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Inclusion of *Myrmecodia pendens* bulb Extract in the Diet Stimulates Immune Response in *Clarias gariepinus* against *Aeromonas hydrophila*

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Abstract

To evaluate the effects of *Myrmecodia pendens* bulb extract (MBE) inclusion on the growth and blood profile of catfish (*Clarias gariepinus*), a group of fish were fed with 1% MBE (T) and compared with a control (C). After 30 days of feeding, the growth, survival and blood profiles were compared between the two groups. Surviving fish from each group were distributed into five subgroups: C without injection (CG1), C with *Aeromonas hydrophila* injection (CG2), C injected with *A. hydrophila* and antibiotics (CG3), T without injection (TG4) and T with *A. hydrophila* injection (TG5). Survival and blood profile post-injection were evaluated for 48 h. After 30 days of feeding, the growth, survival and blood profiles of fish fed MBE was higher than the control. Meanwhile, 24 h post-bacterial injection, fish CG2 showed significantly reduced survival (70%), erythrocytes (2.15 x 10^6 μL^{-1}), platelets (39.48 x 10^3 μL^{-1}) and haemoglobin (4.24 g dL^{-1}). However, increases in leucocytes (49.55 x 10^3 μL^{-1}) and lymphocytes (49.32 x 103 μL^{-1}) were found in fish TG5. This finding demonstrated the usefulness of MBE inclusion in the diet of *C. gariepinus* for improving growth, survival, blood profile and resistance to *A. hydrophila*.

Keywords: *Clarias gariepinus*, *Myrmecodia pendens*, survival, blood profile, *Aeromonas hydrophila*

1. Introduction

One of the most significant pathogens in many fish cultures, causing mass mortality, is Aeromonas species (Hoai et al., 2019). *Aeromonas hydrophila*, an opportunistic Gram-negative pathogen living in freshwater, has been identified as one of the Aeromonas species that causes mass disease outbreaks in fish such as *Siniperca chuatsi* (Chen et al., 2018) and *Carassius auratus* (Linnaeus 1758) (Dharmaratnam et al., 2018). Previous research has revealed that *A. hydrophila* intramuscular injection into *Pangasianodon hypophthalmus* (Sauvage) resulted in a virulent reaction and decreased immune response (Tamamdusturi and Yuhana, 2016).

To tackle mass mortalities of fish cultures, some fish farmers use antibiotics to prevent virulent reactions to A. hydrophila (Sinclair et al., 2016), as commonly used for haemorrhagic septicaemia disease in *Pangasianodon hypopthalmus* (Xuan et al., 2018). Nevertheless, antibiotics are expensive, and their inappropriate application may induce environmental deterioration and negatively affect farming bio-environments (Sinclair et al., 2016). In addition, the increasing market demand for eco-friendly fish products is necessary. As an alternative, researchers have attempted to use various plant extracts as a natural antimicrobial agent.

A potential plant that can be used as an antimicrobial agent source to increase survival and immunocompetence in fish is the ant nest plant (*Myrmecodia* sp). The Myrmecodia species, which has been taxonomically determined as *M. tuberosa* and *M. pendens*, is widely used in West Papua, East Kalimantan and several regions in Indonesia as a herb with significant therapeutic value (Hertiani et al., 2010). Local people have used this species as part of herbal remedies for several medicinal applications, such as ulcers, haemorrhoids, nosebleeds, backaches, allergies, uric acid disorders, strokes, coronary heart problems, Tuberculosis, tumours, cancers and lactagogum (Soeksmanto et al., 2010). *M. pendens*, an epiphyte plant, attaches to large trees and can be found in bubbles underneath the rod (Hamsar and Mizaton, 2012). Previous studies have reported that qualitatively, Myrmecodia contains several important secondary metabolite compounds, namely phenolics, flavonoids, alkaloids, saponins and steroid/triterpenoids (Sari et al., 2017) that are potentially beneficial for medical purposes.

According to Gartika et al. (2018), *M. pendens* bulb extract (MBE) can inhibit and eradicate Streptococcus mutans biofilms. Hertiani et al. (2010) also found that *M. tuberosa* Jack's quorum-sensing has a relationship with the pathogenicity of *Pseudomonas aeruginosa* and

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Staphylococcus aureus. Meanwhile, an ethyl acetate fraction of methanolic extracts of *Myrmecodia tubers* reduced the growth of *Streptococcus sanguis* ATCC 10566 (Fatriadi et al., 2018), and a previous study of Myrmecodia species in fish also found that 0.5–1% Myrmecodia extract is useful for enhancing the growth and blood profile status of *P. hypopthalmus* (Nugroho et al., 2019).

