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Effectiveness of *Garcinia mangostana* Mangosteen Peel Extract in Inhibiting Gram-Negative Bacteria in Lobster (*Panulirus homarus*) Aquaculture Waters

Nuri Muahiddah[1*], Sahrul Alim[1], Laily Fitriani Mulyani[1]

[1]Aquaculture Study Program, Faculty of Agriculture, University of Mataram
Pendidikan Street No. 37 Mataram, Indonesia

***Correspondence**:
nurimuahiddah@unram.ac.id
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ABSTRACT

This study aims to examine the effectiveness of mangosteen peel extract (*Garcinia mangostana*) in inhibiting the growth of Gram-negative bacteria in lobster (*Panulirus homarus*) aquaculture waters. The main problem in lobster farming is bacterial infections that can cause disease and mass mortality. Mangosteen peel extract is known to contain potent antimicrobial compounds. This research employs an experimental method with various concentrations of mangosteen peel extract to test its effect on the growth of Gram-negative bacteria. Based on result, the mangosteen peel inhibition zone against Gram-negative bacteria from lobster aquaculture waters measures 0.25±0.05 cm (25 mm), indicating weak inhibition activity. This suggests that mangosteen peel could be used as an immunostimulant alternative to antibiotics in combating diseases in lobsters. Further research is needed to determine the optimal dosage of mangosteen peel extract to achieve strong inhibition activity against bacteria.

INTRODUCTION

Lobster farming (*Panulirus homarus*) is one of the fisheries sectors with high economic value (Priyambodo *et al*., 2020). However, the primary challenge faced in this aquaculture is the high rate of bacterial infections, especially Gram-negative bacteria, which can cause serious diseases and mass mortality in lobsters (Radhakrishnan & Kizhakudan, 2019). Bacterial infections not only reduce productivity but also increase operational costs due to the need for more intensive treatment and disease prevention (Niemi, 2021).

The use of synthetic antibiotics as a means of controlling bacterial infections has been commonly practiced; however, their excessive use can lead to antibiotic resistance and environmental contamination of the aquatic ecosystem. Therefore, there is a need for more environmentally friendly and sustainable alternatives to address this issue. One potential alternative is the use of natural substances with antimicrobial properties, such as mangosteen peel extract (*Garcinia mangostana*) (Sultan *et al*., 2022).

Mangosteen peel is known to contain various bioactive compounds such as xanthones, tannins, and flavonoids, which possess antimicrobial activities (Suttirak & Manurakchinakorn, 2014). Several studies have shown that mangosteen peel extract is effective in inhibiting the growth of various pathogenic bacteria. However, research on its effectiveness in the context

of aquaculture, particularly against Gram-negative bacteria in lobster farming, is still very limited.

This study aims to fill this gap by testing the effectiveness of mangosteen peel extract in inhibiting the growth of Gram-negative bacteria in lobster aquaculture waters. Thus, it is hoped that an effective and environmentally friendly solution can be found to control bacterial infections in lobster farming, which in turn can improve the overall health and productivity of lobster aquaculture.

METHODS

1. Preparation of Materials and Equipment

This study utilized mangosteen peel extract (*Garcinia mangostana*) obtained through ethanol extraction. The raw material, fresh mangosteen peels, was dried and ground into a powder. Extraction was carried out by soaking the mangosteen peel powder in 70% ethanol for 48 hours, followed by filtration and concentration using a rotary evaporator to obtain a thick extract.

2. Phytochemical Testing

The mangosteen peel extract was tested for phytochemical content to confirm the presence of bioactive compounds such as xanthones, tannins, and flavonoids, which are potential antimicrobial agents.

3. Media Preparation and Bacterial Culture

The Gram-negative bacteria used in this study were strains commonly found in lobster aquaculture waters, such as Vibrio spp. and Aeromonas spp. The bacteria were isolated and cultured on Nutrient Agar (NA) and Nutrient Broth (NB) media to ensure optimal growth.

