Utilization of Moringa Leaf Extract (*Moringa Oleifera*) against the Ectoparasite *Zeylanicobdella* sp. on Grouper Fish In In-Vitro

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Abstract. Grouper is a mainstay commodity for mariculture in Indonesia. One of the obstacles in cultivating grouper fish in floating net cages is the infection of the hirudinea sea leech *Zeylanicobdella* sp. Leech infection causes fish growth to be stunted and they may not even be sold. The aim of this research is to determine the effect of adding moringa leaf extract (Moringa oleifera) and its effective dose which can kill sea leeches. This research was carried out in November – December 2023 for 30 days located at the Pengujan Fish Seed Center (BBI), Bintan Regency, Riau Islands Province. This research includes making extracts using 96% ethanol solvent, qualitatively testing the active content of simplicia, probit analysis of extracts on Artemia salina test animals and testing extracts on the leech *Zeylanicobdella* sp. The graded extract doses used for the LC-50 test on Artemia salina are 20 ppm, 40 ppm and 60 ppm. Meanwhile, the dose for testing on the leech *Zeylanicobdella* sp. namely 10 ppm, 20 ppm and 30 ppm. Determination of dosage for leeches is based on the LC50-24 value. The research results showed that the percentage of water content was 81,73% and the total yield was 9.25%. The results of the phytochemical test showed that 96% ethanol was successful in attracting bioactive compounds such as alkaloids, flavonoids, saponins and tannins. The LC50-24 hour value shows that the dose of Moringa leaf extract that can cause 50% death of test animals is 30,549 ppm and the best dose that can kill *Zeylanicobdella* sp. leeches namely a concentration of 30,549 ppm.

1 Introduction

Sea leech *Zeylanicobdella* sp. is a type of ectoparasite that most often attacks farmed fish such as grouper and sea bass. Fish infected by animals such as leeches or other diseases can damage the fish's organ mechanisms and disrupt physiological processes. These leeches infect parts of the body, eyes, mouth and respiratory cavity. Infection from these leeches can cause wounds on the skin where these wounds can become a place for pathogens to enter and develop [1]. Prevalence of *Zeylanicobdella* sp. in hybrid grouper fish it was reported to reach 100% with an intensity of 21.2 leeches/fish, however the prevalence and intensity of *Zeylanicobdella* sp. varies depending on the condition of floating net cages and fish populations. Large fish tend to have a higher prevalence and intensity of infection with *Zeylanicobdella* sp.

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The existence of these ectoparasites must be eradicated because they can endanger fish farming as a whole. This eradication is usually referred to as parasite control or treatment. Much research has been carried out on this matter, including countermeasures using natural ingredients found in nature. One example is a plant used as a phytopharmaceutical or natural medicine that has secondary metabolite compounds such as Moringa oleifera leaves.

Moringa leaves also known as (*Moringa oleifera*) contain many benefits for people's lives in Indonesia. Moringa leaves contain alkaloids, tannins, flavonoids, antioxidants and saponins. Moringa leaves can also be used as a natural antibiotic for sick fish because of its properties which can stop the survival of pathogens. Traditionally, almost all parts of the Moringa plant, starting from the roots, bark, sap, leaves, fruit, flowers, seeds and seed oil, have been used to cure various diseases [2].

Moringa leaves are only used as a cooking ingredient or as a vegetable in today's society. Therefore, this research was carried out to see and understand whether giving Moringa oliefera leaf extract had an effect on the death of the leech ectoparasite *Zeylanicobdella* sp. as well as knowing the best dosage so that it has the opportunity to become an alternative plant that can be used by fish farmers to control ectoparasites that attack cultivated fish.

2. Materials and Methods

2.1. Time and location

The study was carried out in November of 2023. Research on the identification and qualitative content of the active components in moringa leaf extract was done in the Chemistry lab at Raja Ali Haji Maritime University Tanjungpinang's Faculty of Marine and Fisheries Sciences. Meanwhile, in the Fish Seed Center (BBI) Pengujan, Bintan Regency, Riau Islands Province, LC50 testing on Artemia salina is conducted in addition to leech observation (Zeylanicobdella sp.).

