Immunomodulatory effect and disease resistance from of three Borneo plant extracts to Aeromonas hydrophila and Pseudomonas fluorescens in tilapia, Oreochromis niloticus

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Immunomodulatory effect and disease resistance from of three Borneo plant extracts to *Aeromonas hydrophila* and *Pseudomonas fluorescens* in tilapia, *Oreochromis niloticus*

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Abstract

EH Hardi, RA Nugroho, I W Kusuma, W Suwinarti and Apriza. 2019. Immunomodulatory effect and disease resistance from of three Borneo plant extracts to Aeromonas hydrophila and Pseudomonas fluorescens in tilapia, Oreochromis niloticus. ***Aquacultura Indonesiana, 20 (1): 41-47.*** The objective of this study was to evaluate the immunomodulatory activity and disease resistance of concoction *Boesenbergia pandurata*/B.p, *Solanum ferox*/S.f, and *Zingiber zerumbet*/Z.z extract in tilapia with the concentrations 600 mg/L BP, 900 mg/L SF and 200 mg/L ZZ against *Aeromonas hydrophila* and *Pseudomonas fluorescens*. Immunomodulatory activity was measured by testing the concoction of three plants extract to prevent and treatment pathogen infection (*A. hydrophila* and *P. fluorescens* 10^5 CFU/mL each bacteria). In this research, feed administration method was used with the concoction of extract B.p, S.f, Z.z. The extract was given for 14 days, three times a day (3-5% of fish body weight). The immunomodulatory parameters (white blood cell/WBC, red blood cell/RBC, phagocytic index/IP) and relative present survival/RPS were observed at (4^{th}) week after challenges with bacteria through intramuscularly injection (0.1 mL/fish). In the prevention and treatment groups, the number of WBC and RBC was increased significantly (*P<0.05*) compared to controls without extract. Phagocytic index of fish fed with combined extracts also experienced a significant increase compared to controls. The results of this study indicated that the use of concoction of three extracts provides the best protection (RPS) and diseases infection recovery from *A. hydrophila* and *P. fluorescent*. The conclusion of this research is the concoction of B.p, S.f, and Z.z has an immunomodulatory effect in tilapia and could increase protection and diseases recovery from bacterial infections.

Keywords: *Boesenbergia pandurata*; *Solanum ferox*; *Zingimber zerumbet*; concocction; fish pathogen; immunomodulatory

Introduction

Some of plants extracts has been reported as antibacterial against diseases in aquaculture (Hardi et al, 2016a). The plant extracts can be considered as medicinal herbs, if they have active ingredients which are responsible for various biological activities to inhibit the pathogen (Hardi et al, 2016b). A number of plants has an immunostimulant efficacy to fish and shrimp (Hardi et al, 2017a). The immunomodulator of plant extract in shrimp aquaculture are *Aegle marmelos, Allium sativum, Aristolochia indica, Azadirachta indica, Cassia fistula, Catharanthus roseus, Curcuma longa, Cynodon dactylon, Lantana camara, Melia azedarach, Mimosa pudica, Momordica charantia, Morus alba, Ocimum*

americanum, Phyllanthus amarus, Phyllanthus emblica, Psidium guajava, Solanum nigrum, Tridax procumban and *Tylophora indica* (Limsuwan and Voravuthikunchai, 2008). Similarly, in fish aquaculture industries various herb extracts activity as antibacterial and antiviral activity such as *Calophyllum inophyllum, Clinacanthus nutans, Clinacanthus* sp., *Glinus oppositifolius, Hura crepitan, Momordica charantia, Psidium guajava, Tinospora crispa, Tinaspora cordifolia* (Logambal et al, 2000), *Ocimum sanctum* (Venkatalakshmi and Michael, 2001; Logambal and Michael, 2001), *Azadirachtin* (Dugenci et al, 2003), *Viscum albums, Urtica dioica* and *Zingiber officinale* (Jian and Wu, 2003), *Radix astragalin Hedysari* and *Radix angelicae sinensis* (Jian and Wu, 2003; 2004), *Astragalus radix* and *Scutellari radix* (Yin et al, 2006). The component of crude extract from *B. pandurata, S. ferox*, and *Z. zerumbet* which contain levamisole, flavonoid, steroid, carbohydrate, can inhibit the growth of pathogenic bacteria such as *Aeromonas hydrophila* and *Pseudomonas* sp. that infecting tilapia both in vitro and in vivo (Hardi et al, 2016b; Hardi et al, 2017a and 2017b; Limsuwan and Voravuthikunchai, 2008). The combined use of multiple extracts for disease control has a higher antibacterial activity compared to the use of a single extract. Extract of *B. pandurata, S. ferox*, and *Z. zerumbet* with ratio 1:1:1 has an antibacterial activity to single and combination of *A. hydrophila* and *P. fluorescens* (Hardi et al, 2018). Meanwhile, concoction of *Curcuma longa, Ocimum sanctum*, and *Azadirachta indica* extract combined with a 1:1:1 has a high antibacterial activity ratio in *Goldfish Carassius auratus* that is challenged with *A. hydrophila* infection (Babu et al, 2002). In this paper, we will discuss the immunomodulatory and diseases resistant properties of concoction *S. ferox, B. pandurata* and *Z. zerumbet* extracts in prevention and treatment experiment using *A. hydrophila* and *P. fluorescens* infection in tilapia.

