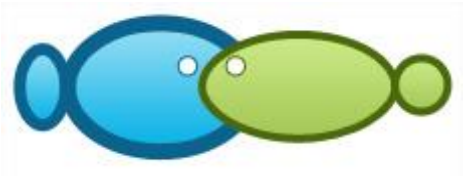

# Dietary supplementation of *Premna serratifolia* and *Punica granatum* enhance innate immune response and disease resistance in striped catfish (*Pangasianodon hypophthalmus*) against *Edwardsiella ictaluri*

[1]Bui T. B. Hang, [2]Nguyen T. Tuan, [1]Tran T. M. Duyen, [3]Kaeko Kamei, [1]Tran T. T. Hoa

[1] College of Aquaculture and Fisheries, Cantho University, Cantho, Vietnam; [2] College of Natural Sciences, Cantho University, Cantho, Vietnam; [3] Department of Functional Chemistry, Kyoto Institute of Technology, Kyoto, Japan. Corresponding author: T. T. T. Hoa, ttthoa@ctu.edu.vn

**Abstract**. This study aims to evaluate the effects of dietary supplementation with *Premna serratifolia* or *Punica granatum* extracts on the immune response and disease resistance of striped catfish (*Pangasianodon hypophthalmus*). A basal diet was supplemented with 1.5% and 3% of *P. granatum* or 1% and 2% of *P. serratifolia* extracts (w/w) and fed to the fish. After 4 weeks of feeding, the numbers of erythrocytes and leukocytes (except for thrombocyte) were significantly higher in fish administered *P. serratifolia* extract compared to control. The serum lysozyme and complement activities were significantly higher in fish of 3% *P. granatum,* or 1%, 2% *P. serratifolia* extract treatments. All plant extract treatments showed significantly higher phagocytic activities compared to the control. Dietary inclusions of *P. granatum* and *P. serratifolia* up-regulated the expression of complement factor B/C2A and interleukin-1β gene in the liver of striped catfish. At the end of the feeding trial, the challenge test with *Edwardsiella ictaluri* showed that the cumulative mortality was lower in all extract supplemented treatments. In particular, the 2% *P. serratifolia* treatment showed the dramatically lowest mortality compared to others. In conclusion, dietary *P. serratifolia* and *P. granatum* extracts could stimulate the innate immune response of striped catfish and enhance disease resistance of fish against *E. ictaluri*.
**Key Words**: bacillary necrosis, growth performance, immune system, plant extracts, striped catfish.

**Introduction**. The striped catfish (*Pangasianodon hypophthalmus*) is one of the important cultured species in Mekong Delta, Viet Nam. In 2020, the production of striped catfish in Viet Nam has reached to 1.5 million metric tons and exported to more than 130 countries (MARD 2021). However, infectious diseases are the main problem in striped catfish farms since pathogenic microorganisms grow rapidly and infect fish in the intensive culture systems, causing heavy losses. In aquaculture, the application of antibiotics and chemotherapeutics is used to control infectious diseases. Moreover, it is expensive and leads to negative side effects including antibiotic resistance, bioaccumulation, and environmental pollution (Jeney et al 2015). To avoid these problems, more environmental-friendly prophylactic and preventive solutions are required and the use of immunostimulants for disease control is a promising method. The herbal extracts contain several active compounds that have been reported to stimulate immune response and control infectious disease. It may be considered as an alternative method to antibiotics and chemicals in aquaculture (Jeney et al 2015; Dotta et al 2018).

*Premna serratifolia* is a widespread genus found throughout Africa, the western Pacific, and Asia. It is one of the important medicinal plants and has been used as a traditional treatment for animal as well as humans. It can be used directly as a therapeutic agent, but also as material for the synthesis of drugs or the extraction of active compounds (Prashant 2016). *P. serratifolia* and derived products have recorded

analgesic (Karmakar et al 2011), anti-bacterial (Paul et al 2015; Rahman et al 2016), anti-oxidant (Selvam et al 2010; Karmakar et al 2011), hepatoprotective (Singh et al 2011) and immunomodulation activities (Gokani et al 2007).

*Punica granatum* is commonly known as promegranate, belonging to the family Punicaceae. It mainly grows in USA, Iran, India, China, the Mediterranean region (Rahimi et al 2012) and also in Viet Nam. *P. granatum* has been used as a traditional remedy for diarrhea, helminth infection, bacterial infection, hemorrhage and respiratory afflictions (Kim & Choi 2009). Several parts from *P. granatum* such as the leafs, bark, roots, fruit, juice, peel and seeds possess various pharmacological properties, including antioxidative (Aviram et al 2004), anti-microbial (Voravuthikunchai et al 2004; Braga et al 2005; Vasconcelos et al 2006), anti-inflammatory, anti-cancer and anti-angiogenesis activities (Rahimi et al 2012).

*P. serratifolia* and *P. granatum* possess several benefits for animal and human health. However, only a few studies of the application of *P. granatum* in aquaculture have been reported (Harikrishnan et al 2010, 2012), and *P. serratifolia* has rarely been explored in aquaculture. This study aims to evaluate the effects of methanol extracts of *P. granatum* and *P. serratifolia* on the innate immune response and disease resistance of striped catfish against the bacteria *Edwardsiella ictaluri*.

## Material and Method

***Fish***. Striped catfish (15-18 g) were obtained from a commercial catfish hatchery in Can Tho province (9°58'12"N, 105°44'14"E), Viet Nam and transferred to the Wetlab of the College of Aquaculture and Fisheries, Can Tho University. The fish were acclimatized for two weeks in an indoor composite tank (2 $m^3$) with aerated freshwater and fed to satiation with pellet feed, without plant extracts, twice a day. The water in the tank was exchanged every two days to maintain water quality. Fish were also examined to determine their health status before starting the experiment and they were confirmed to be healthy.

***Plant extract preparation***. The plants *P. serratifolia* and *P. granatum* were collected from farms in O Mon, Can Tho city, Viet Nam and identified by the Department of Biology, Can Tho University. The whole plants were washed in distilled water. The plants were dried and then ground to powder by using a grinder. The plant powders were extracted following the method of Natarajan et al (2005). The dried powder of *P. serratifolia* or *P. granatum* was soaked in methanol (ration 1:10 w/v), allowed to stand for 5 days at room temperature, and then filtered through a sterile cloth. The filtrate was collected, and the solvent was removed using a rotary vacuum evaporator. The residues obtained after the evaporation of methanol were stored at 4°C for further use.

***Diet preparation***. Four experimental diets were prepared by mixing a commercial diet (Grosbest, 30% crude protein) with crude extract of *P. serratifolia* or *P. granatum* at the required concentration: 1 or 2% (w/w) for *P. serratifolia*, and 1.5 or 3% (w/w) for *P. granatum,* following the method of Harikrishnan et al (2012) with a slight modification. Briefly, the plant extracts (*P. serratifolia* or *P. granatum*) were dissolved with DMSO, sprayed to the basal diet slowly, and then mixed well. The control diet was added with the same volume of DMSO only. The diets were dried in an oven at 30°C for 4 h, then coated with 0.5% squid oil and dried at 30°C for 4 h. Diets were packed and stored at 4°C until use.

***Experimental design***. The experimental design was completely randomized with five feeding groups, including 1 or 2% (w/w) *P. serratifolia* extract, 1.5 or 3% (w/w) *P. granatum* extract, and control (without plant extract)*,* in triplicates. The feeding period was 4 weeks. Striped catfish (17.47±0.94 g) were distributed into 15 plastic 500 L tanks at the density of 30 fish per tank. Fish were fed twice a day at a ratio of 3% of fish body weight per day. A recirculating water system and continuous aeration were used. The water quality parameters were measured daily (temperature 27.77±0.23°C and dissolved

oxygen 6.54±0.47 mg $L^{-1}$) and weekly (pH 7.42±0.26 and total ammonia 0.92±0.17 mg $L^{-1}$). The final weights of fish were measured after 4 weeks of rearing.
The blood of fish (3 fish per tank) was collected for hematological and immunological analyses after feeding for 2 weeks and 4 weeks. Disease resistance against the bacteria *E. ictaluri* was also examined after 4 weeks of feeding.

