Inhibition test of mangosteen peel extract (*Garcinia mangostana* L.) against bacterial diseases of tiger grouper (*Epinephelus fuscoguttatus*)

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Abstract. Cultivation of tiger grouper (*Epinephelus fuscoguttatus*) in Floating Net Cages in several locations in Bireuen Regency has developed. The potential disease issue can be caused by bacteria, viruses, fungi, or parasites. The purpose of this study was to determine the effectiveness of mangosteen peel extract capsules (*Garcinia mangostana* L) against bacterial disease in tiger grouper. This research was carried out in April 2019 at the MIPA Laboratory of Al Muslim University and the laboratory of PT. Centra Proteina Prima, Bireuen Regency. The design used was a completely randomized design with four treatments (0%, 10%, 20%, and 30% mangosteen peel extract capsules) with three replications. The parameters studied in this study including the diameter of the inhibition and the observation of MBC (Minimum Bacterial Concentration). The result showed that the administration of mangosteen peel extract capsules with doses of 10%, 20%, and 30% has no significant effect on reducing disease-causing bacteria in grouper grown on TSA media. Positive results were obtained on positive (+) MBC observations, presumably due to the small dose of mangosteen peel extract capsules given so that it could not kill bacteria grown in dishes with an inhibition range of 0 mm.

1. Introduction

Tiger grouper (*Epinephelus fuscoguttatus*) is one of the local aquaculture commodities cultivated by the people in Bireuen Regency in ponds and in floating net cages. Tiger grouper is also a marine resource commodity that has great potential to be developed in Aceh, especially Bireuen Regency. Cultivation of grouper in floating net cages (KJA) or ponds in several locations in Bireuen Regency has been growing, especially tiger grouper. The increase in cultivation might occur due to the availability of seeds regularly, both in number and size.

With the development of tiger grouper cultivation, there are also obstacles, namely the emergence of disease attacks in cultivated tiger groupers. The diseases might be caused by infection with pathogens, bacteria, viruses, fungi, or parasites. Anxiety can also be due to deficiency or malnutrition, or other
causes [1]. Meanwhile, according to [2], in general, the factors associated with the attack of disease are interactions of 3 factors: the host, pathogen, and the environment or external stressors (unfavorable environmental changes, insufficient hygiene levels, and stress).

Disease always appears as a dynamic process due to the imbalance of the relationship between the host (host), disease body (pathogen), and the environment in tiger grouper cultivation activities. Fish diseases usually arise related to the weak condition of the fish caused by several factors, including the handling of fish, the factors of the feed provided, and unfavorable environmental conditions. At a high stocking density and environmental factors are unsuitable, for example, the low acid content in water, inadequate feed given both in quantity and quality, fish handling is not perfect, the fish will stress and susceptible to disease [3,4,5,6].

In the aquaculture sector, pathogenic bacteria have feared by many fish, shrimp, and shellfish farmers. Because these microorganisms can threaten and even cause mass death in fish and shrimp, this will undoubtedly be very detrimental to grouper cultivation. According to[1], diseases caused by bacteria that often attack grouper are Vibrio sp., Aeromonas sp., Pseudomonas sp., Streptococcus sp., Pasteurella sp. and Mycobacterium sp. According to [7], disease-causing bacteria in tiger grouper isolated from water, floating net cages and culture tanks, and tiger grouper include Pseudomonas sp., Citrobacter sp, Proteus sp, Aeromonas sp, Vibrio sp and Shigella sp. Meanwhile, no data are showing the effectiveness of mangosteen peel extract and its derivatives on these bacteria.

This study aims to control bacterial disease in tiger grouper using herbal/natural ingredients, namely mangosteen peel extract capsules. The content of flavonoid and alkaloid compounds in mangosteen peel extract capsules which act as antimicrobial and antibacterial can control bacterial attack on grouper. There have been many studies on the use of natural medicines to treat several diseases that attack cultured fish. The use of mangosteen peel extract capsules in medicine is currently overgrowing. Therefore the author wants to apply it in fisheries as a medicine to treat bacterial diseases in groupers. With the research results, we hope to help fish farming communities in Bireuen Regency deal with the problem of bacterial disease in grouper fish, especially tiger grouper.

2. Materials and Methods

2.1. Research Time and Location
This research was carried out in April 2019 in the MIPA laboratory of Almuslim University and the laboratory of PT. Centra Proteina Prima, Bireuen Regency. Samples of grouper fish were taken from ponds owned by grouper farmers in Jangka Sub-district, Bireuen Regency.

2.2. Research Method
The method used in this study is an experimental method to treat each medium with several doses of mangosteen peel extract capsules in the inhibitory test method. The research methods included sterilization, preparation of the paper disc method for testing the activity of mangosteen peel extract capsules against bacterial diseases in tiger grouper, bacterial enrichment, and bacterial cultivation, as well as counting the number of colonies using the cup count method. The equipment was sterilized using an autoclave with an air pressure of 1 atm at 121ºC for 15 minutes and 70% alcohol. The mangosteen peel extract capsules sold in packages are opened, and the contents of the capsules are removed, then the powder from the capsule is diluted using 1 ml of 0.7% NaCl according to the doses to be used, namely 10, 20, and 30%. After the solution is ready, put the solution in each Eppendorf, then soak the paper disc with a diameter of 0.1 for 15 minutes. The disc paper was removed from the solution and was ready for the inhibition test [8].