Materials and Methods

1. Time and location of research

This research was conducted from January to March 2018 at Aquatic Microbiology Laboratory, Faculty of Fisheries and Marine Sciences Mulawarman University and Faculty of Forestry Mulawarman University, East Kalimantan.

2. Extraction preparation of *B. pandurata, Z. zerumbet* and *S. ferox*

Three plants were collect from traditional markets in Samarinda, East Kalimantan Indonesia. The extraction process was conducted by using ethanol solution following the basic procedures (Hardi et al, 2017b; Hardi et al, 2016b). The extraction was made at the Wood Chemical Laboratory at the Faculty of Forestry, Mulawarman University.

The extract concoction was prepared by mixing 600 mg/L of *B. pandurata* (B.p), 900 mg/L of *S. ferox* (S.f) and 200 mg/L of *Z. zerumbet* (Z.z) with combination 1:1:1. The concoction extract was given through oral administration.

3. Fish and pathogen bacteria

Fish used in this research were tilapia (*Oreochromis niloticus*) with size of 15±2 g from Tenggarong Seberang Kutai Kartanegara, East Kalimantan Indonesia. The tilapia were kept at the laboratory for 2 weeks before used. The pathogen bacteria used were *A. hydrophila* (EA-01) and *P. fluorescens* (EP-01) from the Laboratory of Aquatic Microbiology Faculty of Fisheries and Marine Sciences of Mulawarman University, and bacteria were grown in BHI (Brain Heart Infusion Broth, DIFCO®) for 24 hours at 28-30 °C, the density used was each of the bacteria was 10^5 CFU/mL. The bacteria combination ratio for challenges was 1:1. Both bacteria was intramuscular injection as 0,1 mL each fish.

4. Prevention and Treatment of fish diseases

The experiments for prevention was performed with the feeding procedure plus the extract administered as much as 3-5% of the fish weight, after feeding treatment for 6 days, a challenge with combined bacteria was administered by intramuscular injection (0.1 mL/fish) on day 7 and the fish were giving with normal feed (without the extract) until day 28

(4th week) after challenges with bacteria. Parameters observation ended in 4th week after injection with bacteria.

The treatment experiment was performed with injecting the combination of bacteria through intramuscullary injection. Three days after injection, fish were fed with the extracted feed until day 9 and the fish were given the normal feed until day 28. The immunology parameters data were collected in week 4 or day 28.

Prevention (A) = fish fed with a concoction extract B.p, S.f, and Z.z and injected with combined bacteria.

Treatment (B) = fish injected with combined bacteria and fed with a concoction extract B.p, S.f, and Z.z.

Control (C) = fish injected with combined bacteria and fed with no additional extract.