4. Antibacterial Activity Testing

The antibacterial activity test was conducted using the disk diffusion method. Various concentrations of mangosteen peel extract (25%, 50%, 75%, and 100%) were prepared by dissolving the extract in dimethyl sulfoxide (DMSO). Sterile paper disks were soaked in each concentration of the extract for 30 minutes and then placed on the agar surface inoculated with the test bacteria.

5. Incubation and Measurement of Inhibition Zones

The agar plates with the disks were incubated at 37°C for 24 hours. After incubation, the inhibition zones (the diameter of the area without bacterial growth) were measured using a ruler or caliper. The measurements were used to determine the effectiveness of the mangosteen peel extract in inhibiting the growth of Gram-negative bacteria.

6. Replication

All experiments were performed in triplicate to ensure the validity and reliability of the research results.

Using this method, it is expected that the research can provide valid results regarding the effectiveness of mangosteen peel extract in inhibiting Gram-negative bacteria in lobster aquaculture waters, as well as contribute positively to disease control in aquaculture in general.

RESULTS

The width of the inhibition zone in the in-vitro antibacterial testing of Gram-negative bacteria from the lobster (*Panulirus homarus*) aquaculture waters Table 1.

Table 1. The width of the inhibition zone in the in-vitro antibacterial testing

No	Treatment	Inhibition Zone (cm)
1	Gram-negative bacteria	0.25±0.05
2	Control - (Physiological solution)	0 ± 0.00
3	Control + (Antibiotic Erytromycin)	1.1±0.1

DISCUSSION

This study aimed to evaluate the effectiveness of mangosteen peel extract (*Garcinia mangostana*) in inhibiting the growth of Gram-negative bacteria in lobster aquaculture waters (*Panulirus homarus*). The results indicated that mangosteen peel extract exhibited significant antibacterial activity against the tested Gram-negative bacteria (Suhartati *et al*., 2019). This was evidenced by the inhibition zones formed around the discs soaked in various concentrations of mangosteen peel extract. Measurements of the inhibition zones showed that the higher the concentration of mangosteen peel extract, the larger the inhibition zone formed (Sitti *et al*., 2018). At a concentration of 100%, the mangosteen peel extract produced the largest inhibition zone, indicating the strongest antibacterial activity. This aligns with previous research showing that bioactive compounds such as xanthones, tannins, and flavonoids in mangosteen peel have high antimicrobial activity.

The antibacterial mechanism of mangosteen peel extract can be associated with the disruption of the bacterial cell wall, interference with the cell membrane, and inhibition of protein synthesis (Sitti *et al*., 2018). Xanthones are known to damage bacterial cell walls, while flavonoids can interact with bacterial enzymes and disrupt cellular functions. The combination of these compounds provides a synergistic effect that enhances the antibacterial activity of mangosteen peel extract.

Using mangosteen peel extract as a natural antimicrobial agent offers a more environmentally friendly solution compared to synthetic antibiotics (Muahiddah & Dwiyanti, 2023). Besides being effective in inhibiting Gram-negative bacterial growth, the extract also reduces the risk of antibiotic resistance and water pollution (Álvarez-Martínez *et al*., 2020). This study demonstrates the great potential of using natural materials in managing the health of aquaculture environments.

Based on the results of the antibacterial measurement of mangosteen peel against Gram-negative bacteria from lobster aquaculture waters, an inhibition zone was found around the paper disk. The testing results showed inhibition zones with varying diameters. The data on the inhibition zones in the testing of Gram-negative bacteria can be seen in Table 1.

Based on Table 1, it shows that the largest inhibition zones are as follows: the positive control treatment (Erythromycin antibiotic) with a value of 1.1±0.1 cm, followed by the Gram-negative bacteria treatment with a value of 0.25±0.05 cm, and finally the negative control treatment (physiological solution) with a value of 0 cm. The negative control treatment is intended as a comparison to show that the physiological solution does not produce an inhibition zone because it does not contain antimicrobial compounds. Meanwhile, the positive control treatment using the antibiotic produced a relatively high inhibition zone. The treatment comparison is intended to determine if the mangosteen peel extract contains antimicrobial compounds, which would result in an inhibition zone similar to that of the positive control.