***Challenge test***. *E. ictaluri* used were obtained from the stock of the College of Aquaculture and Fisheries, Can Tho University. Bacteria preparation followed the method of Hang et al (2013). The bacteria was diluted with saline buffer to $10^5$ CFU $mL^{-1}$ and used for fish challenge. The challenge test was performed randomly with 6 treatments including 2 control diet groups and 4 diet groups supplemented with extract of *P. serratifolia* or *P. granatum.* Each group was injected intraperitoneally with 0.1 mL of 0.85% NaCl solution or 0.1 mL of *E. ictaluri* solution ($10^5$ CFU $mL^{-1}$), according to Hang et al (2013). Each treatment was performed using 15 fish per tank in triplicate. Mortality was recorded daily for 14 days after the challenge. The clinical signs of challenged fish were also observed and recorded daily.

***Growth performance***. The fish was weighed at the start and end of the feeding period to evaluate the growth performance of experimental fish. The weight gain (WG), daily weight gain (DWG), and specific growth rate (SGR) were calculated according to the following equations:

WG (g) = FBW (final body weight) – IBW (initial body weight)

DWG (g/day) = (FBW - IBW)/days of experiment

SGR (%/day) =100 x [ln(FBW)-ln(IBW)]/days of experiment

***Hematological and immunological variables***. After 2 weeks and 4 weeks of feeding, blood was collected through caudal vein puncture using a 1 mL syringe. The proportion of different blood cells and some indicators of humoral immunity in the blood were determined.

***Red blood cell (RBC) count***. Total RBC was counted on Neubauer hemocytometer after staining with Natt-Herrick solution (Natt & Herrick 1952). First, 10 µL of each blood sample was diluted with 1990 µL of Natt-Herrick solution, and then mixed gently for at least 3 min. The suspension was put into the chamber and allowed to settle for 2–3 min before counting under the light microscope. The RBC was counted in 5 of the 25 small areas.

***White blood cell (WBC) count and WBC differential***. An aliquot of whole blood was smeared on a microscope slide by using a smearing slide (cover glasses 24x50, Germany). The slide smear was dried quickly, fixed in methanol (95%, Sigma) for 1-2 min, and stained with Wright and Giemsa (Rowley 1990). Blood cell types were determined following Supranee et al (1991), while blood cell indices were calculated according to Hrubec et al (2000).

***Phagocytic activity***. Phagocytic activity was assayed by the following method of Siwicki & Anderson (1993) with slight modifications by Soltanian & Fereidouni (2016). Briefly, 100 µL of *Saccharomyces cerevisiae* suspension ($10^8$ cells $mL^{-1}$) was added to 100 µL of blood sample in a 1.5 mL microtube. Then, the mixture was incubated at 28°C for 30 min after thorough mixing. After incubation, the tube was mixed gently, and 30 µL of this suspension was smeared on the glass slide. The smeared slides were air dried, fixed with ethanol for 1 min, and stained with Giemsa. The phagocytic cells were counted under the microscope. Phagocytic activation (PA) was determined by enumerating 100 phagocytes per slide. The mean of PA in each slide was calculated as the following formula:

PA = (Number of phagocytic cells with engulfed bacteria/number of phagocytes) × 100

***Lysozyme assay***. The protocol for lysozyme assay was adapted from Ellis (1990) and Milla et al (2010). Briefly, 10 µL of plasma was mixed with 130 µL of lyophilized *Micrococcus lysodeikticus* (Sigma) suspension (0.3 µg $mL^{-1}$) in phosphate buffer (pH 6.2), in a 96-wells microplate. The difference in absorbance at 450 nm was monitored between 0 and 5 min, and used to calculate lysozyme activity in units. One unit represents the amount of lysozyme that caused a 0.001 decrease in absorbance.

***Complement assay***. The alternative complement pathway was assayed using rabbit red blood cells (RRBC, Biomerieux, Craponne, France) as targets following Sunyer & Tort (1995). Briefly, 10 µL of RRBC suspension diluted in veronal buffer (Biomerieux) was mixed with serial dilutions of plasma (60 µL of total volume). After incubation for 100 min at 28°C, the samples were centrifuged at 2000 xg for 10 min at room temperature. The spontaneous haemolysis was obtained by adding 60 µL of veronal buffer to 10 µL of RRBC. The total lysis was obtained by adding 60 µL of distilled water to RRBC. The absorbance was measured at 405 nm. Appropriate calculations served to estimate complement activity (Sunyer & Tort 1995).

***Gene expression***. Extraction of total RNA from the liver was done using RNAzol (Molecular Research Center, USA) following the manufacturer's protocol. cDNA was synthesized using the SensiFAST cDNA synthesis kit (Bioline, Korea) according to the manufacturer instructions. The synthesized cDNA was kept at -20°C until the amplification of the immune genes. The primer sequences and reaction conditions are listed in Table 1. Each 10 µL RT-PCR reaction consisted of 5 µL of 2X SensiFAST SYBR Lo-ROX mix (SensiFAST SYBR Lo-ROX kit, Bioline, Korea), 0.5 µM of each forward and reverse primer and 10 ng of synthesized cDNA. The running condition of the qPCR (preheating at 95°C for 10 min, denaturing at 95°C for 15 s, annealing at 57°C for 15 s, and extension at 72°C for 20 s) was performed in a realtime qPCR machine (LightCycler 480 System, Roche, Switzerland). For the normalization of RNA, gene expression levels were analyzed with $2^{-\triangle\triangle Ct}$ and house-keeping gene; 16S rRNA was used as the reference gene.

Table 1

Primers used for immune gene expression (Sirimanapong et al 2015)

| *Gene name* | *Primer sequence (5'–3')* | *Amplicon (bp)* |
|---|---|---|
| 16S rRNA | F: TATCTTCGGTTGGGGCG<br>R: CCTGATCCAACATCGAGG | 223 |
| Interleukin - 1β | F: CAGAGGCTGAAGCACACTCA<br>R: CCTTGTCCTGCCTGCTGTAA | 148 |
| Complement factor B/C2A | F: CAAGCTAAAAGCCTCCGCT<br>R: AACTGCTAAAAGCCTCCGCT | 110 |

***Statistical analysis***. The data were expressed as mean ± standard deviation (SD). Statistical analysis of data was performed with one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. The significance was established for p-value less than 0.05.

## Results and Discussion

***The growth performance of striped catfish***. The growth performance of striped catfish, after 4 weeks of feeding with a diet containing extracts of *P. serratifolia* or *P. granatum*, is presented in Table 2. Both plant-diet groups showed significant larger growth parameters of weight gain, daily weight gain, and specific growth rate compared to the control diet group ($p<0.05$). The feeding groups of 2% *P. serratifolia* and 1.5% *P. granatum* showed higher growth parameters than other diet groups.

Table 2

The growth performance of striped catfish. Striped catfish was fed the diet supplemented with *P. granatum* or *P. serratifolia* extract for 14 days

| *Diet group* | *Initial weight (g)* | *Final weight (g)* | *Weight gain (g)* | *Daily weight gain (g day$^{-1}$)* | *Specific growth rate (%)* |
|---|---|---|---|---|---|
| 1.5% *P. granatum* | 16.79±0.74$^{a}$ | 34.38±4.85$^{b}$ | 18±4.12$^{c}$ | 0.64±0.14$^{c}$ | 2.61±0.38$^{c}$ |
| 3% *P. granatum* | 16.93±0.4$^{a}$ | 30.4±6.36$^{ab}$ | 13.48±5.58$^{bc}$ | 0.48±0.18$^{bc}$ | 2.39±0.47$^{bc}$ |
| 1% *P. serratifolia* | 17.96±1.43$^{a}$ | 29.25±1.72$^{a}$ | 11.29±1.92$^{b}$ | 0.4±0.04$^{b}$ | 1.84±0.15$^{b}$ |
| *2% P. serratifolia* | 17.73±0.71$^{a}$ | 36.29±2.41$^{b}$ | 18.57±2.08$^{c}$ | 0.66±0.08$^{c}$ | 2.68±0.23$^{c}$ |
| Control | 18.33±1.41$^{a}$ | 26.26±1.9$^{a}$ | 7.93±1.11$^{a}$ | 0.29±0.04$^{a}$ | 1.58±0.13$^{a}$ |

Note: different superscripts show significant differences ($p<0.05$).