Fish pathology anatomy

This parameter was observed to investigate the effect of extract combination to the prevention and treatment of *A. hydrophila* dan *P. fluorescens* infection on tilapia. Measurement was conducted by calculating the percentage of tilapia which experienced fin rot, exophthalmia, and darkness in week 4. The percentage of fish pathology anatomy was calculated according to Hardi et al. (2017 a,b) formula.

$$Fish\ pathology\ anatomy = \frac{total\ of\ fish\ pathology\ in\ days\ 28}{total\ of\ a\ live\ fish\ \ in\ days\ 28} X100\%$$

Phagocytic index (PI)

Phagocytic index is the percentage of macrophage cell. The measurement of phagocytic index was performed on the modification of the methods that previously used by Asimi and Sahu (2013).

$$\text{Phagocytic Index (\%)} = \frac{The\ number\ of\ active\ macrophage\ cells}{The\ total\ of\ macrophage\ cells} X100\%$$

Respiratory burst activity (RBA)

Nitro Blue Tetrazolium (NBT) reagent was used in respiratory burst activity test in accordance with Chakraborty et al, (2014). A 50 µL of fish blood was subtracted, then incubated for one hour at 37 ° C whereas the supernatant was removed. It was proceeded by adding 50 µL of PBS which was repeated for three times. Furthermore, 50 µL of 0.2% NBT was added and then followed by one hour incubation at 37 °C. Two minutes fixation using 50 µL of 100% methanol was performed subsequently, rinsed with 30% (50 µL) methanol for 3 times, then dried and added with KOH 60 µL + DMSO 70 µL afterward. The specimen was then analyzed by ELISA Reader at 540 nm.

Lysozyme activity (LA)

Fish blood sample was drawn from the caudal veins using the injection syringe that has been moistened with anticoagulant. The blood was stored at room temperature for two hours and kept in the refrigerator (4°C) for 24 h. The blood was then centrifuged at 5000 rpm for three minutes; serum obtained was separated. The lysozyme activity test was measured by using 10 µL of serum samples, placed into a microtitre plate and added with 190 µL of *Micrococcus lysodeikticus* (Sigma-Aldrich Chemical) suspension (0.2 mg *M. lysodeikticus*/mL, PBS pH 7.4). This mixture was shaken slowly and incubated at a constant room temperature. After 90 min of incubation, a microtiter plate ELISA Reader with a wavelength of 520 nm[23] was used to analyze lysozyme activity. Relative lysozyme activity (unit) is calculated as follows: Unit = 0.001 absorbance decreasing per min, using a standard curve determined with hens egg white lysozyme (Sigma) in PBS (Díaz-Resendiz et al, 2015).

White blood cells (WBC) and red blood cells (RBC)

White blood cells (WBC) and red blood cells (RBC) were observed at the week 4 (day 28), haematological profiles of fish were observed. Fish was anesthetized using 50 mg MS 222 in dm^3 of water, and the blood was taken through caudal vein, with 1 mL of plastic syringe rinsed with anticoagulant 3% trisodiumcitrate. Total RBC and WBC were determined manually with the improved Neubauer counting chamber, the number of

leukocytes was calculated following method of (Blaxhall and Daisley, 1973).

Diseases resistance

Aeromonas hydrophila and *P. fluorescens* are pathogen bacteria that were used in challenge test. The mortality and diseases resistance (RPS) and survival rate (SR) of fish were collected on the last week of research (4th weeks), and calculated bu using Ellis (1988) equation.

$$SR = \frac{total\ of\ fish\ mortality\ in\ days\ 28}{total\ of\ fish\ mortality\ in\ first\ day} X100\%$$

$$\text{RPS} = 1 - \frac{(Percent\ mortality\ in\ treated\ group)}{Percent\ mortality\ in\ control\ group} X\ 100$$

Data Analysis

All results in the treatment and prevention test were presented in the average and standard deviation of three independent measurements. The parameters in this research such us RBA, LA, WBC, RBC, IP, Lz, SR, and RPS were analyzed using nonparametric. ANOVA (SPSS 16, Inc. USA) was used to determine any significant differences ($P<0.05$) compared to control.

Results and Discussion

Fish which infected by the combination between *A. hydrophila* and *P. fluorescens* can experience fins rot, darkness, and exophthalmia. In the control group, which fish were not given the extract, the recovery rate of the anatomical pathology was 50%, 40%, and 40% respectively, which meant that as many as 50% of the fish were still found to have fins rot, 60% were still found to experience blackened body color, while 60% of fish showed exophthalmos (Table 1).