Although there has been a significant amount of research regarding Myrmecodia species, there has been relatively little focus on the aquaculture field. Thus, it is necessary to perform in-depth research on the application of *M. pendens* in fish, particularly with respect to its role as a therapeutic agent for tackling bacterial pathogens. This study investigated the effects of MBE on the survival and blood profile (including erythrocytes, leucocytes, haemoglobin value (Hb) and platelets (PLT) in *C. gariepinus* when challenged with *A. hydrophila*. Differential leucocytes such as lymphocytes, monocytes and granulocytes, all of which are pivotal blood indices in the immune system of *C. gariepinus*, were also determined during pre- and post-challenge tests.

The immune status of catfish is related to their blood profiles, which are important in monitoring the health status of fish (Shen et al., 2018). Blood profiles such as erythrocytes, leucocytes, Hb, and PLT in fish are pivotal tools that can be used to determine physiological and pathological status in fish (Aliko et al., 2018). Leucocytes or white blood cell counts are generally used as fish health status indicators; they relate to components of the innate immune system and involve immunological function regulation in fish (Velazquez-Carriles et al., 2018). Immunological function related to physiological responses in fish can also be measured by alterations in both total and differential leucocyte counts. These counts have been used as regular indicators in the health status and immune competence of various fish (Jaafar et al., 2016; Aliko et al., 2018).

2. Materials and Methods

2.1. Plant materials

M. pendens bulbs were purchased from a local market. To make a powder, bulbs were dried, cut into small pieces and ground into a powder. This powder was extracted using 95% ethanol for two days, filtrated, evaporated and stored at 4 °C until it was used as a crude extract (MBE). Every 75 g of *M. pendens* bulb powder in 1 L of 95% ethanol produced 18 g crude extract.

2.2. Control and treatment diet preparation

The control diet was a commercial pellet and was obtained from a local commercial market (Hi Pro Vite FF-888). The control diet contained 36–38% protein, 2% lipid, 2% crude fibre, 10% ash and 12% moisture. The treatment diet was prepared by adding 1% MBE to the control diet. The optimum MBE addition (1%) was determined from previous experiments. Both control and treatment diets were repelletised using a mincer, dried in an oven at 50 °C and allowed to cool to room temperature. All diets were stored in a dark plastic bag prior to being given to fish.

2.3. Fish preparation and experimental setup

Six-hundred fish (average initial weight 27.36 g) were obtained from a local breeding fish farm in Samarinda, East Kalimantan, Indonesia. All fish were acclimated at the Animal Physiology, Development, and Molecular Laboratory, Mulawarman University, East Kalimantan for five days. The fish were then randomly distributed into two groups (C and T) with triplicate groups of 40 fish per replicate group. Each fish was then placed in an individual plastic tank (60 L capacity, 40 L of freshwater in each tank). For 30 days (pre-challenge), fish in each group were fed with control or treatment diets at a rate of 3% of body weight three times per day. Temperature, pH, and dissolved oxygen (DO) were checked once a week with a routine thermometer and an HM-7 pH meter (TOA-DKK Corporation, Japan). Nitrate, nitrite and ammonium were also checked weekly using chemical test kits (Salifert test kit™). Siphoning was carried out daily to remove uneaten food and faeces before renewing the water. Forty percent of the water volume was renewed every day and maintained at 40 L of water in every plastic tank.

2.4. Challenge test

On the final day of the feeding trial (day 30), all surviving fish in both control and treatment groups were distributed as follows: the control group was randomly divided into triplicates of three subgroups (30 fish per subgroup): control (C) subgroup fish without bacterial injection (CG1), control subgroup fish with bacterial injection (CG2) and control subgroup fish with bacteria and antibiotic (Gentamycin sulphate 0.1%) injection (CG3). The treatment group of fish was randomly distributed into triplicates of two subgroups (30 fish per subgroup): treatment (T) fish group with no injection (TG4) and with bacterial injection (TG5). All bacterial injections were performed with 20 µL of *A. hydrophila* at 10^6 cfu mL^{-1} intraperitoneally.

2.5. Sampling and analytical procedure

On the first and final days of pre-challenge, the weight of all fish was measured to calculate the initial weight, final weight, body weight gain (BWG), daily weight gain (DWG), average weekly gain (AWG) and specific growth rate (SGR). Feed properties such as feed conversion ratio (FCR) were also determined. At the end of the pre-challenge test, blood profile, such as the number of erythrocytes (10^6 μL^{-1}) and leucocytes (10^3 μL^{-1}), haemoglobin (g dL^{-1}), lymphocytes (10^3 μL^{-1}), monocytes (10^3 μL^{-1}), granulocytes (10^3 μL^{-1}) and PLT (10^3 μL^{-1}) was determined. The survival rate of the fish in each group was noted every week during the pre-challenge test. During the challenge test, the survival and blood profile of fish (N = 3 fish per subgroup) was taken and measured every 24 h interval until 48 h. Blood profile measurement was performed using a Mindray BC2800 Auto Haematology Analyser (Mindray®Shenzhen, China).

