Studies on inhibition of *Morinda citrifolia* leaf extract (*Morinda citrifolia* L) against the growth of *Aeromonas hydrophilla* in vitro

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**Abstract.** *Morinda citrifolia* leaf contains active compounds that function as antibacterial substances, namely saponin, flavonoid, polyphenol, tannin and triterpen. The purpose of this research to discover the effectiveness of the *Morinda citrifolia* leaf extract in order to inhibit the growth of the bacteria *Aeromonas hydrophilla* as a cause of Motile Aeromonas Septicemia disease (MAS) on freshwater fish. This research is experimental research laboratory in vitro. The method used as an antibacterial test is a soft diffused method paper disc. The proportion of *Morinda citrifolia* leaf extract used in this research are 7.5%, 15%, 30%, 60% and negative control (ethanol 96%). The results of the study showed that *Morinda citrifolia* leaf extract is significantly (p<0.05) effective in inhibiting the growth of the bacteria *Aeromonas hydrophilla*. It shows that higher proportions of *Morinda citrifolia* leaf extract then inhibit zone formed when increasingly wide.

1. Introduction

Indonesia has the potential of fishery and aquaculture very potential, one of them is the aquaculture freshwater fish. This time intensive aquaculture system in freshwater fish cultivation activities implemented by fish farmer. This system is done to get maximum production results with widespread minimum land in a short time. This aquaculture system applies a high forbidding sowing solid that caused the fish more susceptible to illnesses.

The disease is vulnerable to attack freshwater fish namely infectious diseases such as Motile Aeromonas Septicemia disease (MAS) or often called with red spots disease on the body of fish. This infectious disease is a disease caused by bacteria *Aeromonas hydrophila*. It is often the problems in the aquaculture activities freshwater fish because bacterial pathogens can cause primary and secondary infections later will cause the death of fish and eventually cause loss for fish farmer.

The Disease MAS began to be known in Indonesia around 1980, where these bacteria cause of disease in various freshwater fish that begins from the West Java Area and finally to spread throughout the region of Indonesia. Motile Aeromonas Syndrome primarily cause disease incultured warm water fishes, like the example Carp (*Cyprinus carpio*), Channel cat fish (*Ictalurus punctatus*), striped bass (*Morone saxalitis*), an large mouth bass (*Micropterussalmoides*). *Aeromonas hydrophila* may also affect a variety of cool water species, but is not only restricted to fresh water environment. *Aeromonas hydrophila* is the cause of frightening diseases in fish farming around the world and is considered to be a major problem, in being the causative agent of Haemorrhagic septicemia and Epizootic ulcerative syndrome (EUS) [1], [9].

Generally tackle disease on aquaculture activities using chemicals or antibiotics. But this time its use has been banned because it has a high risk that, namely can cause the resistance against bacteria, leave oily residues on the body of the fish that can be harmful to human health if consume the fish and also pollute the environment. To overcome this need to search for a replacement alternative more secure, effective and environmentally in controlling disease MAS in freshwater fish.

One of disease control MAS with secure and friendly environment is the use of plants that contains natural active ingredients that are antibacterial. *Morinda citrifolia* one of the plant is as antibacterial. The substance contained in the plant pure Noni juice acts as a antibacterial one namely antrakuinon.
This substance is proven to suppress the growth of bacteria *Pseudomonas aeruginosa*, *Proteus morganii*, *Staphylococcus aureus*, *Basilus subtilis* and *Escherichia coli* [2], [5]. *Morinda citrifolia* has many used by fresh water fish farmer to cope with infectious diseases in fish. The crops are often used to overcome the disease MAS is *Morinda citrifolia* juice. The *Morinda citrifolia* juice proved to be able to cope with the disease MAS on the seed of the nilem [3].

*Morinda citrifolia* leaf is also part of plants which are potential developed to suppress the growth of bacterial pathogens. *Morinda citrifolia* leaf proved to have active substances that can be against the bacteria infection, like *Staphylococcus aureus* [4], *Salmonella sp. And Escherichia coli* [5]. Based on the research [4], *Morinda citrifolia* leaves proved to contain oil industry, fenol triterpenoid saponin, tannin and glycosides that function as antibacterial. With the potential of *Morinda citrifolia* leaf, does not close the possibility if the leaf pure Noni juice can also be used to inhibit the growth of bacteria in the *Aeromonas hydrophila* causes disease MAS In freshwater fish. Therefore, this this research was conducted to see the effectiveness of noni leaves in inhibiting bacterial growth causes disease MAS In freshwater fish in vitro.