### *Hematological parameters*

***Red blood cells (RBC)***. After 2 and 4 weeks of supplementation with *P. serratifolia* or *P. granatum* extracts, RBC numbers of striped catfish of all diet groups ranged between 2.64 and $3.83\times10^{6}$ cells mm$^{-3}$ (Figure 1). Interestingly, RBC of *P. serratifolia* extract diet group was significantly increased compared to the control diet group ($p<0.05$), while oral administration of *P. granatum* extract did not affect the RBC number.

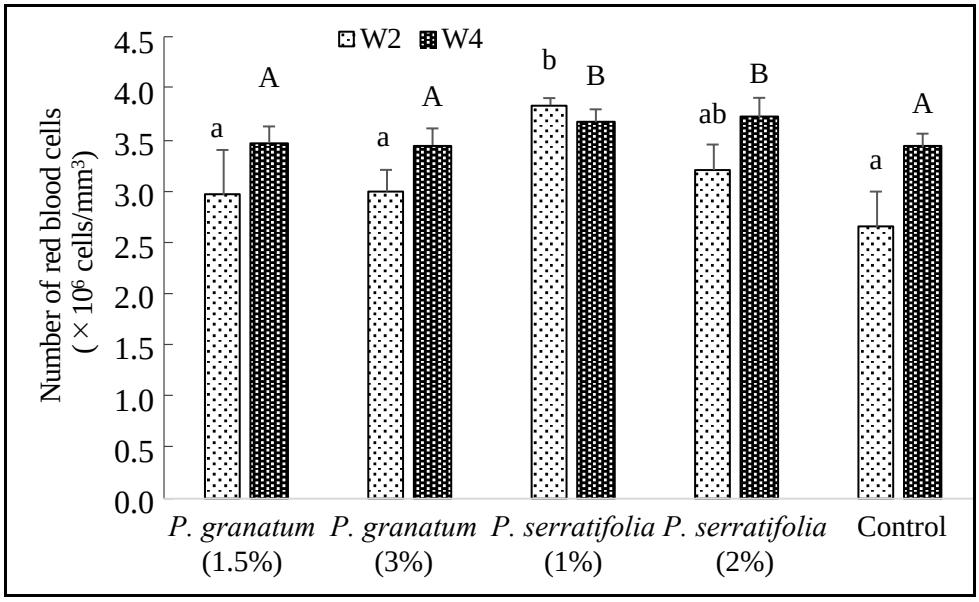


Figure 1. Number of red blood cells of striped catfish fed with supplemented diet of *P. granatum* or *P. serratifolia* extract. Data are the means ±SD. Different letters indicate a statistically significant difference ($p<0.05$).

***White blood cells (WBC)***. By feeding for 2 weeks, the total of WBC in *P. granatum* extract diet group was increased compared to the control, but did not show any significant differences ($p>0.05$) (Figure 2). In contrast, WBC of fish from the *P. serratifolia* extract diet group (1%, 2%) showed significantly higher numbers than that of the control diet group ($p<0.05$). After 4 weeks of feeding, WBC ranged between 257.4 and 315.2 × $10^{3}$ cells mm$^{-3}$. *P. serratifolia* extract diet group had the highest number of WBC, with a significant difference to control group.

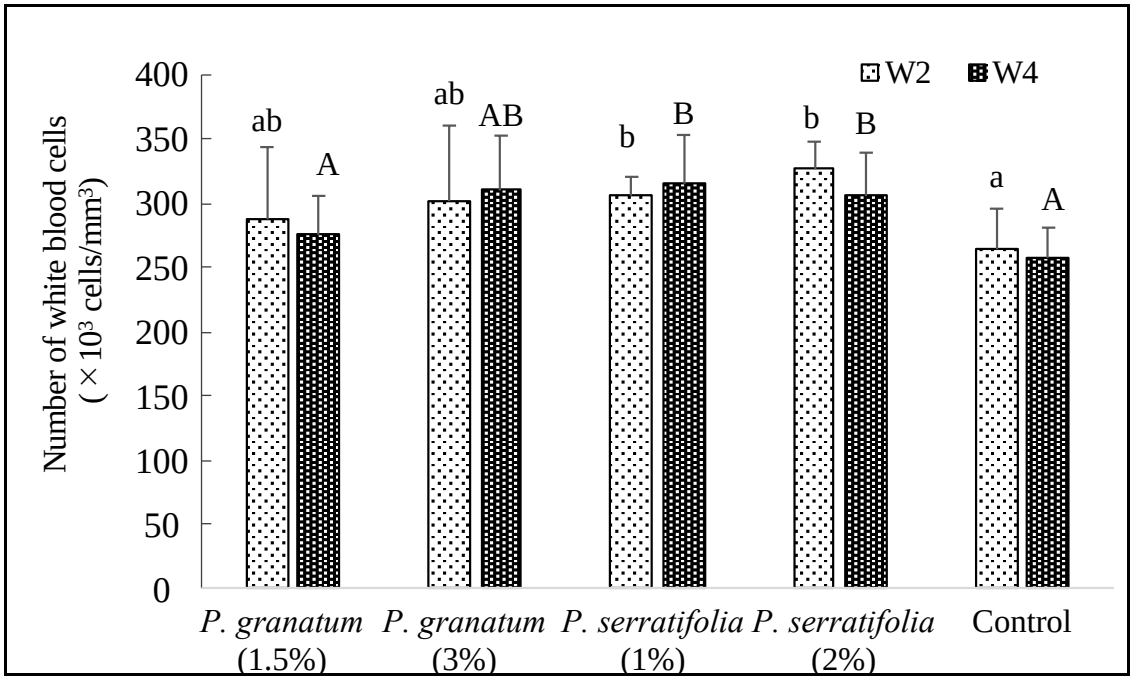


Figure 2. White blood cell count of striped catfish fed with diet supplemented with *P. granatum* or *P. serratifolia* extract. Different letters indicate significant differences ($p<0.05$).

***Monocytes***. After 2 weeks of feeding, both diet groups presented increased monocytes compared to the control group, except for the 1.5% *P. granatum* diet group (Table 3). The number of monocytes from fish fed 1% *P. serratifolia* extract reached the highest values (36.1 $\times 10^3$ cells $mm^{-3}$, $p<0.05$). After 4 weeks of feeding, the numbers of monocytes of all diet groups were significantly higher than those of the control ($p<0.05$).

Table 3

Numbers of white blood cells of different subtype and thrombocyte of striped catfish feeding on *P. granatum* or *P. serratifolia* extract diets for 14 days

| *Diet group* | *Monocytes ($\times 10^3$ cells $mm^{-3}$)* | *Neutrophils ($\times 10^3$ cells $mm^{-3}$)* | *Lymphocytes ($\times 10^3$ cells $mm^{-3}$)* | *Thrombocytes ($\times 10^3$ cells $mm^{-3}$)* |
|---|---|---|---|---|
| After 2 weeks administration | | | | |
| 1.5% *P. granatum* | 22.22±6.96$^{a}$ | 25.05±7.21$^{ab}$ | 216.54±24.14$^{ab}$ | 22.06±6.92$^{a}$ |
| 3% *P. granatum* | 32.94±7.69$^{ab}$ | 22.18±8.13$^{a}$ | 231.52± 1.77$^{ab}$ | 19.23±4.33$^{a}$ |
| 1% *P. serratifolia* | 36.07±9.96$^{b}$ | 31.25±5.8$^{b}$ | 211.35±18.79$^{ab}$ | 23.18±1.9$^{a}$ |
| *2% P. serratifolia* | 31.74±7.82$^{ab}$ | 29.72±6.7$^{ab}$ | 237.59±19.24$^{b}$ | 23.51±5.56$^{a}$ |
| Control | 21.24±8.34$^{a}$ | 22.29±3.96$^{a}$ | 171.34±52.97$^{a}$ | 20.17±8.32$^{a}$ |
| After 4 weeks administration | | | | |
| 1.5% *P. granatum* | 37.96±6.33$^{b}$ | 24.45±11.5$^{ab}$ | 191.12±50.71$^{ab}$ | 27.53±8.48$^{a}$ |
| 3% *P. granatum* | 38.08±7.69$^{b}$ | 23.46±10.49$^{ab}$ | 175.5±82.44$^{ab}$ | 26.25±8.43$^{a}$ |
| 1% *P. serratifolia* | 35.88±5.12$^{b}$ | 31.31±12.54$^{b}$ | 198.02±61.81$^{ab}$ | 26.36±11.19$^{a}$ |
| *2% P. serratifolia* | 36.71±5.53$^{b}$ | 27.11±6.47$^{ab}$ | 232.11±46.77$^{b}$ | 22.61±8.3$^{a}$ |
| Control | 26.06±3.53$^{a}$ | 18.07±5.21$^{a}$ | 143.56±25.29$^{a}$ | 22.39±7.69$^{a}$ |

Note: means in each column with different superscripts have significant differences ($p<0.05$).