2.2. Materials and Methods

This research used a Completely Randomized Design, with 4 treatments and 3 repetitions. Dosage used for LC50 testing in Artemia saline, namely 0 ppm, 20 ppm, 40 ppm, and 60 ppm. Determination of dose concentration based on research [3]. The research design used as follows:

Treatment K: Without additional Moringa leaf extract
Treatment P1: Addition of 20 ppm of extract
Treatment P2: Addition of 40 ppm of extract
Treatment P3: Addition of 60 ppm of extract

Research design for testing the leech *Zeylanicobdella* sp. that is using a Completely Randomized Design (CRD) 4 Treatments 3 times repetition. The doses for this test are 10 ppm, 20 ppm and 30 ppm. Dosage determination based on the results of the LC50 value. The research design used is as follows following:

Treatment K: Without additional Moringa leaf extract
Treatment P1: Addition of 20 ppm of extract
Treatment P2: Addition of 40 ppm of extract
Treatment P3: Addition of 60 ppm of extract

2.2.1. Preparation of Moringa leaves (Moringa oleifera)

The Moringa leaves used come from Air Raja Village, District East Tanjungpinang, Tanjungpinang City. Moringa leaves are used in This research consisted of 1 kg of fresh Moringa leaves. Moringa leaves are then added chop into small pieces and then carry out the drying process. Following completion, moringa leaves are chopped into small bits and dried in an oven set to 90˚C for 45 minutes. The dried Moringa leaves are then ground to form a powder using a blender. The purpose of leaf smoothing Moringa is to make simplicia closer to solvents and makes it easier for the solvent to penetrate to take up more components contained in simplicia.

2.2.2. Maceration of Moringa leaves (Moringa oleifera)

Mix 100 grams of Moringa leaf simplicia into a glass jar size 1500 ml, then 500 ml of 96% ethanol solvent is poured. The solvent is added slowly until the sample is completely wet. Then sample Leave for 3 days, stirring occasionally using a stick stirrer. Maceration is carried out in a ratio of 1:5. After that results. The maceration was filtered using Wattman filter paper no. 42. The results of the maceration were Pour into a 300 ml glass bottle then concentrate using water bath at 40°C until the solution becomes thick like paste.

2.2.3. Qualitative Identification of the Active Ingredient Content of Moringa Leaves

2.2.3.1. Alkaloid Test

Moringa leaf extract was weighed 0.5 grams, put into a test tube, then add 3 drops of Dragendorff's reagent if there is an orange precipitate then it is positive for the presence of alkaloids.

2.2.3.2. Flavonoid Test

Moringa leaf extract was weighed 0.5 grams, put into a test tube with a mixture of 0.1 grams of mg powder and 3 drops of 2N HCl. Color formation orange indicates a positive solution for flavonoids.

2.2.3.3. Saponin Test

Moringa leaf extract 0.5 grams is weighed then added with 2 ml of water and 1 drop of 2N HCl until all parts of the extract are submerged and then shaken strong. If there is foam then the solution is positive for containing saponin.

2.2.3.4. Tannin Test

Moringa leaf extract of 0.5 was put into a test tube and add 1 drop of $FeCl_3$, if it is blackish blue, then it is positive tannin.

2.2.4. LC50 Brine Shimp Lethality Test

Testing using the BSLT method requires Artemia salina as test animals. The first step is to weigh 2.5 g of Artemia salina cysts and hatched in 1.5 L plastic bottles filled with sea

water as much as 1 L and aerated. Leave for 48 hours until the artemia hatch. After hatching, the larvae are ready for toxicity testing. Put 10 Artemia salina into a vial containing 5 ml of seawater and add Moringa leaf extract as much as 20 ppm, 40 ppm and 60 ppm. Death of Artemia in Count after 24 hours and note the number of dead Artemia.

2.2.5. Sea Leech Challenge Test (Zeylanicobdella sp.)

The leeches used in this study were collected manually from tiger grouper contaminated with leeches *Zeylanicobdella* sp. as many as 120 leech tail. The collected leeches are put into the existing container filled with sea water and given gentle aeration, then prepare 12 sized vials 60 ml which has been filled with 50 ml of sea water and Moringa leaf extract in dosage 10 ppm, 20 ppm and 30 ppm then put 10 leeches each into the in the vial using tweezers. The leech is clamped slowly without injure the leech's body. This observation was carried out over a period of 15, 30, 45 and 60 minutes. The next step is to observe the leech's behavior and counting the number of leeches that died in each treatment and replication.