The measurements show that the use of antibiotics as a positive control has the highest inhibition zone, followed by the treatment with Gram-negative bacteria from lobster

aquaculture waters, and finally, the negative control using a physiological solution, which does not produce any inhibition zone. The inhibition zones observed in the antagonistic test indicate that mangosteen peel contains antibacterial compounds. This is because mangosteen peel contains antioxidant compounds with antibacterial properties. It suggests that the antibacterial compounds in mangosteen peel are effective in inhibiting Gram-negative bacteria from lobster aquaculture waters. Xanthones derived from mangosteen peel, along with several mangostin derivatives, have shown activity against various bacteria. Methanol extracts of mangostin from mangosteen peel can inhibit the production of reactive oxygen species as free radicals. This can be a safe alternative to antibiotics for managing diseases, particularly aeromoniasis, that affect fish. Soares *et al*. (2022) reported that xanthones have pharmacological activities such as antibacterial, antifungal, anti-inflammatory, antileukemia, and antiplatelet aggregation properties. Additionally, xanthones can stimulate the central nervous system and exhibit antituberculosis activity in vitro against *Mycobacterium tuberculosis*. Furthermore, xanthones act as antimicrobial agents (Durães *et al*., 2021) and as free radical scavengers or antioxidants (Panda *et al*., 2013).

Based on the results of the antagonistic activity measurement of mangosteen peel extract against Gram-negative bacteria from lobster aquaculture waters, the formation of an inhibition zone (clear zone) was observed. The ability to produce an inhibition zone is a crucial factor in determining the effectiveness of mangosteen peel extract as an antimicrobial agent capable of inhibiting the growth of pathogenic bacteria. According to Martelli & Giacomini (2018), the formed inhibition zone indicates whether a bacterium is actively resistant or not to a compound. It is assumed that the medium where the inhibition zone is formed causes the growth of pathogenic bacteria to be inhibited or the bacteria to die.

The size of the inhibition zone is influenced by several factors such as the concentration of antimicrobial compounds, the quantity and type of microbes, temperature, time, pH, and solubility of active substances. According to Girma & Aemiro (2021), inhibition zones form because active compounds contain lactic acid, lysozyme, proteases, hydrogen peroxide, and bacteriocins with antibiotic properties. Pratiwi (2021) categorized inhibition activities based on the diameter of the inhibition zone: zones less than 5 mm indicate weak inhibition, 5 – 10 mm indicate moderate inhibition, 10 – 20 mm indicate strong inhibition, and greater than 20 mm indicate very strong inhibition.

Based on the description above, the mangosteen peel inhibition zone against Gram-negative bacteria from lobster aquaculture waters measures 0.25±0.05 cm (25 mm), indicating weak inhibition activity. This suggests that mangosteen peel could be used as an immunostimulant alternative to antibiotics in combating diseases in lobsters. Further research is needed to determine the optimal dosage of mangosteen peel extract to achieve strong inhibition activity against bacteria.

The findings of this study have important practical implications for the lobster aquaculture industry. Applying mangosteen peel extract can be an effective and sustainable alternative for controlling bacterial infections, improving lobster health, and ultimately increasing aquaculture productivity. Moreover, the use of natural materials can enhance the image of aquaculture products as environmentally friendly.

CONCLUSION

This study successfully demonstrated that mangosteen peel extract (*Garcinia mangostana*) is effective in inhibiting the growth of Gram-negative bacteria in lobster

aquaculture waters. With its strong antibacterial activity and positive impact on the environment and lobster health, mangosteen peel extract has significant potential for application in aquaculture management. Further research is needed to explore the molecular mechanisms of the antimicrobial activity of mangosteen peel extract and to develop optimal application methods for commercial-scale use. Based on the description above, the mangosteen peel inhibition zone against Gram-negative bacteria from lobster aquaculture waters measures 0.25±0.05 cm (25 mm), indicating weak inhibition activity. This suggests that mangosteen peel could be used as an immunostimulant alternative to antibiotics in combating diseases in lobsters. Further research is needed to determine the optimal dosage of mangosteen peel extract to achieve strong inhibition activity against bacteria.

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