Potential of ginger (Zingiber officinale) crude extract as a chemotherapeutic agent for tiger grouper (Epinephelus foscoguttatus) infected by Aeromonas hydrophila

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Abstract. One week of study was conducted to observe the potential of ginger crude extract in the treatment of tiger grouper (Epinephelus foscoguttatus) infected by a pathogenic bacterium in aquaculture, Aeromonas hydrophila. A total of 135 fish were injected intraperitoneally with 0.1ml of A. hydrophila (10⁶ CFU ml⁻¹) and stock in 15 aquaria (13L). Four concentrations of ginger extract (0.4g/L, 0.8g/L, 1.2g/L and 2.0g/L) were used as the treatment and the control is 0g/L. Water parameters such as dissolved oxygen, pH, temperature and salinity of the treatment tank were in the range of (4.05 – 4.20 ppm), (7.2 – 8.2), (25.6 – 28.8⁰C) and (22-25ppt) respectively. The extract's potential as a treatment agent in the infected fish was recorded in terms of fish survival and physical condition such as the sign of bacterial infection and swimming behaviour. The results show infected tiger grouper treated with a concentration of ginger extract of 0.8g/L can improve the survival rate of fish by 89%. It can be concluded that crude ginger extract can enhance the survival rate and has the potential to be used as a chemotherapeutic agent in bacterial infection of tiger grouper fish.

Keywords. Aeromonas hydrophila, Epinephelus foscoguttatus, ginger extract, survival rate.

1. Introduction
Rapid growth of aquaculture industry and the increasing demand for fish lead to the intensification of cultivation activities, the overdrawing of fish stressors and the worsening of disease risk. Chronic stress has an extreme effect on fish health, causing specific immune responses and defense mechanisms to be inhibited, leading to pathogenic infections in favourable conditions. Synthetic chemicals and antibiotics have traditionally been used as a preventive or prophylactic medium for the treatment of fish diseases. Chemotherapy has until now been the only option to prevent and treat outbreaks of aquaculture disease. However, the use of chemical drugs has several negative effects on both the environment and human beings [1, 2].

Epinephelus foscoguttatus is a tiger grouper commonly belong to family Serranidae and order Perciformes. They are distributed in warm tropical and subtropical sea areas and cultivated marine fish
species of high economic value. However, a skin ulcer disease with high mortality can be occur in the culture that cause by pathogenic bacteria [3]. Aeromonas hydrophila is a gram-negative bacteria and a common pathogen that widely available for fish and shrimp in all kinds of environments. The presence of the colonies can also shift mortality to other species and humans. Aeromonas hydrophila was identified as a virulence outbreak in the United States where the bacteria have been shown to originate from Asia [4].

Zingiber officinale, commonly known as ginger, is a plant in the family Zingiberaceae and has a long history of medicinal use [5]. Ginger contains several phytochemicals among which the most significant groups are non-volatile resins and volatile essential oils [6]. The principal non-volatile pungent constituents of oleoresin are polyphenolic compounds that responsible for their unique pharmacological effects [7]. Several properties of the ginger have been verified in scientific experiments, with emphasis to the antimicrobial activity. Ginger essence oil has been investigated by several in vitro microbiological techniques, in which most of its essential oils presented antimicrobial activity against selected bacteria [8]. In view of reports on Z. officinale antibacterial activity, hence this study was conducted to observe the potential of Z. officinale crude extract in treatment of tiger grouper infected by Aeromonas hydrophila.

2. Materials and Methods

2.1 Plant Preparation and Extraction
Rhizome of Zingiber officinale were bought from local market at Sandakan, Sabah. The ginger peel was removed, cleaned and rinsed in distilled water. The ginger rhizome was cut into small pieces and allowed to oven dried at 38 °C for 48 hours. Dried ginger was weighed and ground into a fine powder in a blender. 10 g of fine grounded ginger powder was measured out using an electronic balance (OHAUS: model NVL2101) and then transferred into a conical flask containing 50 ml of distilled water. It was corked with cotton wool and foil, shaken gently and allowed to stand in room temperature for 24 hours. The content was then transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No. 1 filter paper. Ginger extract was assumed to be 100% concentration and stored in the refrigerator at 5 °C prior to use [9].