2.2.6. Mortality Test

Mortality is the percentage of deaths of test animals in a population. Mortality can be calculated using the formula according to (Christiyanto, 2013) as follows:

$$P = (X//Y) \times 100\%$$

Information:
P: Percentage of deaths (%)
X: Number of dead animals (tails)
Y: Number of animals tested (heads)

2.3. Data Analysis

LC50 Test Data was analyzed using a probit regression equation use MS Excel to obtain the LC50-24 hour value. Influence of materials active herbal against the death of the leech type ectoparasite *Zeylanicobdella* sp. Can seen through challenge test data in analysis with Anova and further tests with Tukey's test.

3. Result, Discussion, Conclusion

3.1. Result

3.1.1. Water Content Analysis

Fresh Moringa leaves and dried Moringa leaves were first weighed using analytical balance to determine the water content. Next, get it The sample yield of fresh Moringa leaves was 1,188 grams and the sample yield was 217 grams dried moringa leaves. The water content contained in the material is 81.73%.

3.1.2. Total Yield

100 grams of Moringa leaf simplicia added with 500 ml of ethanol 96% in the maceration process. So you get a total yield of 100 grams the Moringa leaf sample was 9.25 grams. The percentage of total yield obtained was 9.25%.

3.1.3. Active Ingredients of Moringa Leaf Extract

The results of testing the active ingredient content of Moringa leaf extract are as follows:

Table 1. Test results for the active content of Moringa leaf extract

Sample	Form	Parameter		Result	Unit
Moringa Leaf Extract	Paste	Alkaloid	Dragendorff	+	Deposition
		Flavonoid	Mg powder + HCl 2N	+	Color Vizualitation
		Saponin	HCl 2N	+	Color Vizualitation
		Tannin	$FeCl_3$	+	Color Vizualitation

3.1.4. LC50 Moringa Leaf Extract

The LC50 value is obtained by taking into account the probit value of a material. The probit value is used to estimate the effective dose by determining the death concentration. The results of the toxicity test of Moringa leaf extract using 96% Ethanol solvent are as follows:

Table 2. LC50 Value of Moringa Leaf Extract Against *Artemia salina*

Concentration (ppm)	Log Concentration	% Mortality	Probit
0	0	0	0
20	1,30	40	4,75
40	1,60	53	5,08
60	1,78	70	5,52

The LC50 value based on the probit value obtained is as follows:

Table 3. LC50-24 hour of Moringa Leaf Extract

No	Sample	Regression equation	LC_{50}-24 Hours
1	Moringa leaf extract	Y = 1.5527x + 2.6944	30.549 ppm

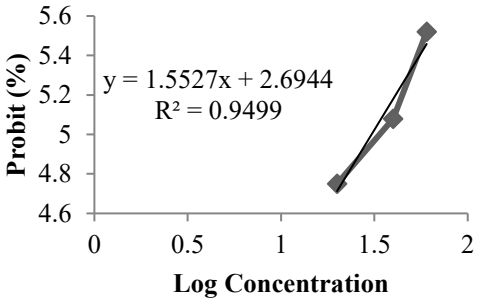

Fig.1. Linear Regression Graph for Moringa Leaf Extract Toxicity Test

3.1.5. Mortality of Zeylanicobdella sp.

Mortality is the percentage of deaths of test animals in a population. The Mortality graph for *Zeylanicobdella* sp leeches. as follows:

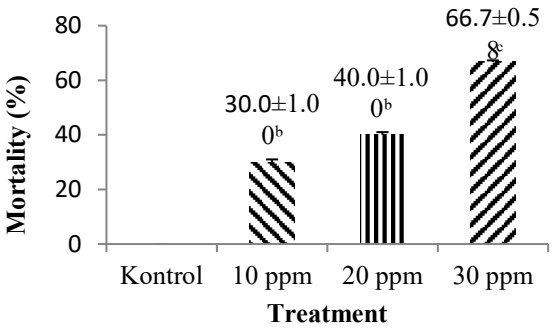

Fig.2. Mortality graph for leeches *Zeylanicobdella* sp.