2.6. Statistical analysis

Data are expressed as means ± standard error (SE) and were analysed using SPSS version 22 (SPSS, Inc., USA). Survival data were firstly transformed to arcsine. Blood parameters such as erythrocytes, leucocyte, Hb and differential leucocytes before the challenge test were analysed with a t-test to determine the significance of differences between control and treatment groups. Meanwhile, two-way ANOVA was used to evaluate survival, erythrocytes, leucocytes, Hb, PLT as well as data regarding the number of lymphocytes, monocytes and

granulocytes in the post-challenge test. A Duncan Multiple Range post hoc test was used to determine significant differences. All test results were reported as significant if P values were less than 0.05.

3. Results

During the pre-challenge test, the temperature, pH and DO value were noted as follows: 28.1 °C ± 0.3, 7.17 ± 0.2 and 8.1 ± 0.3 mg L-1. The nitrate, nitrite and ammonia values in all tanks were below the limits of detection. The growth of both control and treatment groups of fish fed 1% MBE in the diet are presented in Table 1.

Table 1. Growth parameters and Survival of *Clarias gariepinus* fed 1% of *Myrmecodia pendens* bulb extract for 30 days

Parameters	Groups	
	Control	Treatment
Initial weight (g)	27.36±0.11[a]	27.72±0.18[a]
Final weight (g)	50.52±1.00[a]	67.11±0.81[b]
BWG (g)	23.16±0.97[a]	39.38±0.80[b]
AWG (g/week)	2.89±0.12[a]	4.92±0.10[b]
DWG (g/day)	0.41±0.01[a]	0.70±0.01[b]
SGR	1.19±0.03[a]	1.61±0.02[b]
FCR	2.00±0.09[a]	1.18±0.02[b]

Note: Control group = group of fish without *Myrmecodia pendens* bulb extract in the diet. Treatment group = group of fish with *Myrmecodia pendens* bulb extract supplementation in the diet. Mean±Standard error (SE) followed by different letter superscript (a, b) at the same row indicate significantly different ($P < 0.05$). BWG = Body weight gain, AWG = Average weekly gain, DWG = Daily weight gain, SGR = Specific growth rate, FCR = Feed conversion ratio.

After 30 days of the feeding trial, the growth of fish fed MBE in the diet was significantly higher ($P<0.05$) compared to the control group, as shown in the final weight, BWG, AWG, DWG and SGR. The FCR of fish fed 1% MBE in the diet (1.18±0.02) was significantly better than the control group. Significantly higher survival (t-test, $P < 0.05$) (Figure 1) and erythrocytes, leucocytes, Hb, PLT, lymphocytes and granulocytes (Table 2) were also found in fish fed MBE in the diet. However, monocytes were not affected by 1% MBE inclusion in the diet.

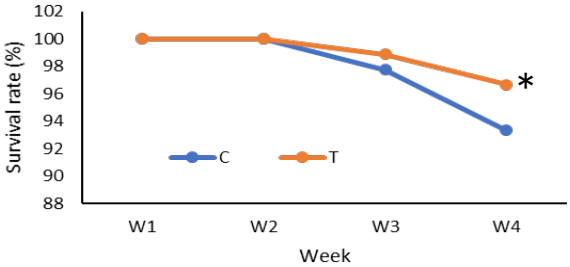

Figure 1. Survival of *Clarias gariepinus* fed 1% *Myrmecodia pendens* bulb extract in the diet for 30 days. * = significantly difference at $P<0.05$. C = control group of fish without *Myrmecodia pendens* bulb extract supplementation. T = Treatment group of fish with *Myrmecodia pendens* extract supplementation in the diet for 30 days.

Table 2. Blood parameters of *Clarias gariepinus* fed 1% of *Myrmecodia pendens* bulb extract for 30 days

Parameters	Groups	
	Control	Treatment
Erythrocyte ($10^6 \mu L^{-1}$)	2.11±0.02[a]	3.34±0.08[b]
Leucocyte ($10^3 \mu L^{-1}$)	38.51±0.06[a]	48.48±0.14[b]
PLT ($10^3 \mu L^{-1}$)	40.84±0.02[a]	41.80±0.05[b]
Hb (g dL^{-1})	5.75±0.03[a]	6.55±0.03[b]
Lymphocyte ($10^3 \mu L^{-1}$)	33.09±0.03[a]	43.39±0.16[b]
Monocyte ($10^3 \mu L^{-1}$)	1.06±0.01[a]	1.20±0.02[a]
Granulocyte ($10^3 \mu L^{-1}$)	1.04±0.005[a]	2.32±0.085[b]

Note: Control group = group of fish without *Myrmecodia pendens* bulb extract in the diet. Treatment group = group of fish with *Myrmecodia pendens* bulb extract supplementation in the diet. Mean±Standard error (SE) followed by different letter superscript (a, b) at the same row indicate significantly different ($P < 0.05$). PLT = Platelets, Hb = Haemoglobin.