Table 1. The anatomical pathology (fins, body, and eyes) recovery of the fish organ of tilapia observation

Group	Fish pathology anatomy	Percentation of pathology recovery (%)
A	Fin rot	100[b]
	Darkness	100[b]
	Exophthalmia	100[b]
B	Fin rot	90[b]
	Darkness	90[b]
	Exophthalmia	100[b]
Control	Fin rot	50[a]
	Darkness	40[a]
	Exophthalmia	40[a]

In accordance with previous studies, the symptoms of anatomic pathology are common symptoms of fish infected with both bacteria, which in tilapia infected with *A. hydrophila* and *Pseudomonas* sp. shows symptoms of anatomic pathology such as bleeding, darkness, fins rot, and exophthalmos (Hardi et al. 2016; Hardi et al. 2017; Toranzo et al. 2005; Yardimci & Aydin 2011; Altinok et al. 2006; Austin & Austin 2007; Hardi & Pebrianto 2012).

In the experiment to prevent infection of *A. hydrophila* and *P. fluorescens* by using a combination of three extracts (Bp, Sf, Z, z) through fish feed, it was found that a combination of three extracts could 100% prevent the emergence of pathology symptoms (fins rot, darkness and exophthalmos) and significantly different to control group ($P<0.05$). Likewise, with healing experiments, the recovery rate of fish from the infection of the two bacteria was 90-100%, which significantly different from controls without extracts ($P<0.05$).

Tilapia infected with *A. hydrophila* and *P. fluerescens* show abnormalities in swimming patterns such as gasping, weakened, and aggressive to touch. Although at the end of the observation on 4th week fish that experienced abnormalities in the swimming pattern were still found, but the result showed that at least 60 % of them were recovered, while 50% of the fish regain their strength, and as much as 40% of the fish returned to normal from their aggressiveness to touch (Table 2). Furthermore, in the prevention of infection by providing a

combination of three extract through fish feed, the recovery rate or the rate of non-emergence of swimming abnormalities as an indication of bacterial infection reached a range of 90-100%. Likewise, in healing experiments; 80-100% of tilapia previously infected with *A hydrophila* and *P. fluorescens* recovered from infection which indicated by no gasping, weakened nor aggressive respond (Table 2).

Table 2. Fish swimming recovery (gasping,weakness and aggressive) of treated fish with concoction three extract

Group	Fish swimming recovery	Percentation of swmming recovery (%)
A	Gasping	90[b]
	Weakness	90[b]
	Aggressive	100[b]
B	Gasping	90[b]
	Weakness	80[b]
	Aggressive	100[b]
Control	Gasping	60[a]
	Weakness	50[a]
	Aggressive	40[a]

Table 3. Immunology respons of tilapia in week 4

Groups	Phagocitycindex (%)	Respiratory burst	lysozyme (μg/L)	WBC (10^4cell/mm^3)	RBC (10^6cell/mm^3)
A	56,83 ±0,76a	0,33 ±0,01[a]	4,67 ±0,29[a]	2,86 ±0,02[a]	7,5 ± 0,2[a]
B	55,00 ±0,50a	0,37 ±0,01[a]	5,30 ±0,26[a]	3,8 ± 0,04[a]	7 ±0,2[a]
C	22,97 ±0,45b	0,26 ±0[b]	3,57 ±0,12[b]	1,4±0,08[b]	2,8 ±0,09[b]

A = fish fed with a concoction extract 1:1:1 and injected with combined bacteria
B = fish injected with combined bacteria and fed with a concoction extract 1:1:1
C = fish injected with combined bacteria and fed with no additional extract
[a,b] Values are the mean for three replicates. Means in the same row with the same superscripts are not significantly different ($P>0.05$).

Table 4. Survival Rate (SR), Mortality, and Relative Percent Survival (RPS) of Tilapia in week 4

Groups	Total Fish At Start of Experiment	Total Fish at The End of Experiment	SR (%)	% Mortality	RPS %
A1	10	8	80,00	20%	75%
A2	10	8	80,00	20%	75%
A3	10	8	80,00	20%	71%
A (Avarage)	10	8	80,00 ±0[b]	20%±3.39[b]	74%±0.02[a]
B1	10	8	80,00	20%	75%
B2	10	7	70,00	30%	63%
B3	10	8	80,00	20%	71%
B (Avarage)	10	7,666666667	77±5.77[b]	23%±0.058[b]	70%±0.06[a]
C1	10	2	20,00	80%	
C2	10	2	20,00	80%	
C3	10	3	30,00	70%	
C (Avarage)	10,0	2,3	23±5.77[a]	77%±0.058[a]	

Tilapia immunity response observed inthis experiment (both prevention and treatment) showed an increase in phagocytic index, respiratory burst, lysozyme, WBC and RBC compared to controls without extracts ($p<0.05$), yet the use of combined extracts for prevention was not significantly different to the use of combined extracts for healing.