3.2. Discussion

3.2.1. Analysis of Moringa Leaf Water Content

The higher the water content of a material, the greater the possibility of contamination by internal biological activity (metabolism) and the entry of destructive microbes. If the water content of the material decreases, the availability of water for physicochemical reactions and the growth of microorganisms will decrease. As a result, food will be more resistant to damage. Water content has a major role because water has its own role in the decay process. Food damage is typically caused by enzymatic, chemical, or microbiological processes, or by a mix of these. Water content, which is measured in a ratio from 0 (complete dryness) to the water saturation value at which all pores are filled with water, is used extensively in the scientific and technical domains. The value can be volumetric or gravimetric (mass), wet basis or dry basis [4]. Measurement of water content is a changeable component in most compounds that can change in relation to weather and temperature [5]. The water content of a food can affect its shelf life, because the lower the water content, the more inhibited microbes are [6].

3.2.2. Total Yield

Yield is the comparison of the dry weight of the product produced with the raw materials [7]. Apart from that, the yield can also be interpreted as the proportion between the extract obtained and the initial simplicia multiplied by 100. The yield results show that there is an influence on the yield obtained on the solvent used. The total yield is influenced by several factors such as simplicia size, solvent type, polarity level and maceration time [8]. The yield calculation itself aims to find out how much extract is obtained from fresh simplicia.

3.2.3. The Active Content of Moringa Leaf Extract

The results of testing the active content of Moringa leaf extract can be seen in Table 3, which shows that the Moringa leaf extract is positive for containing alkaloids with Dragendorff solvent, showing the presence of an orange precipitate. Alkaloid compounds have strong pharmacological properties and physiological activity, so they can be widely used in the field of medicine [9]. Moringa leaf extract was also positive for containing flavonoids with 2N HCl solvent and magnesium powder which produced an orange color. Ethanol solutions are polar so it is easier to extract flavonoid compounds from plant tissue because plants can be dissolved or bound by the solvent based on its

polarity [10]. Apart from that, Moringa leaf extract was also positive for containing saponin which was indicated by the formation of foam in the test solution when shaken. Moringa leaf extract was positive for tannin and showed a blue-black color change. The results of the active ingredient content test in this study are in line with research by [11] which obtained positive Moringa leaf phytochemical test results containing active compounds of flavonoids, saponins, alkaloids and tannins. Toxic activity can be seen from the number of deaths of Artemia salina larvae which is influenced by the presence of secondary metabolite compounds in plant extracts or natural ingredients [3].

3.2.4. LC50 of Moringa Leaf Extract

The LC50 test results for Moringa leaf extract on Artemia salina using 96% ethanol solvent produced a Lethal Concentration value of 50% (LC50), which means that Moringa leaf extract is toxic and can cause up to 50% death of test animals. In Table 5 above, Moringa leaf extract is shown with an LC50 value of 30,549 ppm, which means it is toxic because it is ≤1000 ppm. The LC50≤ 30 ppm value is classified as very toxic, LC50≤ 1000 = toxic and LC50 > 1000 ppm = not toxic [12].

3.2.5. Mortality of Zeylanicobdella sp.

Due to its potential to obstruct the larvae's capacity to feed, alkaloid and flavonoid compounds have a tight relationship to the process of Artemia salina larvae death (antifedants). Certain substances function as stomach poisoning agents because they can instantly interfere with the larva's digestive system upon entering its body. In addition, this substance will also cause disruption to the taste receptors in the larvae's mouth. Consequently, the larvae will not receive the taste sensation, which makes it difficult for them to identify their food and eventually leads to their starved death. Other compounds such as saponins also play a role in the death of Artemia salina by disrupting the feeding ability of the larvae and reducing digestive enzymes. Apart from that, tannin is a polyphenolic compound, which at high doses can act as a toxin for plasma which can cause cell wall damage and collect cell proteins, and at low doses tannin can also interfere with in vitro enzyme multiplication[13].

4 Conclusion

Moringa leaves had 81.73% water content, and 9.25% total yield when dissolved in 96% ethanol solvent was obtained. According to the findings of the phytochemical test, bioactive substances such alkaloids, flavonoids, saponins, and tannins were successfully attracted to 96% ethanol. The LC50 value of Moringa leaf extract is 30,549 ppm, which is toxic to *Artemia salina* at a concentration that can cause 50% of test animals to die. Apart from that, Moringa leaf extract also has an effect on the mortality of *Zeylanicobdella* sp leeches. with the best dose being 30,549 ppm.

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