2. Material and Method

The ingredients in research among others are bacteria *Aeromonas hydrophila*, *Morinda citrifolia* leaf, Natrium Agar media, Aquadest, Alcohol, ethanol, sanitary napkins, aluminum foil. The method used in this research is an experimental method that is done in a laboratory, compiled using five treatment and three times in Deuteronomy. The treatment is done with soaking the discs paper in *Morinda citrifolia* leaf extract with different proportions. The main treatment used as the negative control treatment is (ethanol 96%) and *Morinda citrifolia* leaf extract in various proportion (7.5%, 15%, 30% and 60%). The results of these observations is data tested using descriptive data analysis and quantitative analysis of simple linear regression data. Quantitative descriptive analysis is the analysis of the function that gives the impression of research data described by the descriptive quantitative and presented in the form of tables and graphs.

2.1 *Morinda citrifolia* L. Leaves extraction procedure

706 gram of fresh *Morinda citrifolia* L washed leaves which were neither too old nor young, then dried in 50ºC temperature 18 hours. Afterwards the leaves were mashed by using a blender. Then, the *Morinda citrifolia* L leaves which had become powder form, was extracted using maceration technique. *Morinda citrifolia* L leaves powder was put into a tightly closed erlenmeyer flask with addition of ethanol solvent 96% until the powder submerged for 3 days [6].

After the maceration completed, the powder was filtered with a buchner funnel that had been previously coated with filter paper and accommodated into the eruvier flask to obtain filtrate and residue. Then the filtrate was evaporated using a rotary evaporator at a temperature of 40ºC to obtain a viscous extract. The results of liquid extraction were stored at temperature of 0-4 ºC. According to [6] flavonoids were unstable against the influence of light, therefore a dark storage container is required to avoid the effect of light on the extraction results. Phytochemical test was performed for *Morinda citrifolia* L leaves extract results to determine the content of it’s active compounds.

2.2 Inhibitory Test of Growth the *Aeromonas hydrophilla*

The method used in the bacterial growth inhibitory test is the paper disc diffusion method. The steps to test the growth inhibition of *Aeromonas hydrophila* bacteria in vitro starting from pour Natrium Agar media as much as 12.5-15 ml into a sterile petri dish then leave it at room temperature until it solidifies, then pour 0.1 ml of the bacterial suspension using a micropipette on the solid NA medium and flatten it using a bent pipe. Previously soaking sterilized disc paper (diameter 1.5 cm) into *Morinda citrifolia* leaf extract various concentrations according to negative treatment and control for 24 hours. Laying disc paper on the surface of the media Solid NA which has been spread by bacteria using sterile (tweezers then incubated in an incubator at 37ºC for 24-48 hours, then measuring the diameter of the clear zone formed around the disc paper using a caliper run [7].
3. Results and Discussion
The results of the measurement of the diameter of the clear zone or zone of inhibit the bacteria *Aeromonas hydrophila* in proportion (7.5%, 15%, 30%, 60%) and ethanol 96% as negative control can be seen in the table 1:

| Treatment (Concentration of Morinda citrifolia leaf extract) | Inhibit Zone (mm) |
|------------------------------------------------------------|------------------|
| Control                                                    | 0                |
| 7.5 %                                                      | 3.3              |
| 15 %                                                       | 18.3             |
| 30%                                                        | 27               |
| 60%                                                        | 36.1             |

The diameter of inhibit zone formed in each of the treatments shows on the control using the ethanol 96% could not have formed the inhibit zone, while in proportions 7.5% , 15%, 30% and 60%. Sequentially the average inhibit zone was formed by the increasing of 12.7 mm, 18.3 mm, 27 mm and up 36.1 mm.