***Neutrophils***. By supplementation with *P. serratifolia* or *P. granatum* extract, the neutrophils increased (Table 3). The feeding with 1% *P. serratifolia* was most effective for increasing neutrophil numbers with the significant difference in both 2 weeks and 4 weeks feeding time, compared to the control diet group.

***Lymphocytes and thrombocytes***. By feeding *P. serratifolia* or *P. granatum* extract for 2 and 4 weeks, the lymphocytes were increased compared to the control group (Table 3). The highest numbers of lymphocytes were obtained in treatment with 2% *P. serratifolia.* In contrast, no difference was observed in the numbers of thrombocytes among all groups in both sampling times.

***Lysozyme activity***. After 2 weeks of feeding, serum lysozyme activity of the *P. serratifolia* diet group was enhanced significantly compared to the control group, while that of *P. granatum* diet group was higher than the control, but did not show any significant differences (Figure 3). After 4 weeks of feeding trial, both supplemented diet groups possessed significantly higher lysozyme activities compared to control, except for the 1.5% *P. granatum* group. The highest lysozyme activities were observed in both sampling times by the 2% *P. serratifolia* feeding treatment.

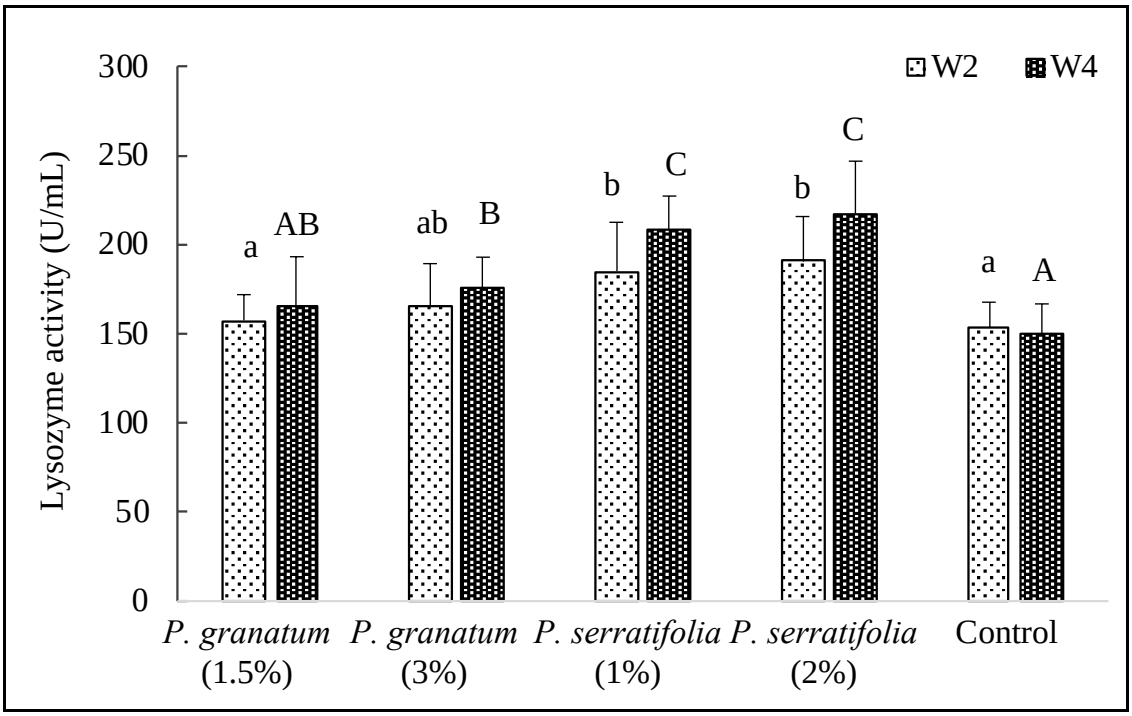


Figure 3. Lysozyme activity of striped catfish fed supplemented diet with *P. granatum* or *P. serratifolia* extracts. Different letters indicate a significant difference ($p<0.05$).

***Phagocytic activity***. After 2 weeks of feeding, the phagocytic activities of extract supplemented groups ranged between 43.1–54.3% (Figure 4), and were enhanced significantly ($p<0.05$) compared to the control group (40.9%), except for 1% *P. serratifolia* diet group. After 4 weeks of feeding, phagocytic activities were increased in all supplemented groups and differed significantly from control ($p<0.05$). The 2% *P. serratifolia* diet group showed the highest phagocytic value and significant differences with other diet groups.

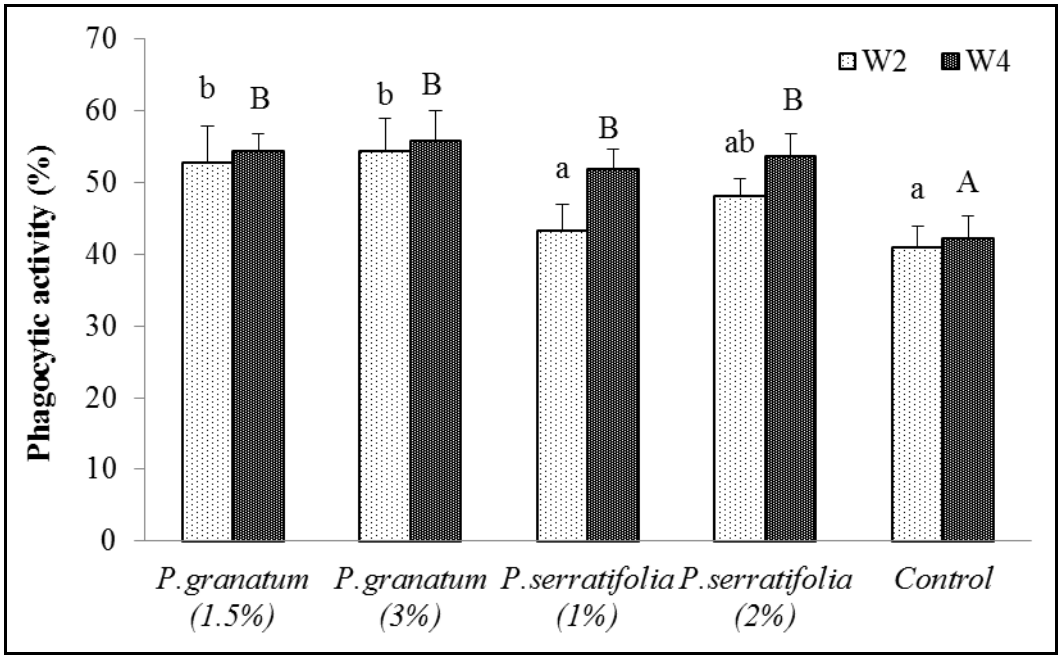


Figure 4. Phagocytic activity of striped catfish fed supplemented diet with *P. granatum* or *P. serratifolia* extract. Different letters indicate significant differences ($p<0.05$).