2.2 Bacterial Strain and Culture
Aeromonas hydrophila that can cause mortality in fish were obtained from Borneo Marine Research Institute, University Malaysia Sabah. The bacteria stock was transferred to Microbiology and Biotechnology Laboratory, Department of Agrotechnology and Bio-industry, Politeknik Sandakan. Prior to in vivo assay, bacteria were recovered from glycerol stocks by streaking on indicated TSB agar medium and incubated at 30°C for 24 hours. Each bacterial pre-culture was prepared by inoculating 5ml of the same broth medium with a single colony and incubated at 30°C while shaking for 16 – 32 h. After incubation, bacterial suspension was then diluted with TSB broth at a cell density of 10^6 colony forming units/ml (CFU mL^-1) [10].

2.3 Challenge Test and experimental setup
A total of 135 tiger grouper fish in an average length of 3-4 inches were bought from local farm, Borneo Aqua Harvest Bhd., Sandakan, Sabah. Fish was acclimatized in a polyethylene tank (500L) for one week prior to the study. Fish were fed ad libitum twice per day and kept with proper aeration in marine water. Experiment was conducted with four treatment groups consist of T1, T2, T3, T4 and TC in triplicate. Each group comprised 9 tiger grouper fish per 13L aquarium and equipped with aeration system. Then, all five groups were injected intraperitoneally with 10^6 cfu ml^{-1} of A. hydrophila suspension (0.1 ml fish^{-1}). Various concentrations of ginger crude extract (0.4g/L, 0.8g/L, 1.2g/L and 2.0g/L) were prepared in the aquarium treatment T1, T2, T3 and T4 respectively. For the TC aquarium, fish did not treat with ginger crude extract. All the fish in all treatment were regularly monitored three times daily. Fish were fed ad libitum twice per day. Uneaten feed was removed from the tank to maintain water quality. Water
changed when necessary, but the ginger extract concentration keep remain the same for each treatment until the end of the study.

2.4 Data Collection and Analysis
Water temperature were taken daily in this study by using a digital thermometer. Dissolved oxygen concentration was measured by dissolved oxygen meter and water pH was measured using pH meter daily. Salinity of the water measured by refractrometer [11]. Survival rate was determined using this formula: SR (%) = (number of fish survived/number of fish injected) × 100. For clinical observation, fishes were examined externally for any injury, infections and diseases. The physical appearance, feeding habit and swimming activeness level of tiger grouper fish were evaluated by using the number of scales that modified from the Heiman-Carver colour rotor [12]. The scale consists of four numbers 1 to 4.

3. Results
Water quality parameters measured throughout this study are summarized in Table 1. Only four basic water parameters measured to maintain the water quality. The dissolved oxygen was set in the range of 3.0 – 6.0 ppm by aeration system with slow air bubble. The water pH was in the range of 7.2 – 8.2 where T3 (0.7g/L) possesses slightly alkaline water pH. Temperature for the treatment tank in this study was maintained in the range of 26 – 29°C. The water salinity is in the range of 22 – 25 ppt since it was adjusted to the salinity used in marine water from the tiger grouper supplier farm. All the water quality reading is acceptable for the tiger grouper fish to live healthily.

| Water Parameter | TC (0g/L) | T1 (0.4g/L) | T2 (0.8g/L) | T3 (1.2g/L) | T4 (2.0g/L) |
|-----------------|-----------|-------------|-------------|-------------|-------------|
| Dissolved Oxygen (ppm) | 3.0-6.0 | 4.0-6.0 | 4.0-6.0 | 3.0-6.0 | 3.0-6.0 |
| pH              | 7.2-7.8 | 7.2-8.0 | 7.4-8.2 | 7.3-8.0 | 7.3-8.0 |
| Temperature (°C) | 26-27 | 26-29 | 26-29 | 26-29 | 26-29 |
| Salinity (ppt)  | 22-25 | 22-25 | 22-25 | 22-25 | 22-25 |

After the ginger crude extract was applied as the chemotherapeutic agent for A. hydrophila infection in tiger grouper fish aquaria, T2 (0.8g/L) show the highest final survival rate with a value of 96%. While the other treatments are 67%, 56%, 56% and 44% for T1 (0.4g/L), T3 (1.2g/L), T4 (2.0g/L) and TC (0g/L) respectively. The ginger crude extract show effectiveness in a certain concentration of application. For the high concentration in T3 (1.2g/L) and T4 (2.0g/L), the mortality of the fish increase such the tiger grouper fish cannot survive in the ginger crude extract immersion. However, the highest mortality is showed in TC (0g/L) which no ginger crude extract applied.