The diameter of the inhibit zone formed are indicators that the *Morinda citrifolia* leaf extract may inhibit the growth of *Aeromonas hydrophila*. Proportions of *Morinda citrifolia* leaf extract 30% and 60% (including in the category of the very strong antibacterial power), proportion *Morinda citrifolia* leaf extract 15% including in the category of strong antibacterial power and proportion *Morinda citrifolia* leaf extract 7.5 % (including in the category of antibacterial power weak).
The results of this research showed that the *Morinda citrifolia* leaf extract proved effective in inhibiting the growth of *Aeromonas hydrophila*. The results of these observations also show that the zone nodes start clearly visible on day two’s incubation period. The clear zone is a guide to the sensitivity of the bacteria to other antibacterial materials used as the test material. It is stated with the width of the diameter of the inhibit zone formed around the paper discs. The higher the proportion of *Morinda citrifolia* leaf extract the more womb antibacterial substance plays an important role in the inhibition of the *Aeromonas hydrophila*, graph is of the relationship between the proportion of *Morinda citrifolia* leaf extract with the diameter of the inhibit zone *Aeromonas hydrophila* can be seen in figure 1

Figure 2. A on the negative control treatment shows no clear zone formation around the paper discs. This illustrates that ethanol 96% as a solvent in the manufacture of *Morinda citrifolia* leaf extract does not affect the power activity in inhibiting the growth of bacteria. While the picture B looks as if it is in the clear zone with a size that is not wide, in picture C visible zone of nodes that is quite clear. While the image of D and E shows the zone nodes more clear and the area around the paper discs.

The formation of the inhibit zone around the paper (picture 2) shows the existence of the activity of antibacterial compounds against bacteria in the bacteria *Aeromonas hydrophila* test. The more the width of the diameter of the inhibit zone formed more bacteria that died, is seen from the clear zone around the paper discs. This proves that the *Morinda citrifolia* leaves have a good capability in inhibiting the growth of the bacteria *Aeromonas hydrophila*. *Morinda citrifolia* leaf also has been proven to inhibit the growth of bacteria Escherichia coli, Salmonella sp [5], and Staphylococcus aureus [4].

According to Daviz and Stout in [4], the diameter of the inhibit zone categorized has the power to inhibit the very strong if the diameter of the clear zone ≤ 20 mm, strong if the zone nodes 10-20 mm, is if the zone nodes 5-10 mm and weak if the zone nodes 2-5 mm. From the results obtained the treatment proportion for *Morinda citrifolia* leaf extract 7.5% have inhibit zone with the category of weak, whereas proportion *Morinda citrifolia* leaf extract 15% has inhibit zone with the category of strong while the proportion *Morinda citrifolia* leaf extract 30% and 60% has inhibit zone with the category is very strong.

![Graph of Linear Regression](image)

Figure 3. Graph of Linear Regression the diameter of the Inhibit Zone formed in each proportion of the *Morinda citrifolia* Leaf Extract

Based on the results of the statistical analysis provided on the linear regression the value, of correlation coefficient (R) of the *Morinda citrifolia* leaf extract of 0.912, was obtained which means that there is a very strong correlation between the proportion of *Morinda citrifolia* leaf extract and the diameter of the zone of inhibit where the *Aeromonas hydrophila* formed. This means that the diameter of the zone of inhibit where the bacteria *Aeromonas hydrophila* formed influenced 83.1% by the
treatment (proportion Morinda citrifolia leaf extract), while the remaining 16.9% were influenced by other factors outside of the treatment. This included factors such as the age of the leaf or plant species or noni used.

Based on figure 3, it can be inferred that the influence of the proportion extract to inhibit zone looks very real by linear (P<0.05). The higher the proportion of extracting the higher is its ability to inhibit the growth of bacteria in the Aeromonas hydrophila. From the results it is known that effective Morinda citrifolia leaf extract inhibits the growth of the bacteria Aeromonas hydrophila in vitro and level of proportion also effects the effectiveness of inhibitor.