In the challenge test, fish in the control subgroup injected with *A. hydrophila* (CG2) showed significantly reduced survival (Figure 2). As can be seen in Table 3, there was a significant decrease in the number of erythrocytes (2.15 x 10^6 μL^{-1}), platelets (39.48 x 10^3 μL^{-1}) and Hb (4.24 g dL^{-1}) in the control subgroup injected with *A. hydrophila* (CG2) at 48 h post challenge. However, increases of leucocytes (49.55 x 10^3 μL^{-1}) and lymphocytes (49.32 x 10^3 μL^{-1}) were found in fish TG5. Meanwhile, fish in the control subgroup injected with *A. hydrophila* and antibiotic (CG3) had relatively constant erythrocytes and monocytes, whereas an increase of leucocytes and granulocytes was observed 24 h post challenge. The Hb of fish in the subgroup CG3 was also gradually reduced until 48 h post challenge. Nevertheless, lymphocytes increased 24 h post challenge. Further, fish fed 1% MBE in the diet and injected with *A. hydrophila* (TG5) showed relatively constant erythrocytes and Hb values. The leucocytes, lymphocytes, monocytes and granulocytes were increased at 24 h post injection.

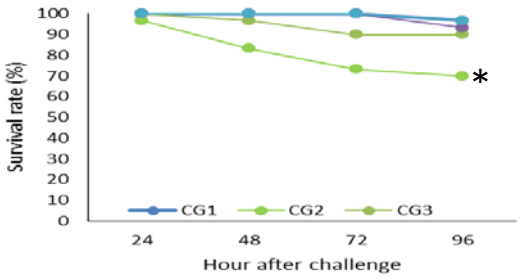

Figure 2. Survival rate of *Clarias gariepinus* in challenge test. Significantly decreased (*) of survival was found in fish groups without *Myrmecodia pendens* extract supplementation and injected with *Aeromonas hydrophila*. CG1 = subgroups fish without bacterial injection, CG2 = control subgroups fish with bacterial injection, CG3 = control subgroups fish with bacteria and antibiotic injection. TG4 = subgroups MBE-fed fish with no injection. TG5 = Subgroups of MBE-fed fish with bacterial injection.

Table 3. Blood parameters and differential leucocyte of *Clarias gariepinus* during challenge test