***Complement activity***. By feeding *P. serratifolia* or *P. granatum* extract for 2 and 4 weeks, serum complement activity of the treatment groups was increased significantly compared to the control, except for the 1.5% *P. granatum* diet group (Figure 5). The highest values of complement activity was observed in both sampling times in the treatment with 2% *P. serratifolia.*

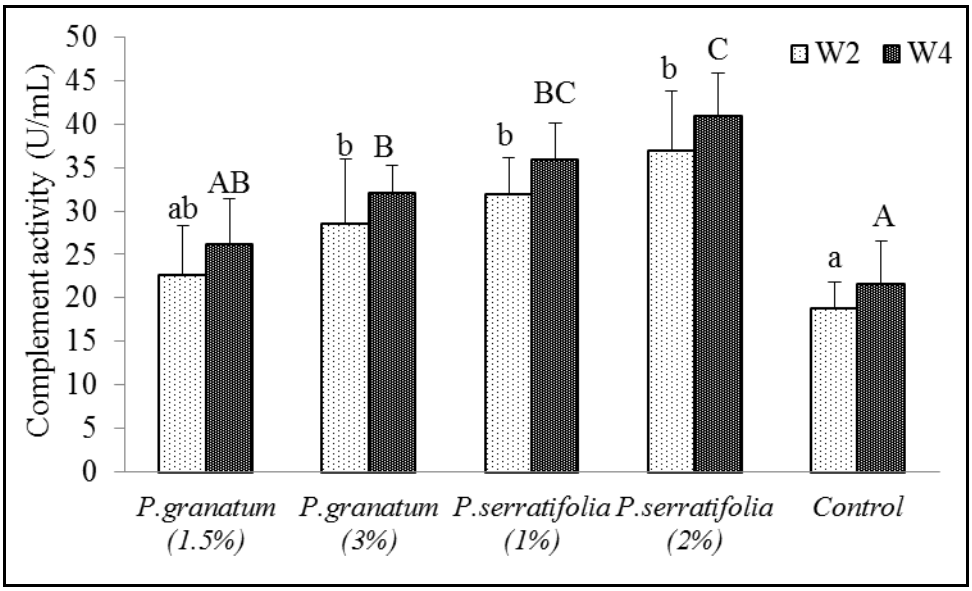


Figure 5. Complement activity of striped catfish fed supplemented diets with *P. granatum* or *P. serratifolia* extract. Different letters indicate significant differences ($p<0.05$).

***Gene expression***. The gene expression levels of complement factor B/C2A (CF) and interleukin -1β (IL) in the liver of fish were presented in Figure 6. Dietary supplementation of *P. granatum* and *P. serratifolia* stimulated significantly CF and IL mRNA levels in fish compared to the control treatment ($p<0.05$). The mRNA levels of CF were significantly up-regulated in fish fed dietary supplementation with 1% *P. granatum*

or 1%; 2% *P. serratifolia* ($p<0.05$) and no significant differences were found in the 1% *P. granatum* treatment. The mRNA levels of IL were up-regulated in fish of supplemented *P. granatum* or *P. serratifolia* treatments, but only the treatment with 2% *P. serratifolia* showed a significant difference compared to the control ($p<0.05$).

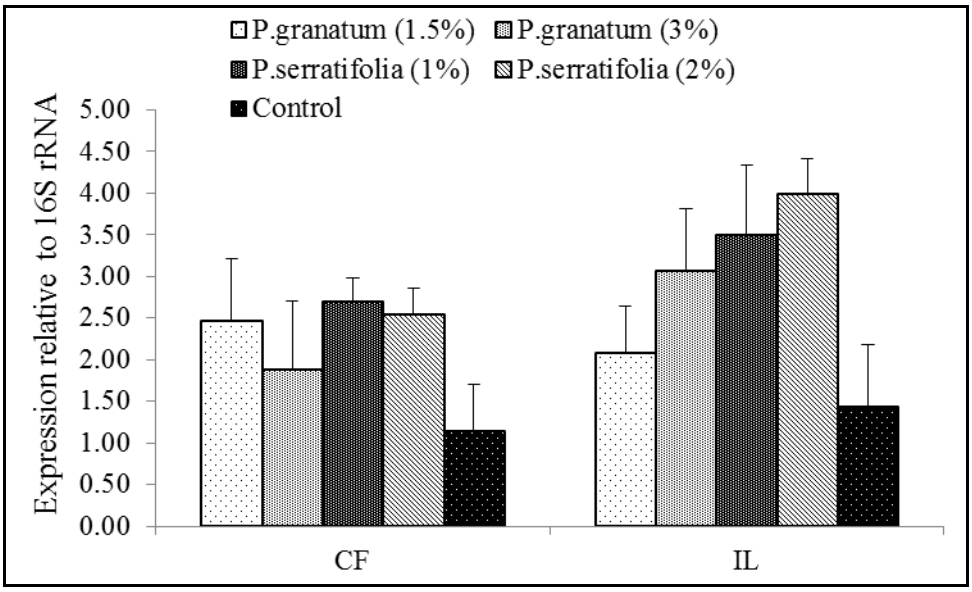


Figure 6. Relative expression of complement factor B/C2A (CF) and interleukin -1β (IL) in liver of striped catfish fed supplemented diet with *P. granatum* or *P. serratifolia* extract.

***Challenged test with E. ictaluri***. After 4 weeks of feeding trial, the challenge test with *E. ictaluri* was performed. On 14 days after injection with *E. ictaluri*, the control diet group showed a cumulative mortality rate of 57.1%, while the cumulative mortality rate decreased in supplemented groups with *P. serratifolia* or *P. granatum* (9.5–38.1%, Figure 7). Especially the 2% *P. serratifolia* diet group had a dramatically reduced mortality to 9.5%, with significant differences to the fish from the control diet group.

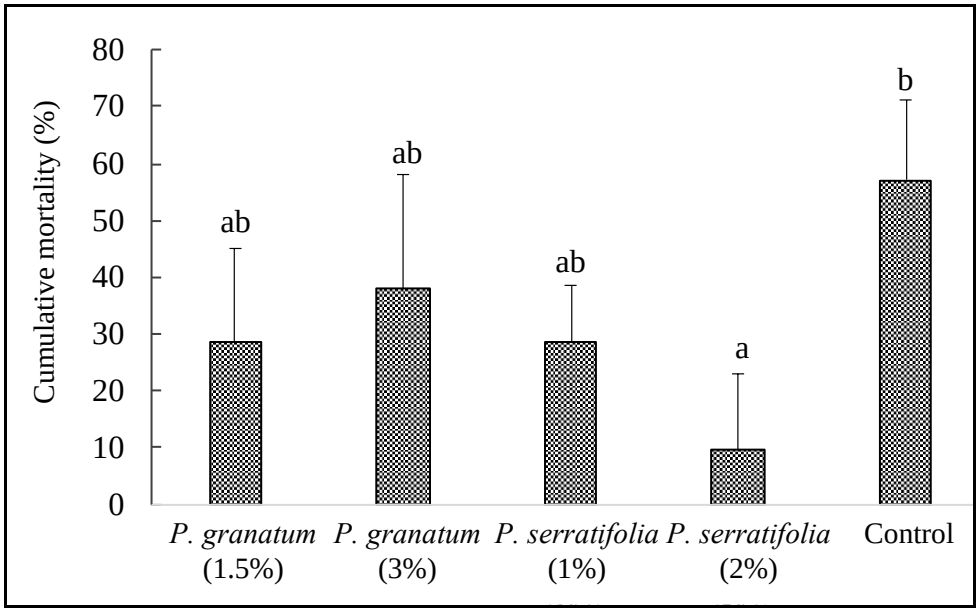


Figure 7. Cumulative mortality rate of striped catfish fed supplemented diet with *P. granatum* or *P. serratifolia* extract. Different letters indicate a statistically significant difference ($p<0.05$).

In this study, we examined the effects of oral administrations of *P. serratifolia* and *P. granatum* extracts on growth performance, immune response and anti-bacterial resistance of striped catfish. By feeding the diet supplemented with *P. serratifolia* or *P. granatum* extract, the growth performance of striped catfish was improved significantly. Our findings are consistent with studies on sturgeon (*Huso huso*) fed diets containing *Allium cepa* (Akrami et al 2015) and on rainbow trout (*Oncorhynchus mykiss*) fed diets containing *Stachys lavandulifolia* (Moghanlou et al 2018). Herbs and phytogenic products could control and limit the growth and colonization of numerous bacteria in the fish gut. This may stimulate the efficiency of utilization of food, enhance the growth performance and improve feed conversion (Reverter et al 2014).