Daily physical examination was done to observe any abnormality of tiger grouper fish. Symptoms of bacterial infection appeared after the second day of tiger grouper challenge by intraperitoneal injection using 1ml of A. hydrophila per fish. The clinical sign of bacterial infection was shown in Figure 2. On the second day of the bacterial challenge, fish skin starts swelling and progressing to lesion. The most common clinical sign observed is redness or white areas on the body. Pop eyes and fin rot also observed at almost fish in all the treatment aquaria. Fish become inactive, show lethargic and keep dormant at the aquarium bottom. After two days of injection, most of the fish show swimming abnormalities where they are swirling slowly and rub their bodies to the aquarium wall. At the beginning of the challenge test, off-feed was observed. Fish were fed on demand during the study.

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Figure 1: Final survival rate of challenged tiger grouper treated with ginger crude extract.

Figure 2: Aeromonas hydrophila associated disease appear after challenge test: (a) fish body swelling and skin progressing to lesion; (b) swollen and contraction on fish muscle and progressing to skin lesion.

Treatment of ginger crude extract shows the healing process in fish where the clinical sign of the bacterial disease was reduced after a few days of treatment. The results of the observation were shown in Table 2. After the tiger grouper fish challenged by A. hydrophila, all the health performance are reduce to the lowest score. Majority of the fish loss appetite. There was less swimming activity and fish become static and inactive. They stay at the bottom of the aquarium. Wounds appear on their progressing to haemorrhage body wall. After seven days of study, most of fish treated in ginger crude extract (0.4g/L, 0.8g/L, 1.2g/L and 2.0g/L) show the best results of physical appearance where no defects or skin lesions were found on their body. Their swimming activity and feeding habit also show some improvement in progressing to normal healthy fish. Compared to control treatment with no ginger crude extract application, their swimming activity and physical appearance performance remained low, and the appetite was lost. Overall of the occurrence of bacterial infection symptoms on fish was shown in Table 3.

Table 2: Score of tiger grouper behaviour and physical appearance

| Treatment   | Day | Swimming activeness against stimuli | Feeding habit | Physical appearance |
|-------------|-----|------------------------------------|--------------|---------------------|
| TC (0g/L)   | 1   | 1                                  | 1            | 1                   |
|             | 2   | 1                                  | 1            | 1                   |
|   |   |   |   |
|---|---|---|---|
| 3 | 1 | 1 | 1 |
| 4 | 1 | 1 | 1 |
| 5 | 1 | 1 | 1 |
| 6 | 1 | 1 | 1 |
| 7 | 2 | 1 | 2 |

T1 (0.4g/L)
|   |   |   |   |
|---|---|---|---|
| 1 | 1 | 1 | 1 |
| 2 | 1 | 1 | 1 |
| 3 | 1 | 1 | 1 |
| 4 | 1 | 1 | 3 |
| 5 | 3 | 1 | 3 |
| 6 | 3 | 2 | 3 |
| 7 | 3 | 2 | 4 |

T2 (0.8g/L)
|   |   |   |   |
|---|---|---|---|
| 1 | 1 | 1 | 1 |
| 2 | 1 | 1 | 1 |
| 3 | 1 | 1 | 1 |
| 4 | 3 | 1 | 3 |
| 5 | 3 | 1 | 3 |
| 6 | 3 | 2 | 4 |
| 7 | 3 | 2 | 4 |

T3 (1.2g/L)
|   |   |   |   |
|---|---|---|---|
| 1 | 1 | 1 | 1 |
| 2 | 1 | 1 | 1 |
| 3 | 1 | 1 | 1 |
| 4 | 3 | 1 | 3 |
| 5 | 3 | 2 | 4 |
| 6 | 3 | 2 | 4 |
| 7 | 3 | 2 | 4 |

