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Growth performance and blood profiles of striped catfish (*Pangasianodon hypophthalmus*) fed leaves extract of *Myrmecodia tuberosa*

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Abstract. Nugroho RA, Hardi EH, Sari YP, Aryani R, Rudianto. 2019. Growth performance and blood profiles of striped catfish (*Pangasianodon hypophthalmus*) fed leaves extract of *Myrmecodia tuberosa*. *Nusantara Bioscience* 11: 89-96. The application of plant extract in the aquaculture field is gaining momentum. This study aimed to determine the effects of *M. tuberosa* Jack leaves extract on the growth performance and blood profiles of striped catfish (*Pangasianodon hypophthalmus*). 400 fish with an initial average weight of 1.54 ± 0.02 g were randomly divided in five groups and fed with different concentration of *M. tuberosa* Jack leaves extract (0.25, 0.5, 1, 2%) and control diet for 80 days. Growth performance, feed conversion rate, feed efficiency, survival rate, morphometric, and hematological profiles of the fish were evaluated at the end of the trial. The results found that fish fed 0.5-1% concentration had significantly higher ($P < 0.05$) growth, feed conversion rate, feed efficiency, morphometric value, survival, white blood cell, hemoglobin, neutrophil, lymphocyte, and monocyte than control. Meanwhile, supplementation of 2% MTE in the diet of fish reduced growth, feed utilization, viscerosomatic index, and morphometric values but increased red blood cell. All the supplemented diets decreased platelet of fish. The findings indicated that supplementation 0.5-1% of MTE is beneficial to the growth and blood profile of fish through the increment of growth indices, feed utilization, white blood cell, hemoglobin, neutrophil, lymphocyte, and survival rate of fish.

Keywords: Blood profiles, growth performance, leaf extract, *Myrmecodia tuberosa*, *Pangasianodon hypophthalmus*

INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) has increasingly become a pivotal commercial species in Africa, Europe and some part of Asia, especially Indonesia. It is also one of the most important fish species currently being cultured both within and outside its natural range of tropical and subtropical environments (Sousa et al. 2013; Yakubu et al. 2014; Da et al. 2016; Gopan et al. 2018). In addition, the striped catfish is relatively resistant to diseases, has high fecundity, and can easily be cultured, these make it an important part of the aquaculture industries in some countries (Haylor and Mollah 1995; Noor El-Deen et al. 2014; Ljubobratovic et al. 2015; Ranjan et al. 2018).

The application of bioactive compound from plant to increase growth and immunomodulatory in fish is becoming a common trend (Emre et al. 2013; Yıldız et al. 2013; Shitole et al. 2014; Rattaya et al. 2015; Soltanian and Fereidouni 2016). Compare to antibiotics and chemical compounds, several advantages of this method have been recognized to include its ability to (i) avoid risk of pathogen resistance; (ii) eco-friendly and without biomagnification; and (iii) minimum residue in the treated fish (Olusola et al. 2013; Reverter et al. 2014; Guardiola et al.

2016; Awad and Awaad 2017). One of the plants extract with such potential is *Myrmecodia tuberosa* Jack leaves extract (MTE).

Myrmecodia tuberosa Jack which is also known as ant-nest is an epiphytic plant with high antioxidant activity and medical value (Hertiani et al. 2010; Soeksmanto et al. 2010; Hanh et al. 2016). Generally, it is often used as a traditional medicine such as a health supplement for the mother's recovery after childbirth and during breastfeeding (Firdausy and Nurlaila 2016). A further report revealed that it enhances lymphocytes proliferation and macrophage phagocytosis activity and that it has antibacterial properties against some pathogens such as *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* (Efendi and Hertiani 2015). Moreover, Sumarto and Sasmito (2013) reported that its extract at $20 \mu\text{g mL}^{-1}$ concentration showed the highest activity in immunity properties, especially in macrophage phagocytosis which is part of leukocyte.

Though some research regarding MTE in medical and pharmacy field has been conducted, the study of growth evaluation and immunomodulatory effects on striped catfish has not been done. This is the first report of the effects of MTE Supplementation in the diet of fish. Therefore, this research was aimed at determining the

growth and immunostimulant effect of the MTE in stripped catfish. To support this study, the phytochemical contents of MTE were qualitatively evaluated.