In aquaculture, hematological parameters are widely used as important tools for monitoring health status in cultured fish (Hrubec et al 2000; Fazio 2019). The number of blood cells can indicate the physiological and immunological change in fish and is used to control fish disease (Oluyemi et al 2008; Gabriel et al 2011). The macrophages, monocytes and granulocytes have been reported as the main elements of the innate immune response (Magnadottir 2006). This study demonstrated that the numbers of RBC, total WBC, monocytes, neutrophils, and lymphocytes increased significantly in striped catfish fed with *P. serratifolia*, while only monocytes increased significantly in fish fed *P. granatum* diet for 4 weeks. The present results have coincided with those of a study on tilapia feeding with propolis and aloe extracts, which caused a significant change in the number of WBC (Dotta et al 2018). Prasad & Priyanka (2011) reported that the RBCs, WBCs, haemoglobin, and PCV of striped catfish increased significantly after *Garcinia gummi-gutta* extract dietary supplementation was performed for 45 days. Harikrishnan et al (2012) also indicated that RBC and WBC levels increased in fish fed with a pomegranate enriched diet. Dietary supplementation of *Ocimum sanctum* to *Labeo rohita* produced a significant increase in RBCs, WBCs, and Hb content (Das et al 2015). These enhanced RBC levels in fish by feeding with different plant extracts may be due to enhanced erythropoiesis (Gupta & Mishra 2014). The increase in red blood cell concentration may help to transport and distribute the oxygen throughout the fish body (Hardi et al 2017). The WBCs play an important role in fish immunology (Ni et al 2014), constituting the cellular component of innate immune response and producing humoral substances (Vetvicka et al 2013). The lymphocytes usually increase when the fish immune system is activated by a pathogen or antigen. These cells are involved in both cellular and humoral immune defense and response for the activation and maintenance of the immune system (Magnadottir 2006). Thus, the increase in RBCs and WBCs may improve fish health and protect the fish against pathogens.

The innate immune system is the first defense line of fish and comprises many components existent in the body before pathological infection (Magnadottir 2006). Lysozyme is one of the innate immune components and plays an important function in the bio-defense system to kill bacteria (Saurabh & Sahoo 2008). The present study found a significant increase in lysozyme activity in fish fed with a diet supplemented with *P. serratifolia* or *P. granatum* extracts. Similarly, Harikrishnan et al (2010, 2012) reported that the serum lysozyme activity was enhanced significantly in fish fed with *P. granatum* enriched diet, which protected the fish from virus and parasite infections. The dietary inclusion of the medicinal plant *Eclipta alba* leaf extract for tilapia showed an increased lysozyme activity (Christybapita et al 2007). Ndong & Fall (2011) recorded that lysozyme activity was enhanced in tilapia fed with 0.5% *Allium sativum*. Similarly, an increase in lysozyme activity was reported in *Astronotus ocellatus*, after the administration of *Dunaliella salina* extract (Alishahi et al 2014). Supplementation of *Excoecaria agallocha* leaf extract stimulated the serum lysozyme activity of tilapia (Laith et al 2017). The enhanced lysozyme activity in treated fish may associate with the increase of WBCs and differentiated leukocytes, hence improved the immune status of experimental fish.

The complement is one of the innate immune responses. It has three different pathways including alternate, classical, and lectin pathways (Sun et al 2010). The complement proceeds to construct the formation of a membrane attack complex, which can destroy pathogens directly (Giri et al 2015). In the present study, serum complement activity was found to be significantly higher after treatment with 3% *P. granatum* and 2%

*P. serratifolia* for 2 weeks. Similarly, the complement activity of *Labeo rohita* fed guava leaf extract supplemented diets was significantly increased after 60 days of feeding (Giri et al 2015). Gobi et al (2016) found that 0.5 and 1% guava leaf extract could stimulate the complement activity in tilapia after 30 days of feeding. Complement activation is usually beneficial to fish (Boshra et al 2006). Therefore, the complement activity of fish fed supplemented diets was enhanced in this study and may provide health benefits to fish.

Phagocytes in blood present an important mechanism in fish innate immune response with the role of preventing infectious diseases. The process involves the internalization, killing, and digestion of invading microorganisms (Panigrahi et al 2005). The findings described in our study illustrate an increase of phagocytic activity in fish administered *P. serratifolia* and *P. granatum*. A previous study has demonstrated that a dose of 1% of plant extracts mixture, *S. trilobatum* and *O. sanctum* stimulated the phagocytic activity in *Mystus keletius* (Subeenabegum & Navaraj 2016). Supplementation of *P. granatum* extracts at high concentration (50 and 100 mg $kg^{-1}$) promote better phagocytic activity in fish after 8 weeks feeding (Harikrishnan et al 2010). These results also agree with a previous study of dietary *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa* extracts (Harikrishnan et al 2009). In this study, the increase of phagocytic activity was correlated with the increase of WBCs as well as monocyte and neutrophil numbers in striped catfish fed *P. serratifolia* or *P. granatum*. These results suggest that supplementation of *P. serratifolia* and *P. granatum* extracts enhanced innate immune response in striped catfish.

The current study demonstrated that the supplementation of *P. granatum* and *P. serratifolia* up-regulated the expression of immune relating genes including complement factor B/C2A and interleukin-1β in the liver of striped catfish. In another study, the expression of anti-inflammatory cytokines (il10 and tgfβ) and other related immune genes (il1β, il8, and il12p40) were detected as up-regulated in the head kidney of rainbow trout fed a diet supplemented with caper (*Capparis spinosa*) (Bilen et al 2016). Sukumaran et al (2016) showed that dietary supplementation with ginger (*Zingiber officinale*) stimulated the mRNA levels of il10 and tgfβ genes and other antioxidant enzyme genes (sod and gpx1) in head kidney and intestine of *Labeo rohita*. The enhancement of immune gene expression (CF and IL) is in accordance with the increasing of complement activity of fish in supplemented treatments. It may stimulate the immune responses leading to the improved resistance of fish to inflammation or infectious diseases.

Active compounds of herbal plants possess therapeutic properties, which are considered promising as functional additives and medicines (Shakya 2016). Many pure compounds have been derived from pomegranate (*P. granatum*), including pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin, and myricetin (Naz et al 2007). Among these compounds, gallic acid showed the highest antibacterial activity against different bacteria species, e.g. *Bacillus subtilis, Shigella, Salmonella, Vibrio cholera*, and *Escherichia coli*. Some publications demonstrated that using medicinal plants stimulated non-specific and specific defense mechanisms of fish against the infection of the pathogen (Giri et al 2015; Ilangkovan et al 2015; Hoseinifar et al 2019). In this study, the efficiency of *P. serratifolia* and *P. granatum* extracts on the immunomodulation in striped catfish were confirmed by the evaluation of the mortality of fish after challenge with *E ictaluri*. The cumulative mortality of control fish was 57.1% during 14 days of *E. ictaluri* infection, while fish administered with the plant extract showed lower mortality (9.5-38.1%). These results are in agreement with previous studies, where the diet supplemented with *P. granatum* extract reduced mortality of olive flounder (*Paralichythys olivaceus*) after infection with *Philasterides dicentrarchi* (Harikrishnan et al 2012) and lymphocystis disease virus (Harikrishnan et al 2010). Similarly, *Carassius auratus* fed crude extracts and purified fractions of *Ixora coccinea* showed an increased survival rate (60-80%) while the mortality of control was 100% 5 days after challenge with highly virulent *A. hydrophila* AHV-1 (Anusha et al 2014). Soltanian & Fereidouni (2016) also revealed that the cumulative mortality of common carp treated with henna extract decreased after being challenged with *A. hydrophila*. Moreover, a similar result was

obtained by Awad et al (2019), where dietary supplementation of hala extract (*Pandanus tectorius*) stimulated disease resistance of rainbow trout against *Yersinia ruckeri*.