Parameters	Time	Control groups			Treatment groups	
		CG1	CG2	CG3	TG4	TG5
Erythrocyte (10^6 µL^{-1})	0	$_1$2.09±0.030^a	$_1$2.13±0.03^a	$_1$2.28±0.02^a	$_1$3.41± 0.166^b	$_1$3.28±0.0541^b
	24	$_1$2.17±0.018^a	$_2$2.22±0.19^b	$_2$2.42±0.03^{ab}	$_1$3.74±0.028^c	$_2$3.52±0.008^c
	48	$_1$2.26±0.008^a	$_1$2.15±0.02^b	$_2$2.10±0.05^b	$_1$3.74±0.038^c	$_{1,2}$3.38±0.067^d
Leucocyte (10^3 µL^{-1})	0	$_1$38.58±0.071^a	$_1$38.43±0.11^a	$_1$37.94±0.664^a	$_1$48.35±0.270^b	$_1$48.62±0.084^b
	24	$_1$38.44±0.2631^a	$_2$45.39±0.89^b	$_2$43.51±0.301^c	$_1$48.78±0.029^d	$_1$48.48±0.35^b
	48	$_1$38.06±0.3781^a	$_2$46.53±0.86^b	$_3$45.84±0.031^b	$_1$48.79±0.042^c	$_3$49.55±0.33^b
PLT (10^3 µL^{-1})	0	$_1$40.84±0.014^a	$_1$40.83±0.045^a	$_1$41.33±0.335^{ab}	$_1$42.83±0.110^b	$_1$41.76±0.020^b
	24	$_1$40.83±0.037^a	$_2$39.15±0.282^{ab}	$_{1,2}$41.84±0.054^c	$_1$42.74±0.063^d	$_2$42.67±0.013^a
	48	$_1$40.75±0.029^a	$_2$39.48±0.384^{ab}	$_{2,3}$41.53±0.280^c	$_1$42.91±0.023^c	$_2$42.38±0.247^a
Hb (g dL^{-1})	0	$_1$5.79±0.02^a	$_1$5.72±0.07^a	$_1$5.66±0.04^a	$_1$6.55±0.035^b	$_1$6.56±0.059^b
	24	$_1$5.49±0.06^a	$_2$5.47±0.05^a	$_2$5.63±0.07^a	$_1$6.72±0.067^b	$_{1,2}$6.53±0.015^c
	48	$_1$5.46±0.06^a	$_3$4.24±0.08^b	$_3$4.68±0.03^c	$_1$6.76±0.011^d	$_1$6.56±0.028^e
Lymphocyte (10^3 µL^{-1})	0	$_1$33.15±0.013^a	$_1$33.04±0.04^b	$_1$33.10±0.07^c	$_1$43.16± 0.056^d	$_1$43.63±0.26^e
	24	$_1$33.15±0.017^a	$_2$43.80±0.66^b	$_2$44.47±0.24^c	$_1$43.53±0.065^d	$_2$49.00±0.01^e
	48	$_2$33.24±0.031^a	$_2$43.19±0.60^b	$_3$48.17±0.30^c	$_1$43.56±0.008^d	$_2$49.32±0.28^e
Monocyte (10^3 µL^{-1})	0	$_1$1.05±0.017^a	$_1$1.08±0.015^a	$_1$1.20±0.034^a	$_1$1.24±0.035^b	$_1$1.17±0.03^b
	24	$_1$1.03±0.0006^a	$_1$1.18±0.017^a	$_1$1.08±0.012^a	$_1$1.37±0.037^b	$_1$1.44±0.23^b
	48	$_1$1.03±0.0009^a	$_1$1.17±0.005^a	$_1$1.14±0.052^a	$_1$1.47±0.018^b	$_1$1.21±0.02^b
Granulocyte (10^3 µL^{-1})	0	$_1$1.04±0.006^a	$_1$1.05±0.068^b	$_1$1.03±0.005^{ab}	$_1$2.21±0.147^b	$_1$2.44±0.021^c
	24	$_2$1.03±0.001^a	$_2$2.71±0.095^b	$_2$2.78±0.031^b	$_2$2.10±0.063^b	$_2$1.86±0.088^c
	48	$_3$1.02±0.0009^a	$_3$2.84±0.062^b	$_3$2.91±0.014^b	$_3$2.31±0.056^b	$_3$ 1.80±0.025^c

Note: Mean±Standard error (SE) follow by different letter superscript (a, b,c,d,e) at the same row indicate significantly different ($P < 0.05$). Meanwhile, different letter subscript (1,2,3) follow by Mean±Standard error (SE) at the same column indicate significantly different ($P < 0.05$). CG1 = subgroups fish without bacterial injection, CG2 = control subgroups fish with bacterial injection, CG3 = control subgroups fish with bacteria and antibiotic injection. TG4 = subgroups MBE-fed fish with no injection. TG5 = Subgroups of MBE-fed fish with bacterial injection. PLT = Platelets, Hb = Haemoglobin.

4. Discussion

Plant extracts containing active phytochemicals and various bioactive ingredient activities such as such as flavonoids, alkaloids, saponins, quinones, triterpenoids, tannins and phenolics have been claimed to be beneficial as a supplementation in the diet of aquaculture species (Afzali and Wong, 2019). The present findings revealed that the supplementation of 1% MBE can significantly enhance the growth at pre-challenge and survival of *Clarias gariepinus*, either pre or post challenge with *A. hydrophila*. Similarly, survival rate of *A. hydrophila*-infected silver catfish (*Rhamdia quelen*) bath with *Hesperozygis ringens* (Benth.) has also been reported (Rosa et al., 2019). According to Nugroho et al. (2019) and Nugroho et al. (2017), the phytochemicals from Myrmecodia may boost the innate immune system and improve survival rates of Siamese fighting fish (*Betta* sp) against *A. hydrophila*. Tilapia (*Oreochromis niloticus*) injected with *Boesenbergia pandurata*, *Solanum ferox*, *Zingiber zerumbet* also had significantly higher survival than controls with no injection (Hardi et al., 2018).

The *Myrmecodia pendens* is abundant in secondary metabolite plant ingredients such as flavonoids (Lestari et al., 2019). Maulida et al. (2018) found that flavonoids have a strong antioxidant property that enhanced superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activity, and reduced malondialdehyde (MDA) levels (Xie et al., 2018). Increases in such antioxidant enzymes may be useful for tackling free radical formation and decreasing lipidic superoxide damage, further increasing the growth and survival of *C. gariepinus*. Besides flavonoids, the biological properties of tannins for boosting fish survival have also been widely reported. For example, tannins from *Terminalia catappa* leaves improved the survival of *A. hydrophila*-infected *Betta* sp (Nugroho et al., 2016; Nugroho et al., 2017). Ashraf and Bengtson (2007) revealed that tannin can reduce harmful chemicals and improve the survival and growth of striped bass (*Morone saxatilis*) larvae. Further, phenolics found in the bulbs of *M. pendens* may also improve survival and immune function stimulation in *C. gariepinus*, while the application of phenolics from liquorice roots (*Glycyrrhiza glabra* L) in diets of Nile tilapia (*Oreochromis niloticus*) improved their survival (El Mesallamy et al., 2015).