1 MATERIALS AND METHODS

Plant material

The *Myrmecodia tuberosa* leaves were collected from Antutan village and Mount White, District of Tanjung Palas, Bulungan, North Borneo, Indonesia, located at 02°15'.43.8" - 02°84'.9.78" North Latitude and 117°29'.60.9" - 117°36'.75.3" East Longitude. To eliminate extraneous matter, the leaves collected were washed with deionized water and immediately dried in an oven at 40°C for 12h. They were later cut and ground to make them powdery using a mill and the powder extracted using ethanol 95% for 3 days (100 gL⁻¹). After filtration, the *M. tuberosa* leaves extract (MTE) liquid was evaporated through the use of a rotary evaporator and stored at 4°C until it was used as a crude extract (Emulsion).

Preliminary phytochemical tests

Phytochemical Tests to detect the presence of possible phytochemicals in the extract such as alkaloid, saponin, steroid, triterpenoid, quinone, phenolic, tannin, and flavonoid were performed using methods as previously used by Nugroho et al. (2016), which were briefly described as follow:

(i) Alkaloid: This was conducted through the addition of 1 mL of Dragendorff reagent along the side of the test tube with 2 mL of the filtrate. Formation of orange or orange reddish-brown precipitate indicates a positive test.

(ii) Test for saponin: 1 mL of extract + 5 mL distilled water was shaken vigorously, the appearance of stable froth (1-3 height) for 15 minutes indicates the presence of saponin.

(iii) Test for steroids and (4) triterpenoid (Liebermann-Burchard): 2 mL extract + 1 mL chloroform + few drops of acetic anhydride + conc. sulphuric acid were added along the side of the test tube. The appearance of a blue or green color indicates the presence of steroids while red or brown color indicates the presence of triterpenoid.

(v) Test for phenols and tannins: crude extract was mixed with 2 mL of 2% solution of FeCl₃. A blue-green or black coloration indicates the presence of phenols and tannins.

(vi) Test for flavonoids: 2 mL extract + conc. hydrochloric acid + magnesium ribbon. The appearance of a pink-red color indicates the presence of flavonoids.

Preparation of basal diet and test diet

Basal diet pellet was bought from a commercial factory (Hi Pro Vite FF-888), containing 38-42% protein, 4-6% lipid, 2% crude fiber, 10% ash and 12% moisture while the test diet was prepared by mixing basal diet pellet with various concentration of MTE (0.25; 0.50; 1; and 2 %), repelletized using a mincer and fed with oven at 60°C for 24h. Dried pellets were then allowed to cool at room temperature, packed and stored in a dark room before use.

Animals and experimental setup

Four hundred stripped catfish with an initial average weight of 1.54 ± 0.02 g were collected from the local fish hatchery in Samarinda, East Kalimantan and acclimated to the experimental conditions for one week. During this period, they were maintained on the basal diet. They were randomly distributed into 20 tanks (125 L containing 100 L fresh water) at a density of 20 fish per tank and assigned to each of the 4 dietary various concentration of MTE and the control group. The tanks were equipped with electric motor pump (Grundfos type NSBasic 4-23) to ensure a constant flow of 0.54 L/min for well-aerated water. For 80 days, fish were fed with various concentrations of MTE at the rate of 3% of their body weight three times per day.

A total of 30-50% of the water in the tanks was replaced with fresh water three times a week to adjust its quality. Temperature, pH, total ammonia nitrate, nitrite, and dissolved oxygen of the water in experimental tanks were measured once a week using routine thermometer, pH meter (Eutech Instrument Cyberscan pH 11), Sera Kit test (Sera test kit™, SERA GMBH, Germany), and dissolved oxygen meter temperature probe (YSI 550A Clandon, Ohio, USA), respectively.

Growth and feed utilization parameters

At the end of 80 days feeding trial, biomass weight, average weekly gain (AWG), body weight gain (BWG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE) and survival rate (SR), was measured to determine the growth and feed utilization of fish fed with various concentrations of MTE. All growth parameters were calculated by using equation previously used by Abdel-Tawwab et al. (2015); Githukia et al. (2015); Havas et al. (2015); Omosowone et al. (2015) as follow:

Body weight gain (BWG) (g) = Final fish weight (g) - Initial fish weight (g)

Average weekly gain (AWG) (g) = Body weight gain (g)/number of weeks

SGR (% / day) = $[(\ln W_t - \ln W_0) / (T_2 - T_1)] \times 100$

Where W₀ is the initial fish weight (g) at time T₁ (day) and W_t is the final fish weight (g) at time T₂ (day).

