial agent in the treatment of fish diseases. Effectiveness of the plant extract used as antimicrobial agent depends on a number of factors that are related to their chemical composition, i.e., the hydrophobicity of the bacteria being tested, duration of exposure, and concentration of bacteria cells [20]. On the other hand, a combination of these plants may increase their effectiveness through synergistic effect [21]. Furthermore, besides their potential as antibacterial agents, plant extracts may boost non-specific immunity of some aquaculture, digestibility, and nutrient availability that ultimately may increase the efficiency of feed conversion and the synthesis of proteins [22].

4. Conclusion
Aquatic plants have the potential to be anti-bacterial biomedicine for treating fish disease. The extent of their anti-bacterial potency is varied depending on the species and the concentration of the plant extract used in the treatment. Each plant species has different anti-bacterial properties for each fish disease bacteria.

Conflicts of interest
The authors declare that there are no conflicts of interest in this study.

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