The existence of antibacterial substances in the Morinda citrifolia leaf extract plays an important role in inhibiting the growth of or killings the bacteria Aeromonas hydrophila [10]. According to in [4], Morinda citrifolia leaf contains a wide spectrum of antakuinon as, flavonol iridoid glycosides and triterpen. The antakuinon substance that was found in the Morinda citrifolia leaf extract is impregnation fenolik which can also inhibit the bacteria with protein denaturation [2], [6] also added that the Morinda citrifolia leaf extract has the highest content of total flavonoid compared 90 to other plants amounting 254mg/100gram fw, flavonoid have microbial active. [2] explain flavonoid is the largest fenol compound in nature that have the nature of microbial on the plants. The activity of antibacterial compounds to microorganisms can be caused by several factors among other disorders of the building blocks of cell walls and can increase cell membrane permeability that can cause loss of components of the building blocks of the cell, make it inactive enzymes and aim or damage the function of the genetic material [5]. The cell wall is the main target that is attacked by an antibacterial substance contained in the Morinda citrifolia leaf extract to ease antibakter compounds into the cell membrane. This is explained by [8] when they explain the antibacterial mechanism on flavonoids and its ability to cause damage to the bacterial cell wall permeability, mikrosom and lysosomes as the interaction between flavonoid with DNA bacteria. The wall of the cell that has the nature of not selectively permeable so easily in the penetration of antibacterial compounds that penetrate the cells that will cause disturbance intergritas cell wall. [2] also strengthened the analysis with his statement that the flavonoid can change the chemical and physical nature of the cytoplasm that contains protein and the denaturation wall of bacterial cells with how to bind to the protein through the hydrogen bond. This activity will interfere with the function of selective permeability, active transport function and control the order of proteins.

Based on screening tests fitokimia, Morinda citrifolia leaf contains essential oil industry, saponin, triterpenoid, fenol, tannin and glycosides. Saponin and tannin include in antibacteria groups which also can interfere with cell membrane permeability microbes. Saponin can cause damage to the cell membrane and cause escaping in various important components in the cell microbes which is a protein, nucleic acids, nucleotides and others [8]. Tannin is capable making inactive adhesin microbes, enzymes and transport proteins in the cell membrane. Oil industry is antibacterial which contains volatile compounds such as the monoterpen and sesquiterpen which is also antibacterial [4].

The diameter of the clear zone formed in every proportion of the Morinda citrifolia leaf extract has the size of the wide and is included in the category of inhibit zone weak, strong and very strong. This is according to [2] and can also be influenced by the acceptance of our extraction method. In this research the method used is the method macerasion with solvent ethanol 96%. Ethanol 96% has high ability in the extract of the content of the plants which has dissolved compounds in it. In addition compounds in the Morinda citrifolia leaf is polar to dissolve easily ethanol also is polar [4] so that suspected compound can be bound perfectly in solvent ethanol.

It has not yet been local communities who have the role of the materials most important in inhibitions the growth of the bacteria Aeromonas hydrophila, the active substances in the Morinda citrifolia leaf extract is suspected to work on its own or together in inhibitions the growth of Aeromonas hydrophila. Flavonoid allegedly has the ability in high antibacterial substances compared to other antibacterial substances. Flavonoid capability in effecting antibacteria among others by inhibiting the function of the membrane of the cytoplasm, inhibits the synthesis of nucleic acids and inhibits the antibacterial activity with how to inhibit the metabolism of energy and also inhibits the consumption of oxygen with how to interfere with electron transport chain respiration. While
according to [4], noni leaves have the womb saponin, flavonoid, polyphenol, tannin and triterpen. The active substance is bactericidal and has its own method in inhibiting the growth of bacteria. The results of our research and the discussion of the Morinda citrifolia leaf extract proved effective in the inhibition of the growth of Aeromonas hydrophila in vitro. However, the activity of the compound in the Morinda citrifolia leaves extract and the type of compounds had the most active role in inhibiting the growth of bacteria Aeromonas hydrophila. Therefore, they still need to be examined and identified with more information.

The results of this research showed that the use of the Morinda citrifolia leaf extract is effective to inhibiting the growth of Aeromonas hydrophila in vitro, and the higher the proportion of Morinda citrifolia leaf extract, the more effective in its inhibition of the growth of Aeromonas hydrophila in vitro.

4. Conclusion
The results showed that in vitro, noni leaf extract proved capable of inhibiting the growth of Aeromonas hydrophila. However, the activity of the compound in the Morinda citrifolia leaf extract and the type of compounds had the most active role in inhibiting the growth of bacteria Aeromonas hydrophila. Therefore, they still need to be examined and identified with more information.

The research also shows that the higher the proportion of Morinda citrifolia leaf extract then it will be more effective at inhibiting the growth of Aeromonas hydrophila.

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