Feed efficiency (FE) = $[(\text{Final fish weight (g)} + \text{dead fish weight (g)}) - \text{initial weight} / \text{Feed consumed (g dry weight)}] \times 100$

FCR = Feed consumed (g dry weight)/Body weight gain.

Viscera index

Viscera and intestines of the fish were collected and weighed to calculate the Viscerosomatic index (VSI) and Intestinosomatic index (ISI) at the end of the trial. They were calculated as follow:

VSI (%) = $100 \times (\text{viscera weight [g]} / \text{whole fish weight [g]})$.

ISI (%) = $100 \times (\text{intestinal weight [g]} / \text{whole fish weight [g]})$.

Morphometric analysis

After 80 days of the feeding trial, morphometric analysis of fish was measured as shown in Figure 1.

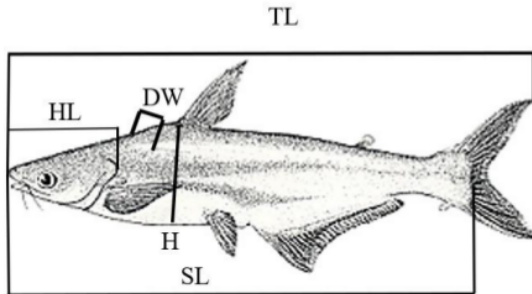


Figure 1. Morphometric measurement. TL = Total length, SL = Standard length, HL = Head length, DW = Dorsal width, H = height

Survival

The survival rate of fish in each tank was recorded at days 0, 40, and 80 in accordance with the equation previously used by Okomoda et al. (2017).

$$\text{Survival rate (SR) (\%)} = (\text{Final number of fish} / \text{initial number of fish}) \times 100$$

Hematological analysis

At day 80, blood samples were collected from five fish in each tank using caudal venipuncture. Total RBC (10^6 per mm^3), WBC (10^3 per mm^3), Hemoglobin (Hb) (g dL^{-1}), hematocrit (Htc), percentage of neutrophil, lymphocyte, and monocyte were determined by using Auto Hematology Analyzer (Mindray BC2800, Mindray® Shenzhen, China).

RESULTS AND DISCUSSION

The results showed that the average of temperature was $22.13 \pm 0.17^\circ\text{C}$, pH 7.35 ± 0.25 , DO 5.70 ± 0.31 ppm, Nitrite 0.17 ± 0.07 ppm, Ammonia 0.14 ± 0.02 ppm, all of which were in the tolerable range for catfish culture (Freelancer, 2015). The findings from the qualitative phytochemicals screening revealed that the MTE positively contained Alkaloid, Triterpenoid, phenolic, tannin, and flavonoid (Table 1) and fish fed with 0.5-1% concentration had significantly higher final weight, BWG, AWG, SGR, FCR, and FE than other groups. However, the 2% concentration reduced all growth parameters (Table 2).

Significantly highest ($P < 0.05$) morphometric value for total length (3.21 ± 0.18 cm), standard length (2.89 ± 0.11 cm), head length (1.15 ± 0.20 cm), dorsal width (1.14 ± 0.21 cm), and height (0.80 ± 0.16 cm) was achieved in the fish fed with 1% MTE (Table 3).

In visceral value, the addition of MTE higher than 1% also reduced VSI while the addition of 0.5% MTE resulted in significantly highest ISI (6.48 ± 0.57) (Table 4). The survival rate of fish fed with 2% MTE shown to be the lowest survival (85%) compared to other groups (Figure 2). It was also discovered that the 0.5-1% concentration showed significantly better WBC, Hb, neutrophil, lymphocyte, and monocyte. Nevertheless, supplementation higher than 1% increased RBC and Htc and all the concentrations decreased fish platelet (Table 5).