The current findings demonstrated that there were significantly reduced erythrocytes and Hb counts

following *A. hydrophila* injection. This result is similar to Ayik (2009), who reported reduced erythrocytes and Hb in rainbow trout (*Oncorhynchus mykiss*) infected with *Pseudomonas putida*. The decreased count of erythrocytes and Hb in both control and treatment subgroups may be due to haemolytic activity caused by *A. hydrophila* infection (dos Santos et al., 2017). According to Yu et al. (2005) and Janda and Abbott (2010), *A. hydrophila* can produce several haemolytic toxins with virulence activities, causing haemolytic anaemia. In contrast, fish leucocytes were found to increase gradually following injection with bacteria. The improvement of leucocytes post injection could be due to their ability to combat bacteria invading the fish immune system. These findings are similar to a past study, which found increased leucocyte levels in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *A. hydrophila* (Ngugi et al., 2015). The biochemical mechanism(s) that lead to MBE-fed fish leucocytes being increased during infection is(are) yet to be clearly determined. Nevertheless, Sujatha et al. (2019) observed that flavonoids have antibacterial properties and can inhibit bacterial growth, reduce the erythrocyte haemolysis counts that are caused by bacterial infection, protect erythrocyte membranes (Asgary et al., 2005), cause erythropoiesis and maintain the heme iron (Shatoor, 2011).

Activated platelets can release cytokines and chemokines, modulating the immune function that relates to pathogenesis (Faggio et al., 2017). The current study found that the PLT of subgroups of MBE-fed fish (TG5) in the challenge test had increased significantly. However, decreased PLT was observed in the control subgroup (CG2) following 48 h post injection. This finding is in accordance with a previous study revealing that *Clarias gariepinus* injected with 2.00 ppm aqueous extracts of *Lepidagathis alopecuroides* leaves showed the highest value of PLT (Gabriel et al., 2009). Phytochemicals from bulb extracts of *M. pendens* containing flavonoids were reported to have various benefits, such as platelet function modulation (Vallance et al., 2019) and provision of antioxidants with positive effects against pathogens. In addition, some flavonoids possess anti-platelet aggregation effects (Faggio et al., 2017).

The lymphocytes, monocytes and granulocytes lead to the production of antibodies and respond to antigens that act as mediators both in cellular and humoral immune processes (Soltanian and Fereidouni, 2016). Fish with and without MBE in the diet were found in the present work to significantly increase lymphocytes, monocytes and granulocyte following 24 h post *A. hydrophila* injection. The lymphocyte counts of all fish subgroups stabilised after 24 h, whereas the granulocytes of the subgroups of MBE-fed fish were constant 48 h post challenge. Based on Sivagurunathan et al. (2011) report, monocytes which play an important role in protecting fish against bacteria via the immune defence system, can transform into macrophages, which phagocytose pathogens at the first recognition site and subsequent infections. Meanwhile, Neutrophils, one of the granulocyte types, are also involved in bacterial phagocytosis activity during initial infections.

5. Conclusion

Myrmecodia pendens bulb extract inclusion in the diet of *Clarias gariepinus* is beneficial for enhancing blood indices and improving immune function. A diet containing 1% *Myrmecodia pendens* bulb extract in the feed of *Clarias gariepinus* is recommended for protecting fish against *Aeromonas hydrophila*. However, further research is required to determine the role of the active phytochemical ingredient in the MBE that is involved in immune function system in fish, including cellular and molecular responses. Finally, field research is also necessary before applying MBE extract as an immunostimulant to tackle bacterial disease in fish cultures.

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References

Afzali S and Wong W. 2019. Effects of dietary supplementation of *Sonneratia alba* extract on immune protection and disease resistance in goldfish against *Aphanomyces invadans*. *Trop Biomed*., **36**: 274-288.

Aliko V, Qirjo M, Sula E, Morina V and Faggio C. 2018. Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment., *Fish Shellfish Immunol*. **76**: 101-109.

Asgary S, Naderi G and Askari N. 2005. Protective effect of flavonoids against red blood cell hemolysis by free radicals., *Exp Clin Cardiol*. **10**: 88.