While Single extract of *B. pandurata* and *Z. zerumbet* effectively inhibited the growth of *A. hydrophila* bacteria both in vitro and in vivo, *S. ferox* extract was more effective in inhibiting *Pseudomonas* sp. both in vitro (Hardi et al, 2016a,b; Jian and Wu, 2004; Yin et al, 2006) and in vivo (Tan and Vanitha, 2004; Hardi et al, 2017a,b). The concoction of the three extracts used were more effective to inhibit both bacteria compared to stand alone extract of those three (Hardi et al, 2018). The concoction of the extract to inhibit pathogenic bacteria proved to be better than a single extract such as the results of Asimi and Sahu (2013) which study the combination of *Ocimum support, T. avicennoides,* and balsam apple (*Momordica balsamina*) (290.6-750.0 lg/mL) against some bacteria, such as *A. hydrophila, Shigella dysenteriae, S. flexneri, S. sonnei, S. bodyii*, and

E. coli. Similarly, the results of the Harikrishnan and Balasundaram (2008) who found that the combination of three extract of *Curcuma longa, Ocimum sanctum*, and *Azadirachta indica* with a ratio 1: 1: 1, effectively suppressing *A. hydrophila* bacteria compared to a single extract.

In the prevention and treatment of *A. hydrophila* and *P. fluorescens* infection, concoction extract of BP: SF: ZZ with a ratio 1:1:1 was effective to improve the non-specific fish immune system (Table 1). In the fourth week after challenge test, the number of WBC and RBC was higher than controls and significantly different ($P<0.05$), as well as phagocytic index of leukocyte cells were higher than controls and significantly different ($P<0.05$). The increase of non-specific immune response was relevant to protecti against pathogenic bacteria infection. Further, both prevention and treatment was also more than 60% which indicated that this substance was effective to protect tilapia from disease (Ellis, 1988; Pasnic et al, 2009).

Phytochemical results showed that the extract of *S. ferox, B. pandurata* and *Z. zerumbet* contains alkaloid flavonoids, steroids and carbohydrates (Jian and Wu, 2004), which are potential components that would normally affect the loss of antibacterial cytokines (Pasnic et al, 2009; Micol et al, 2005). Differences of the substance contained resulted in differences in antibacterial activity against bacteria. The combination of three extract showed an increase in antibacterial ability regarding to prevention and/or treatment of bacterial pathogen infection due to synergism between the three extracts.

Conclusion

The combined extract of *B. pandurata, S. ferox*, and *Z. zerumbet* with the concentration 600 mg/L, 900 mg/L, and 200 mg/L has the potential immunostimulant properties for tilapia to prevent and treat the *A. hydrophila* and *P. fluorescens* infection.

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Reference

Alsaid M, Abuseliana A F, Daud H H, Mustapha N M, Bejo S K, Abdelhadi Y M, Hamdan R H. 2015. Haematological, biochemical and clinical signs changes following experimental infection of *Streptococcus agalactiae* in red hybrid tilapia (*Oreochromis* sp.). Basic Research Journal of Agricultural Science and Review 4(9): 289-295

Asimi, O.A. and N.P. Sahu. 2013. Herbs/spices as feed additive in aquaculture. *Scientific Journal of Pure and Applied Sciences,* 2(8):284-292.

Babu, B., V.K. Jisha, C.V. Salitha, S. Mohan, and A.K. Valsa. 2002. Antibacterial activity of different plant extracts. *Indian Journal of Microbiology*, 42:361–363.

Blaxhall, P.C. and K.W. Daisley. 1973. Routine Haematological Methods for Use with Fish Blood. *Journal Fish Biology,* 5: 577-581.

Chakraborty SB, Horn P, Hancz C, Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. 2014. *Reviews in Aquaculture*. 6(1): 1-19.

Dugenci, S.K, N. Arda N, and A. Candan. 2003. Some medicinal plants as immunostimulant for fish. *Journal Ethnopharmacol*, 88:99-106.