**Conclusions**. This study suggested that dietary inclusions of *P. serratifolia* and *P. granatum* extracts improved the innate immune mechanism of fish, as evident from the increased hematological parameters values, immunological parameters such as phagocytic activity, lysozyme activity and reduced the mortality of fish against *E. ictaluri*. In addition, these results also suggested the important role of plant extracts in an alternative method to replace the use of chemicals and drugs to control fish infectious disease in aquaculture. However, more experiments are still needed regarding the mode and time of administration, the specific mechanisms of action of the plant extracts in fish as well as the environmental evaluation because of their biodegradability.

**Acknowledgements**. This study is funded in part by Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan.

**Conflict of Interest**. The authors declare that there is no conflict of interest.

## References

Akrami R., Gharaei A., Mansour M. R., Galeshi A., 2015 Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish and Shellfish Immunology 45(2):828-834.

Alishahi M., Karamifar M., Mesbah M., Zarei M., 2014 Hemato-immunological responses of *Heros severus* fed diets supplemented with different levels of *Dunaliella salina.* Fish Physiology and Biochemistry 40(1):57-65.

Anusha P., Thangaviji V., Velmurugan S., Michaelbabu M., Citarasu T., 2014 Protection of ornamental gold fish *Carassius auratus* against *Aeromonas hydrophila* by treating *Ixora coccinea* active principles. Fish and Shellfish Immunology 36(2):485-493.

Aviram M., Rosenblat M., Gaitini D., Nitecki S., Hoffman A., Dornfeld L., Volkova N., Presser D., Attias J., Liker H., Hayek T., 2004 Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. Clinical Nutrition 23(3):423-433.

Awad E., Austin D., Lyndon A., Awaad A., 2019 Possible effect of hala extract (*Pandanus tectorius*) on immune status, antitumour and resistance to *Yersinia ruckeri* infection in rainbow trout (*Oncorhynchus mykiss*). Fish and Shellfish Immunology 87:620-626.

Bilen S., Altunoglu Y.C., Ulu F., Biswas G., 2016 Innate immune and growth promoting responses to caper (*Capparis spinosa*) extract in rainbow trout (*Oncorhynchus mykiss*). Fish and Shellfish Immunology 57:206-212.

Boshra H., Li J., Sunyer J. O., 2006 Recent advances on the complement system of teleost fish. Fish and Shellfish Immunology 20(2):239-262.

Braga L. C., Leite A. A., Xavier K. G., Takahashi J. A., Bemquerer M. P., Chartone-Souza E., Nascimento A. M. A., 2005 Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. Canadian Journal of Microbiology 51(7):54-57.

Christybapita D., Divyagnaneswari M., Michael R. D., 2007 Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish and Shellfish Immunology 23(4):840-852.

Das R., Raman R. P., Saha H., Singh R., 2015 Effect of *Ocimum sanctum* Linn. (Tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila*. Aquaculture Research 46(5):1111-1121.

Dotta G., Alves de Andrade J. I., Garcia P., Jesus G. F. A., Mourino J. L. P., Mattos J. J., Bainy A. C. D., Martins M. L., 2018 Antioxidant enzymes, hematology and histology of spleen in Nile tilapia fed supplemented diet with natural extracts challenged with *Aeromonas hydrophila*. Fish and Shellfish Immunology 79:175-180.

Ellis A. E., 1990 Lysozyme activity. In: Technique in fish immunology. Stolen T. C., Fletcher P. D., Anderson B. S., Roberson B. S., Muiswinkel W. B. (eds), SOS Publications, New York, pp. 101-103.
Fazio F., 2019 Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500:237-242.
Gabriel U. U., Akinrotimi O. A., Eseimokumo F., 2011 Haematological responses of wild Nile tilapia *Oreochromis niloticus* after acclimation to captivity. Jordan Journal of Biological Sciences 4(4):225-230.
Giri S. S., Sen S. S., Chi C., Kim H. J., Yun S., Park S. C., Sukumaran S. C., 2015 Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection. Fish and Shellfish Immunology 46(2):217-224.
Gobi N., Ramya C., Vaseeharan B., Malaikozhundan B., Vijayakumar S., Murugan K., Benelli G., 2016 *Oreochromis mossambicus* diet supplementation with *Psidium guajava* leaf extracts enhance growth, immune, antioxidant response and resistance to *Aeromonas hydrophila*. Fish and Shellfish Immunology 58:572-583.
Gokani R. H., Lahiri S. K., Santani D. D., Shah M. B., 2007 Evaluation of immunomodulatory activity of *Clerodendrum phlomidis* and *Premna integrifolia* root. International Journal of Pharmacology 3:352-356.
Gupta S., Mishra P., 2014 Effect of leaf extract of *Eclipta alba* on hematology of *Clarias gariepinus* (Burchell, 1822). World Journal of Pharmaceutical Research 3(3):4860-4870.
Hang B. T. B., Milla S., Gillardin V., Phuong N. T., Kestemont P., 2013 *In vivo* effects of *Escherichia coli* lipopolysaccharide on regulation of immune response and protein expression in striped catfish (*Pangasianodon hypophthalmus*). Fish and Shellfish Immunology 34(1):339-347.
Hardi E. H., Irawan W. K., Wiwin S., Gina S., Sumoharjo A., Lusiastuti M., 2017 Utilization of herbal plant extracts on tilapia in preventing *Aeromonas hydrophila* and *Pseudomonas* sp. bacterial infection. Nusantara Bioscience 9(2):220-228.
Harikrishnaan R., Balasundaram C., Kim M. C., Kim J. S., Han Y. J., Heo M. S., 2009 Innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. Fish and Shellfish Immunology 27(3):508-515.
Harikrishnan R., Balasundaram C., Heo M. S., 2010 Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. Fish and Shellfish Immunology 28(2):354-361.
Harikrishnan R., Kim J. S., Kim M. C., Balasundaram C., Heo M. S., 2012 Pomegranate enriched diet enhances the hematology, innate immune response, and disease resistance in olive flounder against *Philasterides dicentrarchi*. Veterinary Parasitology 187(1-2):147-156.
Hoseinifar S. H., Sohrabi A., Paknejad H., Jafari S. C., Paolucci V., Van Doan H., 2019 Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with *Psidium guajava*: the effects on cutaneous mucosal and serum immune parameters and immune related genes expression. Fish and Shellfish Immunology 86:688-694.
Hrubec T. C., Cardinale J. L., Smith S. A., 2000 Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis niloticus*). Veterinary Clinical Pathology 29(1):7-12.
Ilangkovan M., Jantan I., Mesaik M. A., Bukhari S. N. A., 2015 Immunosuppressive effects of the standardized extract of *Phyllanthus amarus* on cellular immune responses in Wistar-Kyoto rats. Drug Design, Development and Therapy 9:4917-4930.
Jeney G., De Wet L., Jeney Z. Yin G., 2015 Plant extracts. In: Dietary nutrients, additives, and fish health. Lee C., Lim C. E., Gatlin D. M., Webster C. D. (eds), John Wiley & Sons, Hoboken, 355 p.
Karmakar K. U., Soma P., Kumar S. S., Chandra S. M., Kumar B. S., 2011 Assessment of analgesic and antibacterial activity of *Premna integrifolia* Linn. (Family: Verbenaceae) leaves. International Journal of Pharmaceutical Sciences and Research 2(6):1430-1435.