T4 (2.0g/L)
|   |   |   |   |
|---|---|---|---|
| 1 | 1 | 1 | 1 |
| 2 | 1 | 1 | 1 |
| 3 | 1 | 1 | 1 |
| 4 | 1 | 1 | 1 |
| 5 | 3 | 1 | 3 |
| 6 | 3 | 1 | 3 |
| 7 | 3 | 2 | 4 |

Note:
Swimming activeness against stimuli score represent: 1-Static, 2-Slow to respond, 3-Give a bit respond, and 4-Normal respond;
Feeding habit score represent: 1-Loss appetite, 2-Less appetite, 3-Moderate appetite, and 4-Normal appetite;
Physical appearance score represents: 1- Fish skin has wounds and haemorrhage body wall, 2-Reddish fish body with haemorrhage body wall, 3-The skin of fish is pale, and 4-There are no defects or skin lesions.
### Table 3: Occurrence of bacterial infection symptoms on fish

| Treatment | Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-----------|-----|---|---|---|---|---|---|---|
| TC (0g/L) |     | + | + | + | + | + | + | + |
|           |     | + | + | + | + | + | + | + |
| T1 (0.4g/L) |   | + | + | + | + | + | - | - |
|           |     | + | + | + | + | + | - | - |
| T2 (0.8g/L) |   | + | + | + | - | - | - | - |
|           |     | + | + | + | - | - | - | - |
| T3 (1.2g/L) |   | + | + | + | - | - | - | - |
|           |     | + | + | + | - | - | - | - |
| T4 (2.0g/L) |   | + | + | + | + | + | + | + |
|           |     | + | + | + | + | + | + | + |

Symbol:

(+) show the symptom of bacterial infection
(-) does not show bacterial infection symptom

### 4. Discussion

Management of water quality is very important for fish cultivation. A healthy, balanced, and functioning aquaculture system requires optimal water quality. Water quality in this study has been kept in the optimum range to avoid fish stress due to poorer water quality, since water quality problems can promote stress in fish and hence fish disease. For this challenge test study, water in the same concentration of the ginger crude extract was changed for each treatment when necessary to avoid water quality deterioration and microbial contamination increase. Fish was fed ad libitum in order to minimize organic pollution from wastes of fish and feed. Water quality has been maintained because tropical fish disease and mortality are caused by poor water quality [13, 14].

*Aeromonas hydrophila* is opportunistic bacteria that can be found in freshwater to brackishwater. Unlike in freshwater fish species, virulent of this bacteria is more serious. Even this challenge trial in tiger grouper does not show severe hemorrhagic septicaemia symptoms on tiger grouper, but the bacterial infection symptom was observed in fish behaviour and survival. The most common disease that causes by *A. hydrophila* is motile aeromonas septicaemia (MAS). Freshwater and saltwater fish species are susceptible to this disease. The disease is manifested clinically with haemorrhages, ulcerations, abscesses, ascitic fluid and anaemia. Mortality rates are high, and aquaculturists incur substantial economic losses, thereby necessitating timely measures of control for prevention and treatment [15].

Medicinal plants are widely used as phytotherapeutic agents for treating infectious diseases in animals and humans since ancient times. Their use for therapy of bacterial diseases in fish is safe. Plant extract reported to influence various activities such as anti-stress, increase growth performance, stimulate appetite and can be anti-pathogenic in fish and shrimp aquaculture because plants contain alkaloids, terpenoids, tannins, saponins, glycerol, flavonoids, steroids or essential oils [15,16]. This immersion method of disease management using ginger crude extract is applicable. The application of ginger crude extract very practical which no chemical being used in the process of extraction. This chemotherapeutic agent is eco-friendly and provision to green aquaculture production. Since there is so much antibiotic applied in the current aquaculture industry, the approach of using plants in fish disease treatment is highly recommended [2,17].
5. Conclusion
In conclusion, the ginger crude extract has potential as a chemotherapeutic agent in bacterial disease treatment of *Epinephelus fuscoguttatus* if used in an appropriate amount of concentration. This organic extract can improve the survival of fish and reduce the symptoms of bacterial infection. Ginger extract is another alternative treatment that can save on disease management costs because the source is cheap and easy to obtain. Instead using drugs and chemicals that more expensive will bring adverse effects that can affect the environment and endanger other aquatic life species.

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