For bacterial enrichment, it is carried out by taking bacteria from groupers that show bacterial disease symptoms with the characteristics of wounds or ulcers, low appetite, and white or red spots on the outside of the skin using an ossicle and then planted on TSA agar media and incubated. At 37ºC for 24 hours. Bacterial samples were taken using an ossicle, put on TSA agar media (to tilt), and incubated at 37ºC for 24 hours. After 24 hours, bacteria were planted from the enriched colonies, which were diluted...
nine times in a test tube containing 9 ml of NaCl. Then 1 ml of the bacterial colony solution in the enrichment tube was taken and put into a tube containing 9 ml of NaCl in the first dilution stage. From the first dilution tube, 1 ml was taken and put into the next tube and repeated until nine times the dilution to get a total of 108. The response to the presence of anti-bacterial potential was determined by calculating the diameter of the mangosteen peel capsule extract that inhibited bacterial growth. The number of colonies was to determine total colonies grew on the dish, starting from a dilution of $10^0$ to $10^9$.

2.3. Observation Parameters
The parameter was observed in this study: the diameter of the inhibition of the mangosteen peel extract capsule against bacteria in tiger grouper grown on TSA media. The inhibition test was measured to determine the diameter of the inhibitory power produced by the mangosteen peel extract capsule using a caliper. The MBC test was measured to determine the effectiveness of the mangosteen peel extract capsule in killing bacteria in grouper with the dose used.

2.4. Data Analysis
Data analysis was carried out quantitatively based on the number of bacteria [2]. The data obtained were described descriptively and presented in the form of tables and figures.

3. Results and Discussion

3.1. Number of Bacterial Colonies in Grouper
Counting the number of colonies carried out only on the dilution that showed the number of colonies $< 300$ colonies, namely on planting with a dilution of $10^2$ and $10^3$. The number of colonies that grew from planting on a dilution of $10^2$ was 245 colonies, while the number of colonies that grew on a dilution of $10^3$ was 36 colonies. While the results of planting at a dilution of $10^0$– $10^1$ the number of colonies $> 300$ colonies that grew in each container with the number of bacterial colonies could not be counted, while in the $10^4$ dilutions there was only 1 colony of bacteria. With the dilution of *Nigella sativa* on TSA media at a dose of $5 \times 10^3$, it had the lowest number of colonies so that there was a very significant difference with the control [9]. The results of the study showed that bacterial colonies that could be counted were only found in planting $10^2$ and $10^3$ with the number of bacteria growing as many as 245 and 36 colonies(Figure 1 and Table 1).

| No | Dilution | Number of Colonies |
|----|----------|-------------------|
| 1  | $10^0$   | >300 (can not be calculated) |
| 2  | $10^1$   | >300 (can not be calculated) |
| 3  | $10^2$   | 245 colony         |
| 4  | $10^3$   | 36 colony          |
| 5  | $10^4$   | 1 colony           |

Table 1. Results of Calculation of the Number of Colonies from Dilution

The bacterial diseases causing economic losses in seawater fish farming are Vibriosis, Winter ulcer, Photobacteriosis, Pasteurellosis, Furunculosis, Flexibacteriosis, Pseudomodias, Winter disease, Streptococcosis, Lactococcosis, BKD, Mycobacteriosis and Pischaricckettsiosis [3]. Of all the bacteria causing the disease in marine culture, vibriosis is the most common cause of losses in marine fish culture, followed by aeromonas infection and other bacteria. While in the grouper cultivation, the infection disease that is often identified is caused by *Vibrio* bacteria.

The research conducted by [15] showed that one of the predominant bacteria in the gut of the slow-growing orange spotted grouper is *Vibrio*. The percentage of *Vibrio* in the gut of the slow-growing orange spotted grouper was 12.3%, while the fast-growing orange spotted grouper was 3.6%.
Furthermore, the causal agent of skin ulcer disease in juvenile hybrid groupers (Epinephelus fuscoguttatus x Epinephelus lanceolatus) identified as Vibrio harveyii [16].

3.2. Resistance Test
Based on the results of the study, using mangosteen peel extract capsules did not show any inhibition with the resulting inhibitory power being 0 mm in each treatment using mangosteen peel extract capsules at doses of 10, 20, and 30% (Figure 2). Based on Figure 2 that there is no inhibition for each treatment. the mangosteen rind powder capsule used in this research is thought to be not the result of extraction but the dried and powdered mangosteen rind, so that its antibacterial effectiveness is low.

The result of the research conducted by [9], state that the crude extract of mangosteen pericarp did not show activities against F. columnare, E. ictaluri, and S. intae at the highest minimum concentration (100 mg/L) tested.