Table 1. Phytochemical screening test of ethanolic extract of *Myrmecodia tuberosa* leaves

Phytochemicals	Results
Alkaloid	+
Saponin	-
Steroid	-
Triterpenoid	+
Phenolic	+
Tannin	+
Flavonoid	+

(+) Present; (-) Absent

Table 2. Mean \pm SE growth and feed efficiency of *Pangasianodon hypophthalmus* fed dietary *Myrmecodia tuberosa* leaves extract (MTE) for 80 days

Parameters	Groups MTE (%)				
	Control	0.25	0.5	1	2
Initial weight (g)	1.565 \pm 0.005 ^a	1.541 \pm 0.005 ^a	1.531 \pm 0.016 ^a	1.527 \pm 0.013 ^a	1.538 \pm 0.008 ^a
Final weight (g)	3.531 \pm 0.006 ^a	3.527 \pm 0.036 ^a	3.739 \pm 0.049 ^b	3.808 \pm 0.042 ^b	3.356 \pm 0.147 ^a
BWG (g)	1.96 \pm 0.07 ^a	1.98 \pm 0.35 ^a	2.20 \pm 0.45 ^b	2.28 \pm 0.38 ^b	1.81 \pm 1.42 ^a
AWG (g/week)	1.72 \pm 0.006 ^a	1.74 \pm 0.030 ^a	1.93 \pm 0.039 ^b	2.00 \pm 0.033 ^b	1.59 \pm 0.125 ^c
SGR (%/day)	1.017 \pm 0.004 ^a	1.034 \pm 0.012 ^a	1.115 \pm 0.016 ^b	1.142 \pm 0.013 ^b	0.971 \pm 0.049 ^c
FCR	1.27 \pm 0.008 ^a	1.24 \pm 0.022 ^a	1.11 \pm 0.024 ^b	1.07 \pm 0.019 ^b	1.33 \pm 0.074 ^a
FE (%)	78.50 \pm 0.019 ^a	80.48 \pm 0.019 ^a	90.13 \pm 0.07 ^b	93.39 \pm 0.33 ^b	75.91 \pm 0.33 ^a

Note: Different alphabets (a, b, c) indicated significant different means for different treatments at $P < 0.05$. Control group = without *Myrmecodia tuberosa* leaves extract supplementation. BWG = Body weight gain, DWG = Daily weight gain, AWG = Average weekly gain, SGR = Specific growth rate, FCR = Feed conversion ratio.

Table 3. Morphometric value of *Pangasianodon hypophthalmus* fed dietary *Myrmecodia tuberosa* leaves extract for 80 days

Parameters	Groups MTE (%)				
	Control	0.25	0.5	1	2
Total length (cm)	2.68±0.08 ^a	2.48±0.04 ^a	2.55±1.11 ^a	3.21±0.18 ^b	2.46±0.09 ^a
Standard length (cm)	2.50±0.21 ^a	2.15±0.06 ^a	2.09±0.40 ^{ab}	2.89±0.11 ^c	1.99±0.46 ^b
Head length (cm)	0.53±0.01 ^a	0.54±0.02 ^a	0.53±0.02 ^a	1.15±0.20 ^b	0.53±0.01 ^a
Dorsal width (cm)	0.40±0.07 ^a	0.48±0.02 ^a	0.44±0.02 ^a	1.14±0.21 ^b	0.49±0.03 ^a
Height (cm)	0.45±0.07 ^a	0.19±0.008 ^a	0.19±0.01 ^a	0.80±0.16 ^b	0.23±0.01 ^a

Note: Different alphabets (a, b, c) indicate significantly different means for different treatments at $P < 0.05$. Control group = without *Myrmecodia tuberosa* leaves extract.

Table 4. Viscerosomatic and intestinal somatic index of *Pangasianodon hypophthalmus* fed dietary *Myrmecodia tuberosa* leaves extract (MTE) in the diet for 80 days

Parameters	Groups MTE (%)				
	Control	0.25	0.5	1	2
VSI	12.79±0.42 ^a	15.86±0.42 ^b	14.80±0.72 ^{bc}	14.06±0.24 ^c	9.09±0.26 ^d
ISI	1.26±0.11 ^a	5.37±0.51 ^b	6.48±0.57 ^{bc}	4.87±0.41 ^b	4.38±0.18 ^b

Note: Different alphabets (a, b, c) indicated significantly different means for different treatments at $P < 0.05$. Control group = without *Myrmecodia tuberosa* leaves extract supplementation. VSI = Viscerosomatic Index, ISI = Intestinal somatic index.