Ashraf M and Bengtson DA. 2007. Effect of tannic acid on feed intake, survival and growth of striped bass (*Morone saxatilis*) larvae., *Int J Agric Biol*. **9**: 751-754.

Ayik SB. 2009. Hematological parameters and erythrocyte osmotic fragility in rainbow trout, *Oncorhynchus mykiss*, experimentally infected with *Pseudomonas putida*., *JFAS*. **4**: 246-253.

Chen N, Jiang J, Gao X, Li X, Zhang Y, Liu X, Yang H, Bing X and Zhang X. 2018. Histopathological analysis and the immune related gene expression profiles of mandarin fish (*Siniperca chuatsi*) infected with *Aeromonas hydrophila*. *Fish Shellfish Immunol*., **83**: 410-415.

Dharmaratnam A, Swaminathan TR, Kumar R and Basheer V. 2018. *Aeromonas hydrophila* associated with mass mortality of adult goldfish *Carassius auratus* (Linnaeus 1758) in ornamental farms in India. *Indian J Fish*., **65**: 116-126.

dos Santos AC, Sutili FJ, Heinzmann BM, Cunha MA, Brusque ICM, Baldisserotto B and Zeppenfeld CC. 2017. *Aloysia triphylla* essential oil as additive in silver catfish diet: Blood response and resistance against *Aeromonas hydrophila* infection. *Fish Shellfish Immunol*., **62**: 213-216.

El Mesallamy AM, El-Marakby HI, Souleman AM and El-Naby FSA. 2015. Evaluation of phenolic extract of licorice roots in diets of Nile tilapia (*Oreochromis niloticus*). *Egypt Pharmaceut J*., **14**: 117.

Faggio C, Sureda A, Morabito S, Sanches-Silva A, Mocan A, Nabavi SF and Nabavi SM. 2017. Flavonoids and platelet aggregation: a brief review. *Eur J Pharmacol*., **807**: 91-101.

Fatriadi F, Kurnia D and Satari MH. 2018. Antibacterial activity of ethyl acetate fraction from methanolic extracts of ant-plant tubers towards *Streptococcus sanguis* ATCC 10566. *Padjadjaran J Dentistry*., **30**: 190-193.

Gabriel U, Obomanu F and Edori O. 2009. Haematology, plasma enzymes and organ indices of *Clarias gariepinus* after intramuscular injection with aqueous leaves extracts of *Lepidagathis alopecuroides*., *Afr J Biochem Res*. **3**: 312-316.

Gartika M, Pramesti HT, Kurnia D and Satari MH. 2018. A terpenoid isolated from sarang semut (*Myrmecodia pendans*) bulb and its potential for the inhibition and eradication of Streptococcus mutans biofilm. *BMC Complement Altern Med*., **18**: 151.

Hamsar MN and Mizaton HH. 2012. Potential of ant-nest plants as an alternative cancer treatment. *J Pharm Res*., **5**: 3063-3066.

Hardi E, Saptiani G, Kusuma I, Suwinarti W and Nugroho R. 2018. Evaluation of traditional plant extracts for innate immune mechanisms and disease resistance against fish bacterial *Aeromonas hydrophila* and *Pseudomonas* sp. In: IOP Conference Series: Earth and Environmental Science., p 012003.

Hertiani T, Sasmito E, Sumardi and Ulfah M. 2010. Preliminary study on immunomodulatory effect of sarang-semut tubers *Myrmecodia tuberosa* and *Myrmecodia pendens*. *Online J Biol Sci*., **10**: 136-141.

Hoai TD, Trang TT, Van Tuyen N, Giang NTH and Van Van K. 2019. *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture*., 734425.

Jaafar RM, Ohtani M, Kania PW and Buchmann K. 2016. Correlation between leukocyte numbers and body size of rainbow trout. *Open J Immunol*., **6**: 101.

Janda JM and Abbott SL. 2010. The genus Aeromonas: taxonomy, pathogenicity, and infection. *Clin Microbiol Rev*., **23**: 35-73.

Lestari W, Yusry WN, Iskandar SH, Ichwan SJ, Irfan NI and Suriyah WH. 2019. Gene expression of selected apoptotic markers in human oral squamous carcinoma HSC-3 cell line treated with *Myrmecodia pendans* plant extract. *Makara J Health Res*., **23**: 10.

Maulida NF, Yanuaritamala B, Yanuar A, Saputri FC and Mun'Im A. 2018. Optimization of Microwave-Assisted extraction to obtain optimum antioxidant activity and anthocyanin concentration from *Myrmecodia pendens* tubers using response surface methodology. *Pharm Res*., **10**: 253.

Ngugi CC, Oyoo-Okoth E, Mugo-Bundi J, Orina PS, Chemoiwa EJ and Aloo PA. 2015. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. *Fish Shellfish Immunol*., **44**: 533-541.