Ellis, A.E. 1988. In: Ellis AE, editor London: Academic Press 1–19.

Hardi, E.H, G. Saptiani, I.W. Kusuma, W. Suwinarti, A. Sudaryono. 2018. Inhibition of fish bacteria pathogen in tilapia using a concoction three of Borneo plant extracts. *IOP Conf. Series: Earth and Environmental*, 144:012015.

Hardi, E.H., G. Saptiani, I.W. Kusuma, W. Suwinarti, and R.A. Nugroho. 2017. Immunomodulatory and antibacterial effects of *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber zerumbet* on tilapia, *Oreochromis niloticus*. *AACL Bioflux,* 10(2):182:190.

Hardi, E.H., I.W. Kusuma, W. Suwinarti, G. Saptiani, Sumoharjo, A.M. Lusiastuti 2017. Utilization of several herbal plant extracts on Nile tilapia in preventing *Aeromonas hydrophila* and *Pseudomonas* sp. bacterial infection. *Nusantara Bioscience*, 9(2):220- 228.

Hardi, E.H., I.W.Kusuma, W. Suwinarti, Agustina, and R.A. Nugroho. 2016. Short communication: Antibacterial activity of *Boesenbergia pandurata*, *Zingiber zerumbet* and *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp. Nusantara Bioscience, 8(1):18- 21.

Hardi, E.H., I.W.Kusuma, W. Suwinarti, Agustina, I. Abbas, and R.A. Nugroho. 2016. Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of *Aeromonas hydrophila* and *Pseudomonas* sp. *AACL Bioflux,* 9(3):638-646.

Harikrishnan R, Balasundaram C. 2008. In Vitro and In Vivo Studies of the Use of Some Medicinal Herbals against the Pathogen *Aeromonas hydrophila* in Goldfish. Journal of Aquatic Animal Health 20:165–176.

Jian, J. and Z. Wu. 2003. Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, *Pseudosciaena crocea* (Richardson). *Aquaculture* 218:1–9.

Jian, J. and Z. Wu. 2004. Influence of traditional Chinese medicine on non specific immunity of Jian Carp (*Cyprinus carpio* var. Jian). *Fish Shellfish Immunol*, 16:185–91.

Limsuwan, S. and S.P. Voravuthikunchai. 2008. *Boesenbergia pandurata* (Roxb.) Schltr. *Eleutherine americana* Merr. and *Rhodomyrtus tomentosa* (Aiton) Hassk. as antibiofilm producing and antiquorum sensing in *Streptococcus pyogenes*. *FEMS Immunology Med Microbiol,* 53: 429–436.

Logambal, S.M. and R.D. Michael. 2001. Azadirachtin – an immunostimulant for *Oreochromis mossambicus* (Peters). *Journal Aquatice Tropics*. 16:339–47.

Logambal, S.M., S. Venkatalakshmi, and R.D. Michael. 2000. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Hydrobiologia,* 430:113–20.

Micol,V., N. Caturla, L. Pe´rez-Fons, V. Ma´s, L. Pe´rez, and A. Estepa. 2005. The olive leaf extract exhibits antiviral activity against fish pathogenic *Septicemia rhabdovirus* (VHSV). *Antiviral Research,* 66(2-3):129-136.

Pasnik, D.J., J.J. Evans, and P.H. Klesius. 2009. Patogenicity of *Streptococcus ictaluri* to channel catfish. *Journal Aquatic Animal Healthl,* 21 : 184-188.

Díaz-Resendiz, K.J.G., Toledo-Ibarra, G.A., and Girón-Pérez, M.I. 2015. Modulation of Immune Response by Organophosphorus Pesticides: Fishes as a Potential Model in Immunotoxicology. *Journal of Immunolo gy Research*. 213836

Tan, B.K. and J. Vanitha. 2004.Immunodulatory and antimicrobial effects of some traditional chinese medicinal herbs: a review. *Current Medicinal Chemistry*, 11:1423-14

Venkatalakshmi, S. and R.D. Michael. 2001. Immunostimulation by Leaf Extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Journal Aquatic Tropics*, 16:1–10.

Yin, G., G. Jeney, T, Racz, P. Xu, X. Jun, Z. Jeney. 2006. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. *Aquaculture*, 253:39–47.

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