High level of bacterial colony diversity may also be the cause of the low antibacterial activity of the mangosteen peel capsules to inhibit and kill bacteria. The results of the research conducted by [10], which said that mangosteen peel extract at doses of 5%, 10%, 20%, 40%, 60%, and 80% could not inhibit growth and killed Escherichia coli. Meanwhile, based on the results of research conducted by [14], methanol extract from mangosteen rind powder has good antibacterial effectiveness against V. harveyii which is characterized by the formation of an inhibition zone with a diameter of 76 mm (very strong category). As [16] tested the susceptibility of twenty antibiotics against V.harveyii, the results showed that only three antibiotics have a sensitivity against V.Harveyii

![Figure 1. Bacterial Colonies Growing on Plates with TSA Media](image-url)
3.3. Observation of MBC (Minimum Bactericidal Concentration)

The MBC parameter is a parameter that shows the level of activity of giving mangosteen peel extract capsules in inhibiting growth and killing all disease-causing bacteria in grouper planted in plates. In observing the MBC parameters, the observed parameters were that no bacteria were growing in the planting cup after administration of mangosteen peel extract capsules at doses of 10, 20, and 30%. The results of observations of the MBC parameters of mangosteen peel extract capsules against disease-causing bacteria in tiger grouper grown in Petri dishes with a dilution of $10^2$ CFU/ml are as presented in Table 2 below:

| No | Treatment | Inhibition |
|----|-----------|------------|
| A  | 10 %      | +          |
| B  | 10 %      | +          |
| C  | 20 %      | +          |
| D  | 30 %      | +          |

The results of the observations, the administration of mangosteen peel extract capsules at a dose of 10%, 20%, and 30% could not kill disease-causing bacteria in grouper without showing negative results on the growth of disease-causing bacteria in grouper grown on TSA media. The positive results obtained on MBC observations were thought to be due to the small dose of mangosteen peel extract capsules given, so it could not kill the bacteria planted in the petri dish.

In addition, differences in bacterial colonies also affect the ability of antimicrobial compounds in mangosteen peel extract to inhibit and kill bacteria. This is corroborated by the results of research conducted by [12], which said that mangosteen peel extract at doses of 5%, 10%, 20%, 40%, 60%, and 80% could not inhibit growth and kill Escherichia coli bacteria.

The results of research by [11], suggested a 30% dose of mangosteen rind extract was effective in killing and inhibiting the growth of Flavobacterium and Enterobacter bacteria. Based on the differences in research results obtained by several previous researchers, it is suspected that mangosteen peel extract is not effective in inhibiting growth and killing all types of bacterial colonies, but can only inhibit growth...
and kill certain types of bacterial colonies. The content of Xanthones as secondary metabolites found in the mangosteen fruit has an antibacterial effect with varying abilities depending on the bacteria tested. In *S. aureus, P. aeruginosa, S. thypimurium, B. Subillis*, the secondary metabolites of mangosteen were very strong in inhibiting the growth of the bacteria tested, while in *Proteus sp.*, *Klebsiella sp.* and *Escherichia coli* have moderate antibacterial effect. In this test, *Aeromonas, vibrio* and some bacteria that cause disease in fish were not tested [12]. The results of observations of MBC on the cup can also be seen in Figures 3 and 4 visually below:

![Figure 3](image1.jpg)

**Figure 3.** Results of MBC Parameter Testing of Mangosteen Peel Extract Capsules against Disease-causing Bacteria in Tiger Grouper with 0% and 10% Concentrations

![Figure 4](image2.jpg)

**Figure 4.** Results of MBC Parameter Testing from Mangosteen Peel Extract Capsules against Disease-causing Bacteria in Tiger Grouper with 20% and 30% Concentrations

The MBC parameter test which was observed visually showed that the concentration of mangosteen peel extract capsules of 0%, 10%, 20%, and 30% added to TSA media that had been planted with disease-causing bacteria in grouper found the presence of bacterial colonies growing on the cup, so it was concluded that the positive result of the MBC test obtained from the study was (+), which means
that the mangosteen peel capsule extract in the package could not kill disease-causing bacteria in grouper. Research on several types of traditional plant extracts in Thailand has examined their effectiveness against pathogenic bacteria in fish and shrimp, from 16 types of plants only 2 types of extracts from plants Momordica charantia and Psidium guajava showed effectiveness at low concentrations (1.25 and 2.5 ml/mg) the rest showed good effectiveness at a concentration of 10 mg/ml and some did not have inhibitory activity at that concentration. However, from 16 types of plants, mangosteen peel extract was not observed [13].

4. Conclusion
The use of mangosteen peel extract capsules at a dose of 10%, 20%, and 30% could not kill disease-causing bacteria in grouper and did not show negative results on the growth of disease-causing bacteria in grouper grown on TSA media. Positive results were obtained on positive (+) MBC observations, presumably due to the small dose of mangosteen peel extract capsules given, so that it could not kill bacteria grown in dishes with an inhibition range of 0 mm. It is hoped that further research will be conducted on the inhibitory power of mangosteen peel extract capsules using higher doses and different test biota.

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