Nugroho RA, Hardi EH, Sari YP, Aryani R and Rudianto R. 2019. Growth performance and blood profiles of striped catfish (*Pangasianodon hypophthalmus*) fed leaves extract of *Myrmecodia tuberosa*. *Nusantara Bioscience*., **11**: 89-96.

Nugroho RA, Manurung H, Nur FM and Prahastika W. 2017. *Terminalia catapp*a L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp. *Arch Pol Fisheries*., **25**: 103-115.

Nugroho RA, Manurung H, Saraswati D, Ladyescha D and Nur FM. 2016. The effects of *Terminalia catappa* leaf extract on the haematological profile of ornamental fish *Betta splendens*. *Biosaintifika: Journal of Biology and Biology Education*., **8**: 241-248.

Rosa I, Rodrigues P, Bianchini A, Silveira B, Ferrari F, Bandeira Junior G, Vargas A, Baldisserotto B and Heinzmann B. 2019. Extracts of *Hesperozygis ringens* (Benth.) Epling: in vitro and in vivo antibacterial activity against fish pathogenic bacteria. *J Appl Microbiol*., **126**: 1353-1361.

Sari YP, Kustiawan W, Sukartiningsih S and Ruchaemi A. 2017. The potential of secondary metabolites of *Myrmecodia tuberosa* from different host trees. *Nusantara Bioscience*., **9**: 170-174.

Shatoor AS. 2011. Acute and sub-acute toxicity of *Crataegus aronia* syn. azarolus (L.) whole plant aqueous extract in wistar rats. *Am J Pharmacol Toxicol*., **6**: 37-45.

Shen Y, Wang D, Zhao J and Chen X. 2018. Fish red blood cells express immune genes and responses. *Aquaculture Fisheries*., **3**: 14-21.

Sinclair HA, Heney C, Sidjabat HE, George NM, Bergh H, Anuj SN, Nimmo GR and Paterson DL. 2016. Genotypic and phenotypic identification of *Aeromonas species* and CphA-mediated carbapenem resistance in Queensland, Australia. *Diagn Microbiol Infect Dis*., **85**: 98-101.

Sivagurunathan A, Amila Meera K and Xavier Innocent B. 2011. Investigation of immunostimulant potential of *Zingiber officinale and Curcuma longa* in *cCrrhinus mrigala* exposed to *p. Aeruginosa*-haematological assessment. *IJRAP*., **2**: 899-904.

Soeksmanto A, Subroto M, Wijaya H and Simanjuntak P. 2010. Anticancer activity test for extracts of sarang semut plant (*Myrmecodya pendens*) to HeLa and MCM-B2 cells. *PJBS*., **13**: 148.

Soltanian S and Fereidouni MS. 2016. Effect of Henna (*Lawsonia inermis*) extract on the immunity and survival of common carp, *Cyprinus carpio* infected with *Aeromonas hydrophila*. *IAR*., **8**: 247-261.

Sujatha R, Siva D and Nawas P. 2019. Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *WSN*., **115**: 27-40.

Tamamdusturi R and Yuhana M. 2016. Administration of microencapsulated probiotic Bacillus sp. NP5 and prebiotic mannan oligosaccharide for prevention of *Aeromonas hydrophila* infection on *Pangasianodon hypophthalmus*. *JFAS*., **11**: 67.

Vallance TM, Ravishankar D, Albadawi DA, Osborn HM and Vaiyapuri S. 2019. Synthetic Flavonoids as Novel Modulators of Platelet Function and Thrombosis. *Int J Mol Sci*., **20**: 3106.

Velazquez-Carriles C, Macias-Rodríguez ME, Carbajal-Arizaga GG, Silva-Jara J, Angulo C and Reyes-Becerril M. 2018. Immobilizing yeast β-glucan on zinc-layered hydroxide nanoparticle improves innate immune response in fish leukocytes. *Fish Shellfish Immunol*., **82**: 504-513.

Xie J, Wang W, Dong C, Huang L, Wang H, Li C, Nie S and Xie M. 2018. Protective effect of flavonoids from *Cyclocarya paliurus* leaves against carbon tetrachloride-induced acute liver injury in mice. *Food Chem Toxicol*., **119**: 392-399.

Xuan T, Hoang HA and Tam L. 2018. Stability and activity of TG25P phage in control of *Aeromonas hydrophila* in striped catfish pond water. *AJSTD*., **21**: 64-70.

Yu H, Zhang Y, Lau Y, Yao F, Vilches S, Merino S, Tomas J, Howard S and Leung K. 2005. Identification and characterization of putative virulence genes and gene clusters in *Aeromonas hydrophila* PPD134/91. *Appl Environ Microbiol*., **71**: 4469-4477.