Table 5. Hematological profiles of *Pangasianodon hypophthalmus* fed *Myrmecodia tuberosa* leaves extract for 80 days

Parameters	Group (%)				
	Control	0.25	0.5	1	2
RBC ($10^6 \mu\text{L}^{-1}$)	0.91±0.01 ^a	0.91±0.01 ^a	0.91±0.01 ^a	0.92±0.01 ^a	0.97±0.01 ^b
WBC ($10^3 \mu\text{L}^{-1}$)	6.82±0.24 ^a	7.01±0.10 ^a	10.77±0.10 ^b	11.01±0.13 ^b	8.79±0.28 ^c
Hemoglobin (g dL ⁻¹)	5.50±0.19 ^a	5.50±0.10 ^a	6.10±0.25 ^b	6.50±0.10 ^b	6.50±0.11 ^b
PLT ($10^3 \mu\text{L}^{-1}$)	309.00±16.84 ^a	173.00±4.12 ^b	207.00±11.12 ^c	183.00±4.12 ^{bc}	202.00±9.30 ^{bc}
Neutrophil ($10^3 \mu\text{L}^{-1}$)	4.77±0.59 ^a	5.10±0.04 ^b	8.30±0.06 ^c	9.01±0.12 ^d	7.07±0.02 ^c
Lymphocyte ($10^3 \mu\text{L}^{-1}$)	0.88±0.41 ^a	0.55±0.04 ^b	1.25±0.03 ^c	1.42±0.03 ^d	1.19±0.03 ^c
Monocyte ($10^3 \mu\text{L}^{-1}$)	0.48±0.01 ^a	0.52±0.03 ^a	0.65±0.01 ^b	0.50±0.02 ^a	0.51±0.06 ^b

Note: Different alphabets (a, b, c, d) indicated significantly different means for different treatments at $P < 0.05$. Control group = without *Myrmecodia tuberosa* leaves extract supplementation. RBC = Red blood cell, WBC = White blood cell, PLT = Platelet

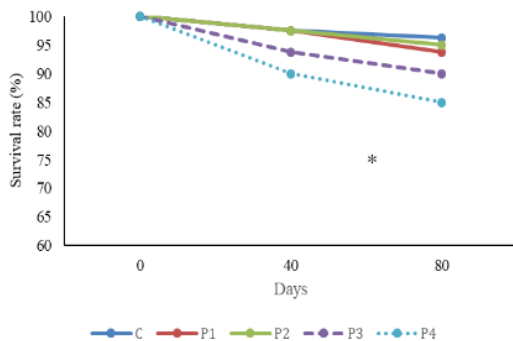


Figure 2. Survival rate (%) of striped catfish (*Pangasianodon hypophthalmus*) fed *Myrmecodia tuberosa* leaves extract in the diet for 80 days. Note: P1 = 0.25%, P2 = 0.5%, P3 = 1%, P4 = 2%

Discussion

The use of plant extracts in aquaculture to promote growth and immunity has been gaining momentum (Soofiani et al. 2016; Abdel-Tawwab, Adeshina, et al. 2018; Adeshina et al. 2018; Rahman et al. 2018; Tan et al. 2018). Plant extracts have several important biologically active compounds such as a flavonoid, alkaloid, triterpenoid, phenolic and tannin (Nugroho et al. 2016; George et al. 2017; Barrett et al. 2018) which was also contained in MTE (Sari et al. 2017 as well as proven in this present study (Table 1).

The MTE supplementation in the diet of *P. hypophthalmus* affected the fish growth status and morphometric value. This might be due to the presence of phytochemical compounds such as flavonoid which is an important secondary metabolite that can act as primary antioxidants because of its antibacterial properties (Rattanachaiakunsopon and Phumkachorn 2007) and as a

growth booster on juvenile *Pargus major* (JI et al. 2007), *Carassius auratus* (Ahilan et al. 2010), *Catla catla* (Kaleeswaran et al. 2011). In fish, free radicals which are naturally produced during metabolism can be scavenged by non-enzymatic antioxidants. The increasing level of free radical that cannot be scavenged by antioxidant may affect the growth of fish. In line with this study, (Izzreen and Fadzelly, 2013) who found flavonoid-containing Green Tea, *Camellia sinensis* 44, led that flavonoid can be used to improve the growth of Nile Tilapia, *Oreochromis niloticus* (Abdel-Tawwab et al. 2010). Apart from this compound, other plant secondary metabolites like triterpenoid, quinone, and phenolic have also been reported to enhance various physiological status like ant stress, growth, appetite, tonic, and immunity (Citarasu 2010; Suman Bhusan Chakraborty et al. 2012; Suman B Chakraborty et al. 2014).