Kim Y. H., Choi E. M., 2009 Stimulation of osteoblastic differentiation and inhibition of interleukin-6 and nitric oxide in MC3T3-E1 cells by pomegranate ethanol extract. Phytotherapy Research 23(5):737-739.
Laith A. A., Mazlan A. G., Effendy A. W., Ambak M. A., Nurhafizah W. W. I., Alia A. S., Jabar A., Najiah M., 2017 Effect of *Excoecaria agallocha* on non-specific immune responses and disease resistance of *Oreochromis niloticus* against *Streptococcus agalactiae*. Research in Veterinary Science 112:192-200.
Magnadottir B., 2006 Innate immunity of fish (overview). Fish and Shellfish Immunology 20:137-151.
Milla S., Mathieu C., Wang N., Lambert S., Nadzialek S., Massart S., Henrotte E., Douxfils J., Mélard C., Mandiki S. N. M., Kestemont P., 2010 Spleen immune status is affected after acute handling stress but not regulated by cortisol in Eurasian perch, *Perca fluviatilis*. Fish and Shellfish Immunology 28(5-6):931-941.
Moghanlou K. S., Isfahani E. N., Dorafshan S., Tukmechi A., Aramli M. S., 2018 Effects of dietary supplementation with *Stachys lavandulifolia* Vahl extract on growth performance, hemato-biochemical and innate immunity parameters of rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology 237:98-105.
Natarajan D., Britto S. J., Srinivasan K., Mohanasundari C., Perumal G., 2005 Anti-bacterial activity of *Euphorbia fusiformis* a rare medicinal herb. Journal of Ethnopharmacology 102:123-129.
Natt M. P., Herrick C. A., 1952 A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poultry Science 31:735-38.Naz S., Siddiqi R., Ahmad S., Rasool S. A., Sayeed S. A., 2007 Antibacterial activity directed isolation of compounds from *Punica granatum*. Journal of Food Sciences 72(9):341-345.
Ndong D., Fall J., 2011 The effect of garlic (*Allium sativum*) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). Journal of Clinical Immunology Immunopathology Research 3(1):1-9.
Ni M., Wen H., Li N., Chi N., Bu Y., Ren Y., 2014 The physiological performance and immune responses of juvenile Amur sturgeon (*Acipenser schrenckii*) to stocking density and hypoxia stress. Fish and Shellfish Immunology 36(2):325-335.
Oluyemi K. G., Adeparusi E. A., Olanrewaju J., 2008 Basic haematogical parameters in African catfish, *Clarias gariepinus* (Burchell, 1822) fed ascorbic acid supplemented diets. Research Journal of Animal Sciences 2(1):17-21.
Panigrahi A., Kiron V., Puangkaew J., Kobayashi T., Satoh S., 2005 The viability of probiotic bacteria in rainbow trout *Oncorhynchus mykiss*. Aquaculture 243(1-4):241-254.
Paul J. A., Selvi B. K., Karmegam N., 2015 Biosynthesis of silver nanoparticles from *Premna serratifolia* L. leaf and its anticancer activity in CCl4-induced hepato-cancerous Swiss albino mice. Applied Nanoscience 5:937-944.
Prasad G. Priyanka G. L., 2011 Effect of fruit rind extract of *Garcinia gummi-gutta* on haematology and plasma biochemistry of catfish *Pangasianodon hypophthalmus*. Asian Journal of Biochemistry 6:240-251.
Prashant Y. M., 2016 Pharmacological potentials of *Premna integrifolia* L. Ancient Science of Life 35(3):132-142.
Rahimi H. R., Arastoo M., Ostad S. N., 2012 A comprehensive review of *Punica granatum* (Pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. Iranian Journal of Pharmaceutical Research 11(2):385-400.
Rahman A., Shanta Z. S., Rashid M. A., Parvin T., Afrin S., Khatun M. K., 2016 *In-vitro* antibacterial properties of essential oil and organic extracts of *Premna integrifolia* Linn. Arabian Journal of Chemistry 9(1):S475-S479.
Reverter M., Bontemps N., Lecchini D., Banaigs B., Sasal P., 2014 Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433:50-61.
Rowley A. F., 1990 Collection, separation and identification of fish leucocytes. In: Techniques in fish immunology. Stolen J. S., Fletcher T. C., Anderson D. P., Roberson B. S., van Muiswinkel W. B. (eds), SOS Publications, New York, pp. 113-136.

Saurabh S., Sahoo P. K., 2008 Lysozyme: an important defense molecule of fish innate immune system. Aquaculture Research 39:223-239.
Selvam N. T., Vengatakrishnan V., Damodar K. S., Murugesan S., 2010 Evaluation of tissue level antioxidant activity of *Premna serratifolia* leaf in paracetamol intoxicated Wistar albino rats. International Journal of Pharmaceutical Life Science 1:86-90.
Shakya A. K., 2016 Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine 4(4):59-64.
Singh C. R., Nelson R., Krishnan P. M., Mahesh K., 2011 Hepatoprotective and anti-oxidant effect of root and root callus extract of *Premna serratifolia* L. in paracetamol induced liver damage in male albino rats. International Journal of Pharma and Bioscience 2(2):244-252.
Sirimanapong W., Thompson K. D., Ooi E. L., Bekaert M., Collet B., Taggart J.B., Bron J. E., Green D. M., Shinn A. P., Adams A., Leaver M. J., 2015 The effects of feeding β-glucan to *Pangasianodon hypophthalmus* on immune gene expression and resistance to *Edwardsiella ictaluri*. Fish and Shellfish Immunology 47(1):595-605.
Siwicki A. K., Anderson D. P., 1993 Nonspecific defence mechanisms assay in fish: I; Phagocytic index, adherence and phagocytic ability of neutrophils (NBT test) and myeloperoxidase activity test. In: Disease diagnosis and prevention methods. Siwicki A. K., Anderson D. P., Waluga J. (eds), FAO-Project GCP/INT/JPA, IFI, Olsztyn, pp. 95-104.
Soltanian S., Fereidouni M. S., 2016 Effect of henna (*Lawsonia inermis*) extract on the immunity and survival of common carp, *Cyprinus carpio* infected with *Aeromonas hydrophila*. International Aquatic Research 8:247-261.
Subeenabegum S., Navaraj P. S., 2016 Dietary supplement of mixture of medicinal plant leaf extracts on immune response of fresh water fish *Mystus keletius.* International Journal of Applied Research 2(2):361-364.
Sukumaran V., Park S. C., Giri S. S., 2016 Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings. Fish and Shellfish Immunology 57:362-370.
Sun Y. Z., Yang H. L., Ma R. L., Lin, W. Y., 2010 Probiotic applications of two dominant gut *Bacillus* strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides.* Fish and Shellfish Immunology 29(5):803-809.
Sunyer J. O., Tort L., 1995 Natural haemolytic and bactericidal activities of sea bream *Sparus aurata* serum are effected by the alternative complement pathway. Veterinary Immunology and Immunopathology 45:333-345.
Supranee C., Chalor L., Praveena K., 1991 Histology of the walking catfish, *Clarias batrachus*. AAHRI, Bangkok, Thailand, 96 p.
Vasconcelos L. C., Sampaio F. C., Sampaio M. C., Pereira M. S., Higino J. S., Peixoto M. H., 2006 Minimum inhibitory concentration of adherence of *Punica granatum* Linn (pomegranate) gel against *S. mutans, S. mitis* and *C. albicans*. Brazilian Dental Journal 17:223-227.
Vetvicka V., Vannucci L., Sima P., 2013 The effects of β – glucan on fish immunity. North American Journal of Medical Science 5:580-588.
Voravuthikunchai S., Lortheeranuwat A., Jeeju W., Sririrak T., Phongpaichit S., Supawita, T., 2004 Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. Journal of Ethnopharmacology 94:49-54.
*** MARD, 2021 [Annual report of striped catfish production in Mekong delta, Vietnam]. 10 p. [In Vietnamese].

Received: 22 December 2021. Accepted: 30 December 2021. Published online: 03 April 2022.
Authors:
Bui Thi Bich Hang, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 St., 94115 Xuan Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam, e-mail: btbhang@ctu.edu.vn
Nguyen Trong Tuan, College of Natural Sciences, Can Tho University, Campus II, 3/2 St., 94115 Xuan Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam, e-mail: nttuan@ctu.edu.vn
Tran Thi My Duyen, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 St., 94115 Xuan Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam, ttmduyen@ctu.edu.vn
Kaeko Kamei, Department of Functional Chemistry, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, 606-8585 Kyoto, Japan, e-mail: kame@kit.ac.ip
Tran Thi Tuyet Hoa, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 St., 94115 Xuan Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam, e-mail: ttthoa@ctu.edu.vn

How to cite this article:
Hang B. T. B., Tuan N. T., Duyen T. T. M., Kamei K., Hoa T. T. T., 2022 Dietary supplementation of *Premna serratifolia* and *Punica granatum* enhance innate immune response and disease resistance in striped catfish (*Pangasianodon hypophthalmus*) against *Edwardsiella ictaluri*. AACL Bioflux 15(2):758-773.