The immunity of fish is related to its blood profile (Satheeshkumar et al. 2012; Soberon et al. 2014; Simide et al. 2016; Moazenzadeh et al. 2017) which are valuable in monitor 26 and assessing fish health (Abbass et al. 2012; Karimi et al. 2013; Kizak et al. 2013; Wang et al. 2014; He et al. 2015; Suely et al. 2016). Blood profiles such as RBC, WBC, Hb, Htc, and PLT are major variables to evaluate the physiological status of a fish. Current research found that groups of fish fed with diet mixed with MTE showed significantly higher RBC, WBC and Hb which are used as an indicator of blood profile in fish and related to innate 42 immune protection and regulation of immunological function in the organisms (Ballarin et al. 2004). Further, the WBC value is generally used as an indicator of fish's health status because it is pivotal to their innate immune defense and functioning (Shiau et al. 2015; Franz et al. 2016). Present research revealed that WBC and Hb of fish fed with 0.5-1% MTE showed significantly higher improvement than other groups. This is similar to past study, stating that plant extracts containing active phytoche 24 als may boost the value WBC and Hb in fish (Barreca et al. 2009; Dotta et al. 2014; Yuniar et al. 2017). In addition, the ethanolic extract of *M. tuberosa* containing phenolic compound helps in improving the immune system (Firdausy and Nurlaila 2016).

However, the mechanism pertaining to increment of blood profiles in fish fed diet supplemented with MTE is not clearly understood. Nair et al. (2002), Lyu and Park (2005) re 32 led that flavonoid from plant extract may modulate Th-1 derived cytokines such as IL-2 (Interleukin 2) and INF γ (Interferon). They may act as biocatalysts in producing WBC as part of nonspecific cellular immunity. Furthermore, they help in reducing hemolysis of RBC and protecting their biological membranes from oxidative damage caused by free radicals (Kitagawa et al. 1992; Asgary et al. 2005). Therefore, this research seems to be in line with past research that MTE containing flavonoids is capable of being an antioxidant which can be used to maintain the heme iron and enhance erythropoiesis (Akah et al. 2008; Uboh et al. 2010; Shatoor 2011).

Furthermore, secondary metabolites from the plant may improve the innate immune system of fish in order to support their survival (Suman Bhusan Chakraborty et al.

2012). Previous research revealed that phytochemicals from plant extract have been successfully used to promote the survival rate of Common Carp (*Cyprinus carpio*) (Mohamad and Abasali 2010); marine ornamental fish (Balachandran and Tissera 2013) and tilapia (*Oreochromis niloticus*) (Akinwande et al. 2011). In addition, Dhanalaxmi and Vastrad (2014) found that cinnamon (*Cinnamomum verum*) which contained active phytochemicals is useful in increasing the survival rate of Nile tilapia after challenged with *Aeromonas hydrophila* (Abdel-Tawwab, Samir, et al. 2018). Current study, however, indicated that *P. hypophthalmus* fed with MTE above 1% significantly reduced ($P<0.05$) survival rate. The highest value of survival rate (Figure 3) was found in 0.5% concentration which does not significantly difference with control group. This might be because of the presence of tannin in the MTE which could be harmful 43 fish at high concentration. Previously, Viswaranjan et al. (1988) reported that tannin which is dominantly present in plants, has negative effects on *Channa striatus* and *Cyprinus carpio* such as decreasing the lipid content on the liver, brain, muscle and protein depletion suffered in their brain. Further, this negative impact may correlate with the increase of mortality.

In conclusions, the *Myrmecodia tuberosa* leaves extract contains phytochemicals such as flavonoid, tannin, alkaloid which support growth and blood profile of *P. hypophthalmus*. A diet supplement with 1% of MTE is recommended to enhance growth and immune function of 25 fish. However, further research needs to be conducted to examine the influence of those plants on fish health (including histological parameters and molecular aspect) as an important step for its large-scale application in aquaculture.

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